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What's New

5.4 New Features

- [Mark Points](#) now has integrated support for spirals; everything previously possible with a single point can now be done with a spiral. This replaces the Spiral Activation feature #1404
- Added [-MarkPoints \(-mp\)](#) script command to mark points from a script. Based on parameters this command can either run the current Mark Point Series, run a saved Mark Point Series, or specify locations and pulse parameters to mark points on the fly #2964
- [Mark Points](#) dialog has been simplified by separating basic features from more advanced features (Mark Point Series) by using different tabs; the preview graph will show what will happen when the Run Mark Points button is pressed based on which tab is active. The basic interface now includes a pictorial representation of how various parameters affect the laser pulses at each point #1404
- [Mark Points](#) and [Point Scan](#) locations can now be moved using the arrow keys when selected in an image window; the point/group selection controls in the Mark Points and Point Scan dialogs have also been updated to allow grid and lines of points to be moved using the buttons provided #1404
- Added an iterations field to [Mark Point Series](#) which allows a Mark Point experiment to be repeated multiple times; previously the entire experiment had to be duplicated multiple times in order to repeat it #2989
- Added a live mode for [Mark Points](#) where interacting with the image window by clicking, and dragging, will expose the area being clicked with some predefined laser power and/or pulse; this can be used as a freehand photo-ablation tool among other things #231
- [Mark Point](#) Series definitions can now be saved to, or loaded from, a file in addition to being part of the [Environment file](#) #3121
- Added support to synchronize a [Mark Point](#) experiment with image acquisition with only one set of galvanometers using a high speed switch to momentarily give control to Mark Points; previously the switch needed to be driven manually by setting up a Voltage Output experiment and calibration of the uncaging lasers was quite cumbersome as the switch state needed to be constantly flipped back and forth manually #434
- When moving the galvos to a custom location, they will now return to that custom location after performing an acquisition, instead of returning to their park position #2262
- Added a ‘Reset to Center’ button to the [Maintenance](#) dialog which will set the custom galvo position to the center of the current field of view; this makes it possible to bring the customer position into the field of view if the previously set custom position wasn’t visible #2262
- Added support for the Single Axis Rotating Nosepiece #3011
- Orbital nosepiece support is now being handled at a lower level so that individual features no longer need to handle it explicitly; as a result the following are now true when using an orbital nosepiece: Z-Series now report step size correctly, Atlas Imaging works, and the stage/focus positions displayed are in the plane of the orbital nosepiece instead of absolute positions which is much more intuitive #3055
- Allowing a custom step size when measuring the piezo focus step response as part of

the piezo calibration process #3023

- Saved [ROIs](#) are now saved/loaded with [environment files](#), including predefined regions (ROIs with a predefined size in pixels, but with no set location on the sample) #762
- An XY stage position can now be recorded as part of an [ROI](#) definition; this is in addition to the ability to record a focus position with an ROI #2250
- [ROIs](#) now inherently store pan location for point scanning acquisition modes which means that it is now possible to scan multiple ROIs in the same field of view which do not appear in the same source image (the image which the ROI was defined on originally) #3034
- Removed some superfluous scan settings: Resonant/AOD X Ratio is now being calculated based on other scan settings and Volts Per Degree has been absorbed by the galvo range/performance settings it affected previously; this change will not affect the end user directly, but will eliminate some extra steps required to ensure [Mark Point](#) and [ROI](#) accuracy in resonant galvo mode #2473, 2983
- The UI associated with managing [ROIs](#) has been completely redesigned to more intuitive and less cumbersome #2285, #3034
- Added new script command, [-EnterROI \(-er\)](#), to enter a region of interest programmatically rather than drawing a region in an image window #2740
- Autoscale [lookup tables](#) option is now being saved/loaded with [environment files](#) #2995
- Added [-SaveEnvironment \(-se\)](#) script command to save the current environment to a file; the saved environment, or portions thereof, can then be loaded later #3093
- Augmented [-PanGalvo \(-png\)](#) script command to pan the scan a specific number of microns when numeric values are provided, and the current objective lens has been calibrated, instead of Volts #3094
- It is now possible to pan the galvos while scanning in resonant galvo mode by using the [-PanGalvo \(-png\)](#) script command #3073
- Including current scan center and current scan amplitude in the metadata for Ultima acquisitions; current X axis pan location is also included for AOD and resonant acquisitions #3161
- Added ability to control the time period displayed during a [Voltage Recording](#) via a new X-Axis button similar to the existing Y-Axes button #3102
- Improved visibility of errors on startup by turning the progress bar red, and keeping the startup splash screen open and scrolling back up to the first problem after the software has loaded; as a result the checkbox to manually keep the splash screen visible has been removed #3122
- Added a feature to [Atlas Imaging](#) to take a single image at all defined X-Y stage locations, using the current Z position. Additionally, these scans, as well as the existing overview scan, can now be saved if so chosen #3015
- Opening [Atlas Imaging](#) in conjunction with [Playback](#) mode now fills in the Atlas Overview window with all images taken at the same Z position #2624
- [Atlas Imaging](#) now supports changing the Optical Zoom even after experiment setup has begun #3151

- Added ability to set a remote access password on the [Edit Scripts](#) dialog to prevent unauthorized access to the system over the network via script commands; the password is user specific to prevent accidental script execution when multiple users are sharing the same system #3135
- Introduced an absolute laser power and PMT gain compensation option for [Z-Series](#), this allows a global table of powers and gains to be set at different focus levels and have Z-Series reference that table instead of specifying specific values; along with these changes the Z-Series UI was revisited to make space for the new controls and unclutter the existing UI #3129
- In addition to the buttons to go to the top middle and bottom slices of a Z-Series a new button was added to go to the selected [Z-Series](#) slice #3130
- System ID numbers and descriptions documented in the configuration file are now saved with each Environment file and data set as well as being displayed in the ‘Help’ -> ‘About...’ dialog; this information will help with service requests as well as providing a reference for which computer/system a particular file came from for sites with multiple systems #3061

5.4 Fixes

- Preventing XML files from being replaced with a corrupted backup file. Additionally there will no longer be a prompt to try loading a backup file if the backup is determined to be corrupt #2675
- [Environment files](#) will no longer be corrupted when setting a saved Mark Point Series to a quick load button without first setting all the quick load buttons to the left #2965
- Running Frames to Average scan parameter will now changes correctly when loading an [environment file](#) which is also changing the acquisition mode #3005
- [Lookup table](#) settings are once again loading correctly from [environment files](#) #2996
- Resizing [ROIs](#) using image window overlays is now a lot easier with handles only resizing in one direction instead of resizing the entire region around the center (and sometimes translating in X and Y as well) #173
- Moving an [ROI](#) will no longer cause it to change size #289
- Fixed a crash caused by including a saved [Mark Point](#) experiment within a [T-Series](#); this occurred when switching to/from camera/SFC mode or when exiting and restarting the program #2992
- No longer displaying an error message when switching from ‘None’, or ‘Crosshair’, to ‘Custom Order’ in the ‘Points/Groups’ column in the [Mark Point Series](#) grid #3007
- Fixed and issue where shutters weren’t being opened when running a saved [Mark Point](#) experiment using different lasers/shutters than the current experiment #3008
- Removed the ‘Other’ option which was appearing in the ‘Points/Groups’ column in the [Mark Point Series](#) grid; the option is used internally and wasn’t meant to be shown to the user #3009
- If communication is not established with the high speed shutter protecting the PMTs it will now show a red X at startup instead of a green checkmark #3078
- Moving the imaging galvos to a custom position using the [Maintenance](#) dialog is now possible without a GPIO box present/configured #3101
- Changed text on [Seal Test](#) start button from Acquire to Run Test to avoid any confusion about data being recorded #3114
- [Seal Test](#) is no longer initialized at startup when no patch amplifier is configured; the only observable change is that the splash screen will no longer show a line for Seal Test #3149
- Fixed some issues opening certain data sets in [playback mode](#); additionally when viewing data sets acquired on a different machine/configuration the laser names displayed in the metadata window will be correct for data sets acquired with more recent versions of Prairie View #3123
- Software will no longer crash when switching in and out of camera acquisition mode for the first time running the software after configuring a camera #3143
- [Mark Points](#) preview will no longer display time spent waiting for a trigger when no trigger is selected; previously if the trigger type was none, but the selection for when to trigger was not, the preview would still show the trigger #3139

- [Mark Points](#) now properly generates an uncaging signal to provide to the high speed shutter controller when synchronizing Mark Points with a Voltage Output experiment; previously the Voltage Output experiment would delay the high speed shutter signal so that the shutter would not close during the uncaging laser pulse and instead close later when nothing was happening #3138
- PMT gain columns no longer appear for a [Z-Series](#) when fastest acquisition is checked; previously the columns were shown, but the values were never changed as the hardware does not support changing PMT gains at the start of specific frames at max speed #2121
- Removed the ‘Software Control’ checkbox from the [Z-Series](#) controls and replaced it with a ‘Manual Z’ toggle button in the [Stage Control](#) section of the [main form](#); the original control didn’t actually have anything specifically to do with Z-Series, wasn’t being updated correctly, and wasn’t actually affecting the Z-Series focus device if it varied from the active Z device selected on the main form #1872, #2123
- Stopping a live scan, or changing settings while live scanning, will no longer hang the software while interlaced line scanning is enabled; this behavior was only observed in resonant mode, but theoretically could have happened in any mode, and also could have affected camera based acquisitions with multiple channels enabled #3150
- Fixed a typo in the message box alerting the user that the piezo hasn’t been calibrated for the selected objective lens #3152
- Eliminated a potential deadlock closing the software at the exact same time the current Environment is being automatically saved #3153
- Playback mode now works correctly on systems with no focus devices configured, the current focus device position also displays as ‘None’, to match the X and Y positions without a stage configured, instead of being left blank #3158
- Preventing an error message on shutdown when no XY stage is configured #3165

What's New

5.3 New Features

- Added a button to automatically adjust the [lookup tables](#) to the image window underneath the existing LUT button which, when enabled, will continuously adjust the lookup tables as new image data is acquired to maximize contrast #198
- Greatly improved the intuitiveness of [image window](#) overlay interactions, especially in cases where multiple activities/overlays are active at the same time; see the [image window](#) documentation for more details on the status bar information which was added #444
- [Line scan](#) cycles are now available within a [T-Series](#) acquisition #887
- Rows/Cycles in the [T-Series](#) grid can now be reordered #1286
- Added support for a motorized [Orbital Nosepiece](#) #2059
- Added ability to automatically zero PMT gains when not scanning; this is a new option in the [Preferences](#) menu #1286
- Improved integration of Nikon's Perfect Focus System so that script commands are no longer necessary; more details can be found in the [Z-Series](#) and [XY Stage tab](#) sections #2051
- Added [summation series](#) feature to Mark Points along with the ability to specify a [custom point order](#) directly in the Mark Point Series #2072
- Added [phosphorescence lifetime](#) (PhLIM) imaging/data acquisition support #229
- Scripts can now be aborted while performing an acquisition, previously aborting a script would first finish the current script command and any acquisition associated with it. Additionally the Pause/Break keyboard shortcut has been added to abort any acquisitions, experiments, and/or scripts in progress #1057
- Tooltips have been added to the line period and frame period displays to show which portion of the period is spent scanning vs. retracing, this is true of the main form and the Line Scan dialog #1078
- Can now use cursor keys during uncaging galvo calibration to make fine adjustments which are tedious using only the mouse #800
- Adjust PMT & Laser checkbox on [z-series](#) tab is now disabled at startup to reduce confusion and increase performance for users that aren't using the feature #2139
- Added password protection option to protect configuration files and scan settings on [multi-user systems](#) #785
- Enabling binning filter in the scan settings dialog, with fewer than four channels enabled, no longer crashes software #2295
- [Script commands](#) can now be used to return values including current scan settings and stage/focus positions #302
- Greatly improved the image data transfer performance of PrairieLink in addition to adding more functionality, and making it easier to use; see the new [PrairieLink](#) section for more details #773, #1462, #2056
- Open in playback mode checkboxes on the [line scan](#) and [point scan](#) dialogs now override the preferences menu option used for other acquisitions (i.e. t-series) #582
- Removing a single [brightness over time](#) region or function no longer requires additional

confirmation, only when clearing all regions or functions is there an additional dialog to confirm the change #858

- Added ability to translate a circular [line scan](#), without resizing the circle, by right clicking and dragging #700
- [Mark Points](#) will now always save metadata when run, for those correlating data acquired with third party software; previously Mark Points only saved metadata when combined with either image data or a voltage recording #1243
- Spectra-Physics lasers: support for MaiTai with DeepSee unit and Insight #2064
- [Spectral unmixing](#) implementation for spectral SFC #2067
- Added support for changing settings during [XZ/YZ](#) live scans #1554
- Coherent laser support: allow Prairie View to take the laser in and out of standby #786
- Support for line scans consisting of multiple separate straight [line segments](#) #191
- Added a [Basic Acquisition Mode](#) for the SFC #2069
- Added support for [SFC widefield](#) bypass accessory to collect widefield image data from the camera normally only available for confocal swept field imaging #2406
- Made improvements to [SFC alignment wizard](#) #2413
- Added support for [dual camera](#) imaging using splitting optics to collect two channels of data simultaneously on two different cameras #2058
- Added a [Multi-pane Spectral View](#) for Opterra Spectral Mode so that all 16 spectral channels may be visualized simultaneously in real-time #2065
- Added an integrated [spectral calibration](#) mode #2066

5.3 Update 1 New Features

- Added support for Photometrics CoolSNAP MYO camera #2287

5.3 Update 2 New Features

- Added support for Bruker 250 µm, 400 µm and 1000 µm objective mounted piezo focus devices #2634, #2859
- Added support for high-speed shutters to protect PMTs while uncaging #2166
- Added support for Hamamatsu ImagEM X2 (C9100-23B) and Hamamatsu ImagEM X2-1K (C9100-24B) cameras #2720
- Added support for Prizmatix UHP-T White LED as a replacement for the traditional transmitted lamp on the Opterra to allow for transmitted light imaging within a confocal imaging sequence #2737
- Acquiring an Atlas Volume now automatically saves the Atlas Overview image into the References folder of the acquisition #2762
- Extended the [-StreamRawData \(-srD\)](#) script command to include an optional parameter specifying the number of frames to buffer, if the number of frames to buffer is greater than zero then all image data will be streamed instead of only buffering the most recent image data; this is useful for applications wanting to process all the image data in real-time opposed to just processing the most recent data to make a decision #2772
- Enhanced the [Opterra Widefield](#) mode by integrating support for automatic LED source switching (when using the XLED), filter wheel sequencing (when using the FLI filter wheel), and frame trigger generation. This also allows max speed Z Series to be run in Widefield mode #2500
- When using the FLI filter wheel, a value can now be set (and is automatically defaulted) to delay the time between frames, thus allowing the desired filter to get fully into position and vibrations to settle #2935
- Relevant scan settings are now being saved/loaded when switching [SFC imaging modes](#), these settings are also preserved between sessions #2811
- Increased maximum number of slices allowed in a [Z-Series](#) from 1,000 to 10,000; improved performance of Z-Series grid updates to compensate for the additional load #2209
- Reimplemented the [Dual ROI](#) scanning feature which has been temporarily removed since version 5.0; this feature allows two separate regions of the sample to be rastered simultaneously #439
- Added support for new preamplifier blades #2880
- Added flimADCResolution (FLIM temporal bit depth) parameter to the XML metadata file in order to make analysis of raw data easier #2906
- Added support for [Nikon Eclipse Ni](#) automated microscope including software control of light path, transmitted light intensity, integrated stage and focus, objective and filter turrets; [script commands](#) have also been made available #2863
- New script command, [-OverrideHardShutter \(-ohs\)](#), added to force a shutter to open, and remain open, for the duration of a scripted experiment #3081
- Improved performance of sequential resonant galvo acquisitions by reducing the delay between them by 38% when using firmware version .57 or later #3085

5.3 Fixes

- False coloring of image data is now performed before the image is scaled to be displayed in the image window(s), previously the false coloring was done on the scaled image which resulted in false colorings like range check not rendering correctly unless the image window zoom was set to 1:1 #2434
- The Image-Block Ripping Utility, and playback mode in Prairie View, will now check the version a data set was collected with before attempting to convert any raw data in order to prevent corruption or loss of data; the raw data format has also changed slightly in 5.3 so that previous versions will fail safely when attempting convert a data set acquired with version 5.3 or later #2283
- Drop down widths for all combo boxes have been standardized to be at least as wide as the combo box control and wide enough to display the longest selection option #2253, #2426
- Channel intensities displayed at the bottom of the image window now update when the image updates in addition to when the cursor moves #2289
- Replaced the giant hand cursor used to click and scroll an image window with scrollbars with a smaller graphic which more accurately points to the selected pixel with the index finger #2245
- When opening a new image windows the buttons along the sides will now be properly highlighted based on which features are currently active #1550
- Changing scan settings while live scanning should no longer occasionally lock up the software (this was especially apparent with a 2P laser configured in spiral acquisition mode) #2315
- Aborting a [Voltage Output](#) experiment with one or more waveform setup dialogs open will no longer hang the software #2437
- Synchronizing a [Mark Point](#), [Voltage Output](#) and/or [Voltage Recording](#) experiment with multiple repetitions of a [Z-Series](#) within a [T-Series](#) will only run the Mark Point, Voltage Output and/or Voltage Recording experiment once instead of every repetition, to be consistent with other T-Series acquisitions; this fix also eliminates a problem where synchronizing with a specific frame which isn't in the first repetition would cause the T-Series to wait indefinitely until aborted #2286, #2270
- Fixed a number of issues regarding [T-Series](#) timing information displayed when using multi-track Parameter Sets, Spiral and/or camera acquisition modes #2510
- Required disk space calculations for [T-Series](#) in Camera and SFC acquisition modes are no longer grossly overestimated when the photoactivation dwell time was larger than .4 us #2374
- When running an [T-Series](#) with multiple repetitions at all stage locations, the stage location is now explicitly reset each repetition in case the stage/focus move during the repetition; before this fix multiple iterations of a Z-Series would drift as each stack gets acquired from a different starting position #2478
- Fixed an uncommon issue where frame triggers would stop being counted on systems with a GPIO box and an SFC or camera configured; a workaround was to choose not to

generate any triggers under the preferences menu and then set up the triggers again as desired #2531

- SFC [high speed filter wheel controls](#) are now hidden when leaving SFC acquisition mode, previously these controls were obstructing settings for camera acquisition mode #2422
- SFC Analog Z focus control now works with multiple cameras and splitting optics #2483
- When using [dual splitting optics](#), either two channels with one camera or two cameras each acquiring a channel, [SFC trigger lines](#) will now correctly work when two exposures are required to acquire all the enabled channels (this is true when both channels 1 and 3 are active, or both channels 2 and 4 are active); additionally frame acquisition times written to the metadata are now also correct under the same conditions #2353, #1843
- Removed the option from the [Nikon Eclipse Ti Controls](#) to explicitly retract the currently objective lens prior to moving the turret to another position, this is handled implicitly by the microscope itself and eliminates an issue where the wrong objective would be selected if there wasn't enough focus travel available for the current objective to escape #125, #1889
- Fixed an issue where the list of objectives available on the [Nikon Eclipse Ti Controls](#) dialog wasn't being populated correctly at startup #126
- Fixed a potential deadlock when switching objectives on the [Nikon Eclipse Ti Controls](#) dialog while live scanning #1559
- Switching to camera mode no longer enables Dodt detector controls when no Dodt detector is configured #2468
- Parameter Set selection control, in the [Scanning](#) section of the main form, is now enabled in camera mode making it possible to use [Parameter Sets](#) outside of a T-Series #2467
- Hamamatsu cameras are now being properly uninitialized, previously this could have resulted in the software to hang on shutdown or fail to reenter camera mode after switching to a different acquisition mode #2474
- Preamplifier filter bank selection drop down is no longer shown when no preamplifiers are configured #2272
- Setting both the [Z-Series](#) start and stop positions near the same limit will no longer hang up the software #1858
- Using the Goto start, middle, stop buttons on the [Z-Series tab](#) will no longer move multiple focus devices when more than one focus device is configured #2512
- Dragging a cursor along a freehand line scan is now much easier, the cursor will snap to the nearest point instead of forcing the mouse cursor to follow the line with a lot of resistance #1326
- Circle, Spiral and Lissajous line scan definitions are now properly restored from previous session on start-up #2345
- State of scan continuous checkbox on line scan dialog is now correctly checked or unchecked correctly (based on setting from previous session) on start-up #2323
- Number of pixels displayed on line scan dialog is now correct after exiting playback mode #1719

- Fixed a typo in the file extension displayed when saving a line scan profile CSV file #2277
- Reworded [raw data conversion](#) during acquisition option to be more accurate, previously it incorrectly mentioned it could affect data integrity #2336
- Drawing elliptical regions is now handled the same way throughout the software, click the center and drag outward to enlarge #261
- Dragging the photoactivation mask transparency slider up/down now properly updates the overlays #2294
- Buttons to reorder scripts, and script categories, in the script editor dialog now work correctly under all circumstances #2349
- Changed the way scripts are saved and loaded to eliminate some issues caused by various fields getting out of sync #1565, #1566
- Stage and focus positions are now displayed as 'None' when no device is configured instead of '0.00000' #2277
- Moving to the selected, previous, or next [stage position](#) now works after moving the stage/focus manually #204
- Multiple repetitions of a point scan at the crosshair location are now correctly acquired in a single acquisition instead of being split up with a delay between them (this was only an issue with uncaging galvos configured); they are still being correctly split into multiple acquisitions when simultaneously uncaging #2268
- Scan until stopped option on the point scan dialog is working again #2267
- Point scan feature is disabled more consistently between acquisition modes which don't support it: SFC, camera, resonant galvo, and AOD modes #651
- Custom point orders containing descending ranges (i.e. 10-1) are now interpreted correctly on the mark points and point scan dialogs #2389
- [Mark Points](#) will now open the hard shutter after acquiring a reference image on systems without uncaging galvos #2508
- [Mark Points](#) will no longer briefly attempt to drive the imaging galvos to their center position in the middle of an experiment when only one set of galvos is preset/configured #2498
- Uncaging calibrations are now correctly interpreted in resonant galvo mode, this was especially noticeable when panning and/or using ROIs and affected both [Mark Points](#) and [Spiral Activation](#) #2470, #1809, #1051
- A newly created uncaging calibration from the previous session will now correctly load the next time the software is run instead of reverting back to the previous uncaging calibration #2314
- The endpoints of the [line profile](#) tool overlay now have priority over the cursors, so it is no longer possible to drag the line really short and be unable to interact with it #2244
- Locking the computer, or having a screen saver pop-up during an acquisition will no longer crash the software when unlocking the computer, or returning from the screen saver #622
- Context menus for the channel and LUT buttons now work for the [Atlas](#) image window just like other image windows #2243, #2239

- Opening [Atlas Imaging](#) no longer hides the lookup table form #2237
- Minimizing Prairie View while [Atlas Imaging](#) is active no longer hangs up the software #2236
- LUT button is now correctly highlighted green on the [Atlas image](#) window when the lookup table form is open #2238
- [Atlas Imaging](#) now opens with all previously enabled channels enabled in the Atlas Image window #2395
- [Atlas Imaging](#) acquisitions now appropriately wait for the stage to reach its target position before beginning to acquire data #2394
- Returning from a screen saver or locked computer with [Atlas Imaging](#) open no longer crashes Prairie View #1552
- [Atlas Imaging](#) is now more responsive, regardless of image resolution #1533, #1497
- Software control of Physik Instrumente piezos (PI-E662 and PI-E665) works again #2290
- Removed option to sum samples for each pixel from the [dwell time controls](#), and [point scan](#) dialog, as it was possible to overflow a 16-bit TIFF file and lose data; to increase contrast and/or dynamic range use the [lookup tables](#), [DAQ/camera gain](#), and/or [preamplifier gain](#) instead #872
- Creation of new [Parameter Sets](#) is now possible after loading an environment file without any Parameter Sets defined #2523
- DAQ Gain parameter now works properly when included in [Parameter Sets](#) #2530
- If a [Parameter Set](#) fails to load for any reason a warning will appear at the bottom of the main form, this can occur when attempting to change image resolution and dwell time in the same Parameter Set #2462
- Changing a [Parameter Set](#) that was previously added to, and then removed from, the T Series no longer displays an error #2399
- Secondary laser beam route parameter is no longer accessible in [Parameter Sets](#) when the feature is not configured #2459
- Limiting manual entry of parameter set values to valid values for all parameters which aren't camera/SFC acquisition mode specific #1950
- Warning and error messages displayed at the [bottom of the main form](#) no longer disappear immediately #2030
- Scan rotations entered by the user are now consistently being clipped between -180 and 180 degrees, values outside that range are offset by a multiple of 360 degrees so that they are within the specified range for display purposes #2466
- Recalculating BOT data in playback mode no longer leaves the live scan button enabled occasionally when aborted and restarted quickly; if the live scan button was pressed in this state, the software would crash #1564
- Copying a corrupted configuration files folder from a previous version will no longer pop up error messages on shutdown #2271
- Helios ND filter positions are now displayed in the playback metadata window when applicable #1065
- Opening the Notes dialog for the first time using the F8 keyboard shortcut no longer

locks up the software #2522

- Helios ND filter combo boxes now update when the filter is changed using a script, Parameter Set, Template or Environment #2485
- [SetAcquisitionMode](#) script command is no longer allowed within a T-Series as it wouldn't be factored into the timing calculations #2472
- Aborting an acquisition before any frames are collected, usually while waiting for a start trigger, will no longer write XML metadata or TIFF files for a frame which was never acquired #2145
- BOT, Line Scan, and LUT dialogs are now opening on startup if they were open in the previous session; additionally many windows which were opening prior to the splash screen disappearing are no longer doing so #430
- Lookup tables histograms no longer flicker to zero while live scanning #2016
- Lookup table intensity mapping indicator (yellow plot) no longer plots incorrectly when raising the brightness and zooming in #324
- Pressing the auto adjust button on the lookup table dialog multiple times no longer produces different results #325
- Greatly improved performance communicating with Device Control, most noticeably this eliminates lag dragging PMT and laser sliders #2516
- Aborting an acquisition using a [photoactivation](#) mask after changing [image resolution](#) will no longer result in incorrectly blanking the laser #2501
- Using [photoactivation](#) masks with [regions of interest](#) (ROIs) will no longer change the optical zoom to 1; this change wasn't reflected in the optical zoom display on the main form #2479
- Fixed raw data conversion fix for camera-only systems where no focus devices are configured #2321
- Improved communication with Spectra-Physics lasers #164
- Preventing unexpected wavelength changes when using Spectra-Physics lasers #1030, #2292
- Improved power-on and warm-up control of Spectra-Physics lasers. #2062
- Certain versions of the Spectra-Physics MaiTai caused laser to go into standby mode unexpectedly #2257
- Spectra Physics MaiTai: fixed error message when clicking status update button in 2P laser tab #2258
- Revisited drawing freehand lines for [XZ/YZ Scanning](#); it is now possible to draw straight line segments, extend an existing line or translate the entire line (much like freehand [Line Scans](#)) #1881
- Fixed incorrect Z position data in OME Tiff for multiple Z-Series repetitions #2401
- PhysicalSizeZ in OME Tiff meta data no uses Z step size to work with PSF report generator #2105
- Fixed incorrect .ome.ome.tif extension for saved live scan images #2127
- [Line Scan](#) data sets opened in [playback mode](#) now also display image data in the Line Scan dialog #2223

- Voltage recording raw data is now being converted correctly when opening a previously unconverted data set in playback mode #2529
- The Z-position drifts between multiple repetitions of an XZ/YZ non-fastest acquisition in a T-Series which does not keep the hard shutter open (not max speed) #2296
- Playback of voltage recording data in a T-Series with multiple iterations now works correctly #2441
- Exported CSV for changed and recalculated line scan profiles in playback mode is no longer incorrectly scaled #2324
- The main window now displays the correct pixel size width for non-square fields of view #2373
- Stop triggers now work when: in SFC or camera acquisition mode, closing the hard shutter between images, using a multi-track Parameter Set, or any other acquisition where max speed was not checked and not enabled automatically to improve performance #1555
- Stop triggers now work with asynchronous photoactivation (note: SFC box trigger input is not supported) #1967
- No longer creating precalculated MIP images for Line Scans split across multiple TIFF files #2198
- Line scan profiles crash for line scans split amongst multiple Tiffs fixed #2453
- Fixed a situation which might cause incorrect timestamps in recalculated BOT data #2486
- No longer generating incorrect galvo drive waveforms after shutting down Prairie View immediately following a freehand/circle/Lissajous or spiral Line Scan #2308
- [FLIM settings dialog](#) now properly snaps to other windows, remembers last location, and is made translucent by ghost mode #2423
- Acquiring a reference image in FLIM acquisition mode, for a line scan specifically, no longer crashes the software #2311
- Pixel photon counts displayed at the bottom of the image window in FLIM acquisition mode are no longer multiplied by a multiple of the current dwell time #2284
- Custom Output controls on the [Misc tab](#) are now working again #2322
- Possible responses to the exit prompt, “Are you sure you want to exit?”, have changed from Ok/Cancel to Yes/No #2235
- [Nyquist Sampling](#) feature now works correctly when changing the laser type between 1P and 2P #246
- Improved spectral accuracy and removed spectral banding artifact by introducing real-time interpolation algorithms #2063
- Fixed an issue where if not live scanning, changing a spectral bin in Spectral Mode did not update the image window data until the subsequent scan #2119
- Status updates displayed at the bottom of the main form once again include the save path for all acquisition types, previously some acquisition types, such as T-Series, BOT and Line Scan, were missing this information #2320

5.3 Update 1 Fixes

- [Voltage Recordings](#) included within a [T-Series](#), or [Line Scan](#), will now respect the [Automatically Convert Raw Files](#) option in the [Preferences menu](#) and wait until the end of the acquisition to convert raw data when appropriate #2542
- Running a [Mark Points](#) experiment within a [T-Series](#), on a system not configured with uncaging galvos, will no longer re-enable controls before the T-Series finishes #2542
- Performing multiple iterations of a [T-Series](#) containing a [Mark Point](#) experiment, synchronized with one or more voltage recordings, will no longer overwrite voltage recording data for previous iterations (this was also a problem for multiple Mark Point experiments synchronized with voltage recording in the same T-Series without multiple iterations) #2542
- Acquisition specific scan settings (galvo, resonant galvo, SFC photoactivation, AOD plunger settings) have been migrated into the [Environment file](#) to: take advantage of the auto-save feature, protect system specific scan settings, and make the rest of the settings user specific; as a result these settings can no longer be edited in the Configuration Utility #2600
- Moving the stage/focus manually after [adding a stage location](#) will no longer move back to that position after modifying another parameter (i.e optical zoom or dwell time) #2554
- Fixed some UI issues related to moving rows around within a [T-Series](#) #2544
- Inserting imaging cycles into a [T-Series](#) will now properly update the T-Series timing information; adding imaging cycles was working correctly #2605
- Changes to the Execution Order setting in the [T-Series Preferences](#) dialog will now be loaded properly when bringing up the dialog instead of getting changed back to the default value #2626
- [XZ/YZ](#) image window overlays no longer change the mouse cursor to resize or rotate icon when mousing over different parts of a rectangular handle #2563
- [Preamplifier gain](#) slider button is now called ‘Reset’ instead of ‘Zero’ since a multiplicative gain of zero doesn’t make sense; contact a Bruker Fluorescence Microscopy representative about configuring the UI to display 1 as the minimum gain setting instead of 0 #2559
- Mentioned use of the Pause/Break keyboard shortcut, used to abort any acquisitions, scripts, and/or experiments in progress, in the Keyboard Shortcuts dialog accessible through the [Help menu](#) #2556
- [SetState](#) script command is no longer allowed within a T-Series as it wouldn’t be factored into the timing calculations #2551
- [SetAcquisitionMode](#) script command no longer throws an exception when trying to flip between galvo mode and SFC/camera mode #2558
- Ensuring all parameters of a [Parameter Set](#) are applied properly by applying perquisite parameters first, previously a warning would be displayed that not all parameters had been applied #2545
- Editing values in the active [Parameter Set](#) manually no longer changes the parameter as it is being entered; the change will not take effect until <Enter> is pressed, or the cell

loses focus #2543

- Selecting a Parameter Set on the left side of the [Parameter Sets](#) tab, after having no Parameter Set selected, will no longer truncate Parameter names in the Parameter Selection section in the center #2461
- Copying [Parameter Sets](#) now works correctly, previously the copy needed to be renamed to work properly #2460
- Updated reference to legacy Label Groups feature in Single Scan button tooltip to mention multi-track [Parameter Sets](#) instead #2458
- Correctly saving/loading settings on the SFC [Triggers tab](#); previously any channel that had previously been enabled, even if disabled later, would be re-enabled on startup #2340
- Times in the [linescan profiles](#) plots, but not the CSV files, were incorrect. This could lead to data points being plotted out of order #2590
- AOD plunger scan settings are now being applied correctly, previously dwell time, optical zoom, and image resolution failed to change to the correct value in some circumstances #2594
- Resolved an issue upgrading from an older versions of software with a resonant galvo configured, in some cases there was an exception attempting to record the scan rotation for galvo mode #2595
- [Notes](#), [Seal Test](#), [Voltage Output](#), [Voltage Recording](#), [Spiral Activation](#) and [Atlas Imaging](#) now properly save/restore whether or not they are open at shutdown and start-up as well as when saving/loading an [Environment file](#) #2548
- Closing Prairie View with [Atlas Imaging](#) open no longer results in an extra image window being opened the next time Prairie View is started #2561
- Any changes to the [notes](#) are now saved on exit, or when saving an [Environment file](#), previously any modification made to the notes since the last acquisition were not saved #2593
- Multiple repetitions of a [Line Scan](#) within a [T-Series](#) with multiple iterations no longer fails #2547
- Opening a post-processed [spectral data set](#) in [playback mode](#) will no longer load as an unprocessed spectral data set #2604
- Stop triggers now supported for [Line Scans](#) in a [T-Series](#) #2439
- Improved support for changing scan parameters such as resolution, dwelltime, rotation and zoom during an [XZ/YZ](#) live scan #2581, #2582
- Piezo motion wasn't properly aborted for [XZ/YZ](#) live scans during which resolution, device or number of lines was changed to or from one #2579
- Sometimes after shutdown [XZ/YZ](#) scans were restricted to XZ only the next run of Prairie View #2580
- Incorrect waveform generation for segmented [Line Scans](#) was resolved #2571
- Improved response time changing [Line Scan](#) settings for spiral and Lissajous line types; other related changes made in this release also fixed a potential issue where a line scan waveform was being used to collect full frame data #2615

- [Voltage Recording](#) settings moved to environment file #2217
- [Seal Test](#) settings moved to environment file #2218
- Speedup for uncalibrated, unidirectional multi-frame [XZ/YZ](#) scans #2252
- Render errors no longer occur when using [Atlas Imaging](#) #2622
- The Clear All button in [Atlas Imaging](#) no longer hides the scale bar #2621
- [Atlas Imaging](#) now updates its overview window more frequently when not scanning, including after generating a montage and clearing all locations #2576
- Cycling through controls in [Atlas Imaging](#) using the Tab key now follows a more linear fashion and clicking the Generate Montage button no longer gives focus to the Clear All button #2575
- The preview function in [Atlas Imaging](#) no longer acquires images that contain motion artifact #2574
- The scale bar in [Atlas Imaging](#) is now positioned such that the entire bar and associated text is visible #2568
- The overview window in [Atlas Imaging](#) no longer automatically zooms in when moving the stage location near the center #2567
- Changing the color of a channel in any image window, including [Atlas Imaging](#), now pops up the color selection dialog next to the corresponding channel button #2534
- Setting the current XY stage location to (0,0) using the “0” button in the Stage Control section of the Main Form while using [Atlas Imaging](#) no longer shifts the overview window #2429
- [Playback mode](#) can now be entered while using [Atlas Imaging](#) without error #2428
- The preview function in [Atlas Imaging](#) no longer causes the overview window to resize #2427
- The interpolation/extrapolation used in [Atlas Imaging](#) no longer returns values far outside the expected range #2265
- The default values for z ranges in [Atlas Imaging](#) are now set to the current z motor position #2138
- The Take Snapshot button in [Atlas Imaging](#) no longer causes an error if no previous scan had taken place #2633
- [Atlas Imaging](#) montages are now correctly setting the GridIndex parameters in the metadata so that the Prairie Reader plugin interprets each location appropriately #1568
- Support for dragging yellow image position indicator overlay between multiple [line segments](#) #2572
- Changing the focus device on the [Z-Series](#) tab, after setting the start and stop limits, when multiple focus devices are configured, will no longer display/goto the wrong middle position #2629
- Setting [Z-Series](#) start and stop limits before visiting the Z-Series tab will no longer ignore the limits being set #2334
- [Line Scan](#) yellow image position overlay is now drawn on top of all other line scan related overlays so it is obvious when an endpoint which previously obscured the position overlay is selected #2412

- Switching between the [Basic Acquisition](#) and Advanced Acquisition methods now correctly returns camera controls appropriately #2549
- The [Basic Acquisition](#) form size is now correct when switching back and forth from Advanced Acquisition #2546
- Collapsing and expanding groups in the [Basic Acquisition](#) form no longer causes the Advanced Opterra Control window to show #2540
- Defining an acquisition in the [Basic Acquisition](#) without any [Parameter Sets](#) defined no longer causes an error #2539
- [Line scan](#) profiles line boundary constraints are correctly enforced again #2635
- Stage and/or focus devices will no longer take off when moving the device after just starting the system and then changing a scan setting in Prairie View; this was easiest to reproduce when zeroing the stage and focus in the software before shutting down the system #2251

5.3 Update 2 Fixes

- Headers in [Voltage Recording](#) data files (.csv) are now correct, previously the header names were incorrect in cases where the enabled channels had changed without restarting the software #2754
- Fixed an issue where dragging a PMT gain slider could lock up the software for an indeterminate amount of time #2767
- Fixed an issue where dragging the preamplifier offset and/or gain sliders could lock up the software for an indeterminate amount of time #2780
- Software no longer crashes when attempting to uncage a single point; this occurred using either the Mark Point at Crosshair/Mark Selected Point section of the [Mark Points](#) dialog or the burn spot option when performing an [Uncaging Galvo Calibration](#) #2666
- The [Mark Points](#) preview, at the bottom of the Mark Points dialog, is now correct when synchronizing uncaging of points with both a [Voltage Recording](#) and [Voltage Output](#) experiment #2810
- [Line Scan](#) overlay captions at the bottom of the image window now correctly identify the activity as Line Scan, additionally using the Ctrl+Tab keyboard shortcut to change overlays interaction priorities now works again, as does clicking in the line scan dialog to give Line Scan overlays the highest priority #2412
- [Line Scans](#) with multiple repetitions, which are also synchronized with a [Voltage Recording](#), [Voltage Output](#) or [Mark Points](#) experiment each repetition, will no longer crash the software while setting up the second repetition #2755
- Resolved a random (~4% chance) problem when shutting down where the current [Environment file](#) could be in the process of being auto saved; this caused the software to not shut down properly and could also result in corrupted Environment files #2676
- [Environment files](#) no longer grow to enormous sizes when one or more Parameters are configured for the [Parameter Display](#) dialog, this was causing the software to load slower and slower over time #2674
- Channel 2 data is now displayed in [MIP \(Maximum Intensity Projection\)](#) mode with non-classic pseudocolor coloring selected #2673
- Depth based coloring in [MIP \(Maximum Intensity Projection\)](#) mode now works correctly again; this was broken during 5.3 development #2672
- Fixed a number of software crashes related to enabling/disabling channels while using [MIP \(Maximum Intensity Projection\)](#) mode or exiting playback mode while MIP mode was enabled #2671, #2637. #1388
- Enabling [MIP \(Maximum Intensity Projection\)](#) mode for a dataset containing a significantly large number of frames/slices no longer resizes the image window to be unusably small, making it impossible to disable MIP mode #2636
- Selecting a sequence in the playback metadata window no longer occasionally throws up an error message #2748
- Fixed [BOT Drive Signals](#) which synchronize an analog output with the brightness of a particular Brightness Over Time region, they had been broken since before version 5.0 #2649

- The state of the ‘Zero/Restore PMT Gains With Imaging Shutter’ option under the [Preferences](#) menu is now properly saved/restored between sessions, additionally the PMT gains used for the last acquisition of the previous session are used for the first acquisition of the next session when this feature is enabled #2714
- Displaying line scan updates at the bottom of the main form when acquired as part of a T-Series, previously these updates were only shown in the line scan dialog #2713
- Updating line scan images at the bottom of the line scan dialog when line scan data is acquired as part of a T-Series, previously this image data was never shown #2713
- No longer displaying multiple updates for each part of a longer line scan broken up into multiple TIFF images #2713
- Fixed a number of issues pertaining to defining a manual laser power gradient for a [Z-Series](#), the most noticeable of which is that values are no longer reset to zero after editing another cell #2256
- Yellow line scan position marker is now removed from the image window(s) in addition to the line scan dialog images when starting a new line scan acquisition; previously the marker was only removed from the line scan dialog images #2524
- Opening the [Fluorescence Unmixing](#) dialog under the Tools menu, with no unmixing channels enabled, no longer corrupts the images displayed in the image windows #2318
- Status updates displayed at the bottom of the main form now include the save path for aborted acquisitions #2320
- Line period in Camera and SFC modes is now displayed as ‘Not Applicable’ instead of ‘0 ms’ #2276
- Multi-channel FLIM acquisitions, where router channels are mapped to different display channels, now work as expected; for example the default mapping of router channel 3 to display channel 2 required display channel 3 to be active in order to collect data on channel 2 #2722
- Script commands can now be executed during all acquisitions by using the [DoNotWaitForScans \(-dw\)](#) command, previously this was only allowed while running a T-Series or live scanning #2723
- Raw data streaming performance via [PrairieLink](#) or [script command](#) has been increased by returning at most two frames of new data in one call instead of only one #2756
- Now properly restoring from a backup environment on shutdown if an exception is thrown while saving an environment file; previously the file was left open and it was not possible to replace it, but the backup would be used properly at startup #2727
- Ctrl+Tab keyboard shortcut for image window overlays is no longer popping up an error message when only one overlay is shown and more than one image window is open #2728
- The [Ratio](#) image window can now be opened at the same time as the [Line Scan](#) dialog without crashing the software #2729
- Acquiring a z-stack at all stage position from the [Z-Series](#) tab will now correctly position the z-stack in relation to the saved stage/focus positons #2725
- Set current Z location to zero button is now correctly disabled at startup for focus

devices which do not support it #2504

- Tear out windows, such as the T-Series tab which can be popped out of the main form and places elsewhere on the screen, are now properly displayed in the Windows taskbar when the software starts up #2457
- Warning message about various types of line scans not being allowed in the current acquisition mode will no longer appear when exiting playback mode in camera or SFC modes #2451
- The line scan dialog is now closed when exiting playback mode when using the SFC or camera acquisition modes, previously the dialog was left open and it was possible to attempt to acquire camera images as line scan data #2451
- Fixed several bugs with the contrast gain and multiplication (EM) gain controls when using a Hamamatsu camera #2720
- Software will no longer crash when changing to a camera with fewer readout ports available #2730
- Fixed 'Bulb' mode triggering for the Opterra when using a Hamamatsu ORCA Flash4.0 V2 (C11440-22CU) or ORCA Flash4.0 LT (C11440-42U) version of the Flash4.0 camera model #2724
- Using high binning values in SFC and/or camera mode no longer sizes the image incorrectly, which led to jumbled image data #3119
- Fixed a bug where entering SFC mode more than once caused the Gain and EM Gain controls to not be available for use on the user interface #2736
- Pressing the “0” button in the Stage Control section of the Main Form prior to opening [Atlas Imaging](#) no longer causes Prairie View to freeze #2726
- Pressing the Z “0” button in the Stage Control section of the Main Form prior to opening [Atlas Imaging](#) no longer causes an error message to be displayed #2804
- Using [Atlas Imaging](#) with an inverted Z device now performs Z series in a direction consistent with the desired series #2744
- The Z ranges used for extrapolated locations in [Atlas Imaging](#) when generating a montage from only 2 locations are now correct #2745
- Opening [Atlas Imaging](#) and then loading an environment which doesn't have Atlas open no longer causes error messages nor loss of image windows #2749
- Opening and closing [Atlas Imaging](#) multiple times and then pressing the “0” button in the Stage Control section of the Main Form no longer causes the Atlas Overview window to shift erroneously #2758
- [Atlas Imaging](#) acquisitions now use the Z step size entered at the time the acquisition is started, instead of that saved when the first stage location was added #2763
- [Atlas Imaging](#) Overview window overlays now continue to update after closing and reopening Atlas Imaging; this was also a problem after loading an [Environment file](#) #2818
- Performing an acquisition while [Atlas Imaging](#) is open now continuously and persistently updates the Overview window with an image from the current location #2362
- [Atlas Imaging](#) now uses the entered overlap for generating a montage, instead of being locked at 15% #2136

- Opening [Atlas Imaging](#) for the first time no longer clears all previously defined stage locations #2975
- Interpolation of focal depths in [Atlas Imaging](#) when using single planes now works with multiple Z devices #3116
- Closing and Reopening Prairie View with [Atlas Imaging](#) open no longer starts up with the bottom slice automatically defined as the current stage position plus 1 um #3118
- Image intensities displayed at the bottom of the [Line Scan](#) dialog are now correct for freehand line scans where the minimum dwell time for line scans exceeded the minimum dwell time for raster scanning; additionally the intensities are now correct in playback mode #2603
- Changing the dwell time after acquiring an image, or acquiring channels with different dwell times, will no longer cause the pixel intensity information at the bottom of the image window(s) to be off #2732
- Changing the dwell time after acquiring an image, or acquiring channels with different dwell times, will no longer cause the brightness of the image window(s) to change unexpectedly; this was mainly a concern when using a multi-track [Parameter Set](#) or when exiting [playback mode](#) #2733
- Aborting an acquisition waiting for an input trigger will no longer start driving the galvos/lasers for a brief period of time while the acquisition is terminated #735
- [Look Up Table \(LUT\)](#) histogram data is no longer cleared out for frozen or disabled channels, instead the previous histogram remains until new data is available #2536
- [Z-Series](#) laser powers displayed in the grid, for a laser in attenuation [power calibration](#) (% of max power), are no longer 100x larger than expected; the laser power used during acquisition was always correct #2247
- Dodt mirror will now be automatically moved out on shutdown to allow the lamp house to be used prior to restarting the software #2606
- [Z-Series](#) timing information displayed on [T-Series](#) tab is now correct for multiple repetitions of a fastest acquisition Z-Series collected at max speed when using a Bruker piezo in calibrated mode, previously it was grossly overestimated in this case #2742
- Fixed a potential crash starting an acquisition, this was originally observed starting a [Z-Series](#) #2743
- Stage will no longer take off when starting a [Z-Series](#) with the orbital nosepiece enabled, previously the X and Y positions were doubled #2753
- Pressing the [2P Laser](#) status buttons for a Coherent Chameleon laser which isn't connected will no longer pop up an error message, but will instead display "No Response" #2760
- Timing information for [T-Series](#) cycles is now correct when imaging an ROI in resonant galvo mode #2761
- "No ROI" cycles in a [T-Series](#) are now respected and will force subsequent imaging cycles to acquire whole frames #2764
- Controls on the [Mark Points](#), [Voltage Recording](#), [Voltage Output](#), [Seal Test](#) and [Maintenance](#) Dialogs will no longer be enabled in error during a [T-Series](#) after a Mark

Point, Voltage Output, or Voltage Recording #2542

- Warnings in the [T-Series](#) grid (red triangles in the corner) are now correctly updated when moving rows up/down #2544
- Resolved a few issues caused by adding [Parameter Set](#) cycles to a [T-Series](#) when no Parameter Sets have been defined for the current acquisition mode #2791
- Disallowing [script commands](#) that can change scan parameters within a T-Series, [Parameter Sets](#) can be used to achieve these behaviors; additionally the warning message displayed when a prohibited script command is found is much more informative #2551
- Inserting new cycles into a [T-Series](#) now correctly updates the timing information for Z-Series, Line Scan and Mark Point cycles #2605
- It is now possible to close the [line scan](#) dialog, using the form close button in the upper right, during an acquisition such as a live scan #2747
- Ignoring travel limits for stage and focus devices which have the reset option enabled, this option allows the user to arbitrarily set a zero position; since the majority of devices cannot be polled for their limits it is not possible to support both settings #2334
- Corrected a few misspellings of the word acquisition in the UI and XML files #2799
- The laser power in the [Basic Acquisition Channel Definitions](#) can now be updated in real time while scanning #2489
- The [Basic Acquisition](#) interface now makes use of [Environments](#) and [Templates](#) to restore saved acquisition definitions #2650
- Loading an [Environment](#) while using the [Basic Acquisition](#) interface no longer causes one additional image window to be opened #3068
- Loading an [Environment](#) while using the [Basic Acquisition](#) interface no longer displays a warning about the existence of an Area Mask when area masks are not in use #3070
- The preference of [Basic Acquisition](#) v Advanced Acquisition interface is now remembered between sessions of Prairie View #2069
- Minimizing and restoring the main control window when using the [Basic Acquisition](#) interface no longer causes the Advanced SFC control window to reappear #2069
- The camera controls in the [Basic Acquisition](#) interface have been changed from drop down menus to text labels, as the camera to use is decided by the hardware available and not easily changed by the user #2069
- The advanced forms in the [Basic Acquisition](#) interface are now modal and always open next to the button that causes them to show #2069
- The camera temperature readout in the [Basic Acquisition's Camera](#) section no longer shows the units twice #2069
- Adding channels to live scan while using the [Basic Acquisition](#) interface now updates the channels to scan immediately #2069
- Using the [Basic Acquisition](#) interface now maintains the correct number of image windows for each channel defined #2069
- When using multiple cameras in the [Basic Acquisition](#) interface, enabling and disabling the camera split no longer leaves two channels to acquire on the second camera #2069
- The [Basic Acquisition](#) interface no longer shows the camera and filter block columns if

they do not apply to the type of acquisition being set up #2069

- The piezo voltage tweak field has been removed from the [Basic Acquisition](#) interface to reduce confusion #2069
- An “Optimal” button has been removed to the confocal settings on the [Basic Acquisition](#) interface to calculate and select the optimal aperture for the given objective and imaging channels #2069
- The stage position indicators in the [Basic Acquisition](#) interface no longer cuts off text for multiple Z devices #2069
- The home stage button in the [Basic Acquisition](#) interface is no longer visible until a home position has been set #2069
- The zero Z axis button in the [Basic Acquisition](#) interface has been replaced with the ability to store and return to a home position #2710
- The button to change save path in the [Basic Acquisition Definition](#) section now allows the user to choose the correct directory #2069
- The [Basic Acquisition Sequence](#) section now shows the grid lines for the table used to enter timing parameters #2069
- Clicking the group/ungroup button in the [Basic Acquisition Sequence](#) section without a sensible group of acquisitions (or groups) selected no longer causes an error #2069
- The start button in the [Basic Acquisition Sequence](#) section is no longer able until a valid acquisition has been added to the sequence #2069
- The [Basic Acquisition](#) interface now includes the ability to set up input triggers for time-lapsed experiments #2069
- Adding an acquisition to the [Basic Acquisition Sequence](#) no longer clears out the defined acquisition #2069
- Double clicking on an acquisition name in the [Basic Acquisition Sequence](#) and then clicking elsewhere without changing the name no longer causes an error #2069
- Stopping a live scan by means other than the stop scan button (e.g. removing all channels from the live scan, starting an acquisition) while using the [Basic Acquisition](#) interface now changes the live scan button text to reflect the end of the live scan #2069
- Linking channels in the [Basic Acquisition Channel Definitions](#) in one imaging mode no longer links them in all imaging modes #2651
- Removing and then adding back more than one channel from the [Basic Acquisition Channel Definitions](#) no longer causes duplicate channels to appear in the [Acquisition Channels](#) section #2652
- Highlighting a stage location in either the image or table used to show stage locations in the [Basic Acquisition](#) interface now updates the other appropriately #2653
- Changing imaging parameters in the Advanced interface will now carry over to the [Basic Acquisition](#) interface, where appropriate #2660
- The [Parameter Set](#) created using the [Basic Acquisition](#) interface is now created and carried over to the Advanced interface whenever the interface is switched #2661
- The Z controls in the “Z at Multi XY” tab in the [Basic Acquisition](#) interface now update appropriately when stage locations are selected via the table or image #2663

- A button has been added in the [Basic Acquisition](#) interface to move to previously defined stage locations, when setting up an acquisition that uses multiple stage locations #2664
- The table in the “Z at Multi XY” tab in the [Basic Acquisition](#) interface now updates with the Z series parameters for each stage location #2665
- The orientation of the image used to visualize multiple stage locations in the [Basic Acquisition](#) interface now matches that of [Atlas Imaging](#) #2667
- When using the [Basic Acquisition](#) interface to acquire a [Spectral Imaging](#) dataset, the galvo is no longer left in a sweep mode other than normal #2668
- Setting Frame Averaging in the [Basic Acquisition](#) interface now applies to both live scans and upcoming acquisitions #2669
- The Stage “0” button has been removed from the [Basic Acquisition](#) interface, as it is only necessary for advanced experiments #2670
- Using the FLI filter wheel and switching between the [Basic Acquisition](#) interface and the Advanced interface no longer leaves the checkbox indicating that different emission filters will be used per channel unchecked, even though it will be used #2681
- When using the FLI filter wheel, the emission filter wheel will now be returned to its previous position after an acquisition has completed #2682
- It is no longer possible for the FLI filter wheel to be in a position other than what is reported at startup (Requires SFC Scan Control firmware version 1.217 or later) #2612
- Selecting a different emission filter on the Opterra now continues to work after a scan has been started #2918
- The calculated time to pad for filter wheel movement (when using the FLI filter wheel) is now correct when the filter wheel has more or less than 6 positions #3018
- Exiting Prairie View while using the bypass accessory on the Opterra no longer uses those settings while switching to confocal mode on startup #3021
- Opening the advanced dialogs from the [Basic Acquisition](#) interface no longer causes them to open in the Advanced interface #2683
- The [Basic Acquisition](#) interface now loads with a default Position selection of Current and highlights the Temporal selection to indicate that a selection must be made before the acquisition can be started #2684
- A button has been added to the [Basic Acquisition](#) interface’s [Acquisition Definition](#) section to explicitly add the currently defined acquisition to the sequence #2685
- The positions of the Z and XY controls on the “Z at Multi XY” tab of the [Basic Acquisition](#) interface have been swapped to follow the streamlined workflow procedure #2686
- The “New (Blank)” option has been removed from the [Basic Acquisition Definition](#) section and replaced with a button which explicitly clears all parameters which had been previously set up #2687
- The [Basic Acquisition](#) interface now includes the ability to set up input triggers for time-lapsed experiments #2688
- The ability to choose units for the [Basic Acquisition Sequencer](#)’s interval and duration has been added to match the ability of the time series table #2689

- Switching between the [Basic Acquisition](#) interface and the Advanced interface now correctly returns control to the visible scrollbars and labels #2549
- The automatic timing parameters in the [Basic Acquisition](#) interface are now calculated to correctly match the selected units #2701
- Running a sequence of acquisitions from the [Basic Acquisition](#) interface is no longer setting up the necessary [Parameter Sets](#) erroneously, which led to the sequence appearing not to run #2691
- Adding synchronized events in the [Basic Acquisition](#) interface to a defined acquisition which includes multiple XY locations now fills the drop-down menu with the applicable locations immediately #2716
- The “MDA Current” [Parameter Set](#) is no longer created in cases which do not necessitate it, including saving environment files, saving acquisition definitions from the [Basic Acquisition](#) interface, and changing acquisitions in the [Basic Acquisition Sequence](#) #2731
- Loading an [Environment](#) or [Template](#) now replaces, instead of appends to, the currently defined [Basic Acquisition Sequence](#) with that in the environment/template #2752
- The [Basic Acquisition](#) interface now correctly remembers the Z range used when saved in an environment or template and centers that range around the current position when loading the environment or template #2650
- Setting Z Series limits in the [Basic Acquisition](#) interface now correctly calculates and maintains the position of the stop slice as close as possible to the desired stop position #2849
- Saving a defined acquisition in the [Basic Acquisition](#) interface no longer includes an unnamed category, which caused an error when used #2650
- Selecting a new position from the dropdown menu in the [Basic Acquisition](#) interface now immediately updates the relevant tables and images in the acquisition tabs #2856
- Using the [Basic Acquisition](#) interface now checks for the need of a multi-track parameter set before setting max speed, instead of only acquiring the first channel defined #2857
- Collecting a Z Series using the [Basic Acquisition](#) interface now checks for the need of a multi-track parameter set before allowing Fastest Acquisition to be enabled #3044
- Using the [Basic Acquisition](#) interface without a transmitted lamp connected to the Nikon Ti-E base no longer displays an error #2860
- The Perfect Focus controls in the [Basic Acquisition](#) interface now command the Perfect Focus hardware to move to the appropriate position #2861
- Checking the autosave button in the [Basic Acquisition](#) interface no longer increments the file iterator by two #2940
- Using the [Basic Acquisition](#) interface to set up a timelapse experiment now calculates the minimum allowable interval #2946
- Entering an interval and duration into the time table of the [Basic Acquisition](#) interface now re-calculates the first-entered parameter to match the rounded number of repetitions #3064
- Using the [Basic Acquisition](#) interface without time selected can no longer continue to repeat the acquisition more than once #2951

- Setting up channels using the [Basic Acquisition](#) interface no longer allows two channels to be in the live scan which share an illumination source but differ in intensity #2953
- The [Basic Acquisition](#) interface now only presents the option for fastest acquisition for devices that support it #2955
- Setting a Z series in the [Basic Acquisition](#) interface using the “Set Middle and Range” option now correctly shifts the Z series to the correct planes #2957
- Adding stage locations from the “Z at Multi XY” tab in the [Basic Acquisition](#) interface now respects the enabled state of a focal lock device (e.g. Nikon’s Perfect Focus) #2959
- Changing the PFS sliders in the [Basic Acquisition](#) interface and then typing a value into the textbox now sends the updated value to the focal lock device #2961
- The PFS controls in the [Basic Acquisition](#) interface now respond to changes in the PFS offset more often #2963
- Looping the time table in the [Basic Acquisition](#) interface and then turning off looping no longer leaves the table looping behind the scenes #2966
- Synchronized events in the [Basic Acquisition](#) interface, specifically those after the first event, now run on the correct repetition #2968
- Changing parameters for an area mask in the [Basic Acquisition](#) interface no longer asks to delete the mask from the T Series with every change #2969
- When loading the [Basic Acquisition](#) interface with an area mask saved, the message indicating that a mask needs to be defined is no longer shown multiple times #2971
- The saved laser intensity for area masks when using the [Basic Acquisition](#) interface is now correctly restored when starting Prairie View #3051
- Using the [Basic Acquisition](#) interface and generating a montage using [Atlas Imaging](#) no longer causes error messages to be displayed #2977
- The Resource selections in the Events tab of the [Basic Acquisition](#) interface are now updated anytime an event of the selected type is saved, deleted, or modified #2979
- The interval of acquisitions entered in the Time tab of the [Basic Acquisition](#) interface is no longer ignored when synchronized with an area mask in the Event tab #2981
- Multiple events can now be synchronized with one entry in the time table of the [Basic Acquisition](#) interface #2986
- The process of switching from the [Basic Acquisition](#) interface to the Advanced interface has been significantly sped up #2988
- Switching from the [Basic Acquisition](#) interface to the Advanced interface no longer assigns lasers to channels that have been disabled #2991
- The controls for area masks when using [Basic Acquisition](#) no longer allow different values to be entered for laser, intensity, or dwell time #2994
- The time table in the [Basic Acquisition](#) interface now immediately calculates values when loading a time table and changing one of the values #2998
- The [Basic Acquisition](#) interface now loads with the time table sized appropriately for the data it contains #3000
- Defining stage locations in the [Basic Acquisition](#) interface now appropriately listens to the preference for aligning Z series to stage locations, so Z series don’t run offset from

their defined locations #3002

- Updating stage locations when using the [Basic Acquisition](#) interface no longer changes the highlighted row in the stage location table to the first row #3004
- The [Basic Acquisition](#) interface integration with the bypass accessory has been improved to be more robust and functional #3020
- Adding, removing, and changing the order of channels in the acquisition section of the [Basic Acquisition](#) interface now correctly enables, disables, or re-orders channels for the upcoming acquisition #3026
- The parameter set used behind the scenes in the [Basic Acquisition](#) interface is now forced to update prior to shutdown, eliminating the possibility of saving an outdated parameter set #3028
- Switching between the [Basic Acquisition](#) interface and the advanced interface now only changes the exposure time if all defined channels share the same exposure time #3030
- The [Basic Acquisition](#) interface aperture selection no longer contains duplicate entries for each aperture #3035
- Switching between imaging modes in the [Basic Acquisition](#) interface no longer causes channel definition selections to be lost #3038
- The title of section 2 in the [Basic Acquisition](#) interface now reads, “Define Image Channels / Live Scanning,” to better show that the channels will be used for the upcoming acquisition #2711
- The [Basic Acquisition](#) interface now only allows selection of configured imaging modes (Confocal, Widefield, and Spectral) #2719
- Opening the [Basic Acquisition](#) interface now correctly restores the appropriate channels to be included in an upcoming live scan #3053
- The “Sequencer” section of the [Basic Acquisition](#) interface has been removed, as it did not provide functionality that fit within the basic workflow methodology the interface provides #3126
- Filter turret positions are no longer attempted to be set on Zeiss microscopes as part of a [Parameter Sets](#) #2949
- Loading a [Spectral Imaging](#) dataset into [Playback mode](#) now immediately updates the spectral window’s histograms to display the spectral information from the dataset #2392
- Saving a [Spectral Imaging](#) dataset from [Playback mode](#) now defaults the save location to the dataset location #2586
- Using the XLED no longer causes an error on startup of Prairie View #2831
- Clicking the “Zero” button on the XLED controls no longer causes an error message to be displayed #2848
- Using the XLED with less than 4 channels enabled no longer allows the illumination to get out of sync with its corresponding channel #2874
- The XY Home button in the [Stage Control](#) section of the main form now works correctly after using the manual stage controls, previously this only worked if the motors were moved with the software buttons #2834
- Improved wording of the [Frame Trigger Output Selection](#) menu under the [Preferences](#)

menu for clarity #2836

- Fixed a couple issues with [interlaced scan patterns](#) introduced in version 5.3, these issues were resulting in errors saving the data as well as saving black images instead of the actual image data when an interlaced scan pattern was enabled #2840
- [T-Series](#) timing information, the total time and estimated time fields specifically, now take [Voltage Output](#), [Voltage Recording](#), and [Mark Point](#) experiments into account when synchronized with an imaging cycle; previously this time was omitted even if the synchronized experiment was longer than the time spent imaging #2829
- The y-axis caption on [Voltage Recording](#) plots is now updated every time a channel is enabled or disabled, previously changing channel states more than once every half a second resulted in the caption being wrong #2208
- The [Seal Test](#) ‘Acquire’ button is now properly disabled when running a [Voltage Output](#), [Voltage Recording](#), or [Mark Point](#) experiment; previously it remained enabled and pressing it during a live Voltage Recording made it impossible to stop the recording #2210
- When using an AxoPatch 200B patch amplifier with [Seal Test](#), the telegraphed gain no longer fluctuates between correct and incorrect values #2972
- The labeling of channels selected in the [Voltage Recording](#) units selection, when read from an AxoPatch 200B, has been made consistent with that of [Seal Test](#) and the associated hardware #2973
- The software will now startup successfully after unconfiguring a feature for which the controls had been [torn off of the main form](#) and put into their own window, for example tearing off the [Z-Series](#) tab and then unconfiguring all the focus devices, or tearing off the [2P Laser](#) tab and then unconfiguring all the 2P lasers #2255
- The software will now startup successfully with no focus devices configured #2847
- Added configuration utility option to hide the [interlaced scan pattern controls](#) to declutter the UI a little for users not interested in the feature #2497
- Fixed an issue where the [interlaced scan pattern controls](#) were incorrectly displayed for unsupported configurations when changing acquisition modes out of either SFC or camera mode #2497
- Save path file iteration text boxes can now display values up to 199999, previously 10000. This limit is also enforced behind the scenes whereas previously the iteration would continue to rise past 10000 and the textbox wouldn’t update; iterating past the maximum value wraps back around to zero #2862
- Reworded [playback](#) toolbar window to use the word sequence to refer to a sequence of images within a data set; previously the sequence selection controls referred to sequences of images as datasets #2868
- Loading a [T-Series](#) definition containing [Voltage Output](#), [Voltage Recording](#), or [Mark Point](#) experiments which aren’t defined on the system will now correctly ignore those experiments; previously the T-Series grid displayed the names of the non-existent experiments and in some cases would crash when attempting to start the T-Series #2869
- No longer showing channel LEDs in [playback metadata window](#) when no LEDs were used during the acquisition #2883

- Fixed an issue where failing to configure preamplifier blades contiguously, starting with the first slot, resulted in software crashes #2888
- Exiting [playback mode](#) while playing through a multiple sequences of a data set (i.e. [Z-Series](#) over time), especially while displaying MIP projections, will no longer display an error message and cause playback not to work properly next time #2891
- Configuring an SFC Analog Z device will no longer cause the software to crash on startup #2898
- [Functional Mapping](#) no longer requires its image to be exactly the same size as the image in the image window at the time an acquisition was started. This corrects erroneous color wrapping in the color bar, identification of which point was clicked in the image, drawing of the region to zoom on, and setting limits of panning movement #2922
- Now able to control an LED transmitted light source on a [Zeiss microscope](#) #2937
- It is not possible to load a freehand line definition using the Load button on the [Line Scan](#) dialog, additionally loading an environment file will now correctly update the freehand line definition; as part of these changes the values related to line scans are stored with higher precision within XML files and the original freehand line definition is stored with each data set in addition to the interpolated points at each scanned pixel #2911
- Fixed multiple issues with [calibrated 2P lasers](#) including crashes at startup and malfunctioning laser power controls #4046, #3047, #3048
- Fixed an issue starting Prairie View after upgrading from a much older version of the software (version 4.x or earlier); due to compatibility breaks in version 5 it is highly recommended to upgrade to version 5.0 prior to updating to a later version, in order to prevent this type of issue #3049
- Galvo and laser drive signals no longer stop unexpectedly in the middle of a longer acquisition, this issue generally only affected acquisitions containing thousands of frames #3057
- Piezo tuning dialog, for 250, 400 and 1000 micron travel piezos, will no longer miscalculate frequencies when the piezo has been modified to accommodate a longer calibrated waveform #3058
- Changing advanced settings on the piezo tuning dialog, for 250, 400 and 1000 micron travel piezos, will now be applied as soon as they are accepted; previously it was necessary to accept the changes, close and reopen the dialog and then switch objectives #3059
- Eliminated a potential deadlock modifying scan settings; this issue was observed while changing the scan rotation and zoom in galvo mode #3072
- Pockels lag scan setting is now respected properly in resonant galvo mode, previously the setting was clipped at half of the retrace time #3076
- Checking that the Y range minimum value is always less than the Y range maximum value for [Voltage Recording](#) plots; previously setting the minimum value to be higher than the maximum value caused the Voltage Recording feature to no longer work after restarting the program #3077

- Fixed a number of issues causing 5.3 to not work correctly in AOD mode when upgrading from an older version #3038
- Eliminated a potential deadlock aborting a [T-Series](#) containing multiple saved [Z-Series](#) #3087
- Fixed some issues loading software without a stage and/or focus device configured, also no longer displaying PMTs or Laser loading information in the splash screen for systems without any configured #3088
- [T-Series](#) timing information is now correct when using an [interlaced scan pattern](#) #3090
- Preventing some error messages from popping up when interacting with [Line Scan](#) overlays; this issue was especially prevalent when working with line scan profiles and freehand lines #3092
- Fixed a couple issues imported custom waveforms for a [Voltage Output](#): imported values were being clipped at 10, even if the unit conversion would have resulted in a voltage less than 10, and it was not possible to import negative values, they were interpreted as being positive #3112, #3113

5.3 Update 3 Fixes

- Resolved some communication issues with the high speed shutter controller which caused the software to connect to another USB device and cause both devices not to communicate properly #3137
- Fixed an initialization bug for the Motorized Orbital Nosepiece. Also, lowered the threshold for registering a position change down from 0.25 degrees to 0.02 degrees and changed the yaw and pitch steps to be floating point and apply to the currently reported position (ignoring the software threshold) #3140
- [Line Profile](#)/Measurement tool now works correctly after restarting Prairie View with it open #3144
- Eliminated a source of error when calculating pixel sizes included in data set metadata; this error measured less than 10 nanometers and predominately affected the pixel width calculations for resonant galvo and AOD acquisitions, but affected all galvo based acquisitions to a lesser extent #3146
- Enabling the “Frame Rate” display from the “Display” menu now correctly updates the frame rate after any scan is finished #3148
- Using an ROI on a Hamamatsu camera in either SFC mode or Camera mode with a long Max Speed acquisition no longer causes the acquisition to stop before collecting the desired number of images #3154

Introduction

Congratulations on your purchase of a Bruker Fluorescence Microscopy System. This manual is intended to provide the information necessary to operate Prairie View software for image collection, scanner control, and synchronizing imaging with electrophysiological or photo-stimulation events.

Prairie View is the software that controls all scanning, image collection, photo-stimulation, voltage output, voltage recording, and synchronization functions of Bruker Fluorescence Microscopy systems. Several operational modes are available to control various system configurations, including Galvo, Spiral, Resonant, AOD, FLIM, SFC, and Camera modes. In addition to the controls described here, many hardware settings are configured in the Prairie Configuration Utility, which is accessed via its own icon on the desktop or within the Prairie directory. Consult Bruker Fluorescence Microscopy support personnel before making any changes to the configuration utility settings, as those changes may cause the system to malfunction.

Conventions Used in this Manual

When referring to a specific button, icon, or check-box a bolded font is used:

Press **Single Scan** to collect an image.

Specific key strokes are denoted with bolded font within angle brackets:

Press <**Enter**> to continue.

Menu strings will be denoted with angle brackets to define each sub-menu:

Go to File>Preferences>Z-Series to edit these options.

When describing a specific action that the user is to take, particularly as a part of a sequence of actions, numbers are used to delineate the steps:

1. Press **ROI**
2. Click and release the mouse once at one corner location of the ROI.
3. ...

When discussing acquisitions that involve more than one active channel, each channel's data is referred to as an "image" and the set of images for all active channels for a given time point is referred to as a "frame".

The computer on the imaging system is an integrated part of the imaging workstation, which consists of many components. Bruker Fluorescence Microscopy manufactures and sells computers that are designed to fully support the laser scanning instruments and many other devices from Bruker Fluorescence Microscopy and other hardware vendors. In addition to having the correct hardware capabilities, each computer from Bruker Fluorescence Microscopy comes configured with the correct operating system, drivers, and support applications needed to run Prairie View software.

It is important that the user back up the Prairie software directory (C:\Program Files\Prairie\) and configuration directory (C:\ProgramData\Bruker Fluorescence Microscopy\Prairie View) regularly in addition to backing up any data that has been acquired. In the event of a catastrophic computer failure, an up-to-date backup of the Prairie software directory and configuration folders will allow the user to resume operation of the system in the shortest amount of time. Many systems have complex hardware configurations that change over time and if the software configuration is lost it may take hours of support to reconfigure the system to a fully operational state.

Quick-Start Guides

These are short guides to getting started with some common tasks in Prairie View. For more information, refer to the relevant sections of the manual.

Note that hardware referenced in these quick-start guides is for the Ultima In Vivo system or the Ultima BX51/BX61 system. Steps may differ for other platforms, including Ultima FN1 and Ultima Examiner systems.

1. Turn on all electronics, including the camera, laser(s), control boxes, and microscope, and start the computer
2. Double-click the desktop icon to start Prairie View; a dialog box will appear and report progress as the software loads and establishes connections with various components
3. Turn on the ultra-fast laser and open the laser cavity shutter; depending on laser manufacturer and system configuration, this may be done manually or via Prairie View or other software
4. Find your sample using transmitted or epi-fluorescence light and the eyepieces (these steps may vary based on the microscope body design)
 - a. Select the light path and/or optical port that directs light to the eyepieces; for In Vivo systems, push the trinoc plunger in to the “Bi” position
 - b. Move the epi-dichroic (or reflector turret) filter wheel position to a cube that allows the desired transmitted or epi-fluorescence excitation wavelengths to reach the sample
 - c. Put a sample on the stage and focus through the eyepieces
5. Configure the system for laser scanning
 - a. Turn off the transmitted or epi-fluorescence light. Make sure the epi-fluorescence mercury lamp is completely shuttered or off if present on the system. The shutter mechanism provided on the front of the epi-dichroic turret is NOT an adequate shutter, as it leaks light from the lamp house that will flood the PMTs and introduce noise into the image.
 - b. Pull the trinoc plunger out to the “LSM” position
 - c. Move the epi-dichroic (or reflector turret) filter wheel to position 1 to put the Primary Dichroic in the light path
 - d. Close the light box door
 - e. Turn off the room lights
 - f. Tune the laser to the desired wavelength; depending on laser manufacturer and system configuration, this may be done manually or via Prairie View or other software
6. Select the objective lens you are currently using in the list of calibrated objectives in the Prairie View main control window; this list does not change the objective, but rather tells Prairie View which objective you are using

7. In the Image window, click the channel names along the left side of the window to activate the display channel(s) corresponding to the PMT(s) you will use to acquire your image. Multiple channels can be displayed in a single window or in separate windows; click the **New Window** button to open additional Image windows. A typical configuration with dual upper detectors utilizes a filter cube to direct red light to Channel 1 and green light to Channel 2; some system configurations may differ from this arrangement.
8. Click the **Live Scan** button located in the upper right region of the Prairie View main control window
9. In the Laser, PMT, DAQ tab, adjust the HV slider of the desired PMT channel(s) to an appropriate level. Side-on multi-alkali PMTs do well around 700, while GaAsP detectors perform better above 800; adjust accordingly for your sample.
10. In the Laser,PMT,DAQ tab, adjust the Pockels laser power slider until your image appears; the hard shutter will not open until the laser slider value is greater than zero
11. Continue to adjust the laser power and PMT voltage sliders along with the image size, focus (z-level), optical zoom, pan, scan rotation, etc, to get the desired image. Optionally, adjust image display settings (click the **LUT** button on the Image window to access controls).
12. Click the **Stop Scan** button located in the upper right region of the Prairie View main control window

3-Channel Confocal Imaging

1. Turn on all electronics, including the camera, laser(s), control boxes, and microscope, and start the computer
2. Key on the laser launch and toggle the shutter switch to the open position
3. Double-click the desktop icon to start Prairie View; a dialog box will appear and report progress as the software loads and establishes connections with various components
4. Find your sample using transmitted or epi-fluorescence light and the eyepieces (these steps may vary based on the microscope body design)
 - a. Select the light path and/or optical port that directs light to the eyepieces; for In Vivo systems, push the trinoc plunger in to the “Bi” position
 - b. Move the epi-dichroic (or reflector turret) filter wheel position to a cube that allows the desired transmitted or epi-fluorescence excitation wavelengths to reach the sample
 - c. Put a sample on the stage and focus through the eyepieces
5. Configure the system for laser scanning
 - a. Turn off the transmitted or epi-fluorescence light. Make sure the epi-fluorescence mercury lamp is completely shuttered or off if present on the system. The shutter mechanism provided on the front of the epi-dichroic turret is NOT an adequate shutter, as it leaks light from the lamp house that will flood the PMTs and introduce noise into the image.
 - b. Pull the trinoc plunger out to the “LSM” position
 - c. Move the epi-dichroic (or reflector turret) filter wheel to an open position (often position 2)
 - d. Close the light box door
 - e. Turn off the room lights
6. Select the objective lens you are currently using in the list of calibrated objectives in the Prairie View main control window; this list does not change the objective, but rather tells Prairie View which objective you are using
7. Configure the confocal detectors
 - a. In the Laser, PMT, DAQ tab, click the green bar to the left of the Lasers panel to reveal the Confocal panel
 - b. Choose a pinhole size from the drop-down menu

- c. Choose the dichroic mirrors and band pass filters from the drop-down menus for each position in the confocal detection path
8. In the Image window, click the channel names along the left side of the window to activate the display channel(s) corresponding to the PMT(s) you will use to acquire your image. Multiple channels can be displayed in a single window or in separate windows; click the **New Window** button to open additional Image windows.
 9. Click the **Live Scan** button located in the upper right region of the Prairie View main control window
 10. In the Prairie View Laser, PMT, DAQ tab, adjust the HV slider of the desired PMT channel(s) to an appropriate level. Side-on multi-alkali PMTs do well around 700, while GaAsP detectors perform better above 800; adjust accordingly for your sample.
 11. In the Prairie View Laser, PMT, DAQ tab, adjust the laser power slider to slowly increase power on the desired laser line(s) until your image appears; the hard shutter will not open until the laser slider value is greater than zero
 12. Continue to adjust the laser power and PMT voltage sliders along with the image size, focus (z-level), optical zoom, pan, scan rotation, etc, to get the desired image. Optionally, adjust image display settings (click the **LUT** button on the Image window to access controls).
 13. Click the **Stop Scan** button located in the upper right region of the Prairie View main control window

Collecting a Z-Series

A Z-Series is defined by four parameters: a start position, a stop position, a step size, and a number of slices. Defining any three of these parameters allows the fourth to be calculated. Click the Calculate radio button in the Z-Series tab next to the one parameter to have calculated. The following steps describe setting the start and stop positions of the image stack and choosing the step size.

1. Get in image of your sample using the steps defined in the Multi-photon or 3-Channel Confocal Quick-Start Guide
2. Use **Live Scan** and/or **Single Scan** (see the Scanning Controls section of this manual) to locate the sample
3. Use the up/down software buttons to navigate to the top or bottom of the desired Z-Series
4. Set the current position as the start of the Z-Series by clicking the **Set Start** button in the Start Position area of the Z-Series tab, or by clicking the  button in the Stage Control section of the main control window
5. Use the up/down software buttons to navigate to the desired stop position of the Z-Series
6. Set the current position as the stop of the Z-Series by clicking the **Set Stop** button in the Stop Position area of the Z-Series tab, or by clicking the  button in the Stage Control section of the main control window
7. Enter the desired step size into the Step Size field in the Z-Series tab
8. Confirm that the **Save Path** and file name point to the intended location of the images
9. Click the **Start Z-Series** button

The user can move to a previously set or calculated Start, Middle, or End position by clicking the corresponding **Goto** button in the Z-Series tab.

Photoactivation Masks

Photoactivation masks allow the user to define custom areas of the field of view to be scanned. The scans are performed by the imaging galvanometers using one or more lasers on the imaging path. Scan settings (Image Size, Dwell Time, etc.) are defined in the main control window, and laser settings are defined in the Photoactivation dialog.

1. Get in image of your sample using the steps defined in the Multi-photon or 3-Channel Confocal Quick-Start Guide
2. Click the  button on the Image window to open the Photoactivation dialog
3. Click to choose a laser palette; use the **Edit** button to change the laser power(s) associated with the palette. If using lasers at current settings, use the laser slider(s) in the main control window to set laser power
4. Choose a brush, ellipse, rectangle, or polygon shape and draw a mask on the Image window where laser power should be applied
5. Click **Save Mask** and name your mask
6. Close the Photoactivation dialog
7. Define a T-Series to apply the mask in the T-Series tab
 - a. Click **Image Sequence** and define images to be acquired before the mask is applied
 - b. Click **Photoactivation** and choose the saved mask
 - c. Click **Image Sequence** and define images to be acquired after the mask is applied
8. Click the **Start T-Series** button to begin the photoactivation and acquisition
9. After the T-Series is complete, click the arrow buttons in the Playback dialog to watch a playback of the experiment

Alternatively, the mask can be applied to Single Scans without using a T-Series. Masks can also be applied in three dimensions to Z-Series. More information is available in the [Photoactivation](#) section of the Prairie View manual.

Basic Point Photoactivation

All alignment and calibration must be done before performing this procedure; details are provided in the Prairie View manual. Point Photoactivation/Uncaging is carried out with the Mark Points feature of Prairie View software.

1. Configure the system with glass to allow light from the photoactivation laser to the sample. This configuration varies from system to system, and will be described during initial installation and training by Bruker Fluorescence Microscopy personnel.
2. Collect an image of your sample, as described in the Multi-photon or 3-Channel Confocal Quick-Start Guide
3. Mark points in the Image window
 - a. Open the Mark Points Controller window by selecting Mark Points from the Applications menu or by clicking the **Mark Points** button on the Image window
 - b. Right click in the Image window to drop points on the image and/or use the buttons in the Mark Points Controller window to move points and add points, lines, and grids of points to the image. Adjust the point density for each line and grid using the + and - buttons in this panel.
4. Choose a calibration file by clicking the ... button in the Mark Points Controller window or by choosing Load Uncaging Calibration from the File menu
5. Configure the stimulation in the Mark Points Controller window
 - a. Below the Mark Point Series table, click **Clear All Rows** to remove previously-defined experiments
 - b. Click **Add New Row** to add a cycle to the Mark Point Series table
 - c. Choose a point, line, grid, or group of points for the cycle
 - d. Define the Initial and Inter Point delay times, Duration of laser stimulation, number of Repetitions of the cycle, Uncaging Laser and Uncaging Laser Power to use for the cycle
 - e. Optionally, define start triggers and synchronization with [Voltage Recording](#) and [Voltage Output](#) modules
6. Click **Run Mark Points** at the bottom of the Mark Points Controller window
7. Use **Live Scan** or **Single Scan** to collect an image of the sample after the experiment

*Alternatively, the Mark Points experiment can be embedded in a T-series. More information

about this option is available in the [Mark Points](#) section.

Acquire an Image

1. Begin imaging with the steps described in the Multi-photon or 3-Channel Confocal Quick-Start Guide
2. Images can be made from a single frame or by averaging frames in one of two ways:
 - a. Average during a **Live Scan**: Check the box next to **Running Frame Average** and choose from the drop-down menu a number of frames to average
 - b. Average during a **Single Scan**: In the drop-down menu next to **Average Every N Frames**, choose a number of frames to average
3. Stop the live scan by clicking **Stop Scan**; from here you can work with the most recent images still in the Image windows, or click **Single Scan** to collect another image
4. Adjust the Look Up Table for each channel by clicking the **LUT** button on the Image window and adjusting the graphs in the new window that appears; right clicking on this button will bring up options to reset or automatically adjust the look up table

Save an Image

1. Define a save path in the “Misc” tab of the Prairie View main control window
 - a. Click ... to navigate to the desired folder (this path is also used to save T-Series and Z-Series files)
 - b. Type a name for your images in the file name field. The default name references the date and time the software was started, followed by a counter that automatically increases by one after each file is saved. If you change the default Base-Directory name and want that new name to be the default setting, go to Preferences > Preserve User-Modified File Names
 - c. Check the disk box next to **Single scan** if you want every Single Scan to be saved; this prevents loss of data but fills disk space quickly
 - d. Check the disk box next to **Live scan** if you want the last frame of every Live Scan to be saved
2. To save an image currently displayed in the Image window (from a live scan or a single scan), click on the **Snap** (camera) button on the left side of the Image window; this saves the current image as a 16-bit data file to the save path defined in the main form. If any overlays are present on the image, the exact displayed view (including display zoom) and overlays will be saved as an 8-bit TIFF file.

1. Go to File > Load Image(s)
2. Navigate to the directory and folder for the image you want
3. Open the XML file for the image

Main Control Window Overview

Most of the controls of Prairie View are accessed through the Main Control Window. Some sections may have extra controls accessible through a green bar located to the left of the section. Selected buttons are shaded green. The controls present in this window depend on system configuration. The example shown below is from an Ultima (point scanning) system.

Click on the image for more information on a specific topic

File Preferences Tools Display Electrophysiology Applications Templates Help

Image Resolution

64 x 64	512 x 512
128 x 128	1024 x 1024
256 x 256	2048 x 2048

Image Size: 512 x 512

FOV: 181.3 x 181.3 μm

Pixel Size: 0.354 x 0.354 μm

Dwell Time Per Pixel [μs]

<	Min 0.8	Current 4.4	>
---	---------	-------------	---

Optical Zoom [mag]

<	1.00	1.00	Reset
---	------	------	-------

Scan Rotation [deg]

<	Fine 0.00	Reset
---	-----------	-------

Stage Control

X = 0.00 | Y = 0.00
Z = 0.00, 0.00

Position None Defined

XY Step Size [μm]	Z Step [μm]
5.00	User Defined

XY Home [μm]	Z Home [μm]
Not Set	Set

Z Device Piezo

Panning Control

Coarse	Reset
<	>

Scanning

Live Scan Single Scan

Running Average Average Every
2 Frames 1 Frames

Save Path
... SingleImage-03032016-1048 129

Shutter Control Soft Hard

Acquisition Mode Galvo

Line Period: 2.762 ms

Frame Period: 1417.021 ms

Frame Rate: 0.706 fps

PA Mask None

Parameter Set Current Settings

Objective Lens 60x

Image Windows

New Smaller Fit 1:1 Larger

Power/Gain Z-Series T-Series 2-P Laser XY Stage Parameter Sets Misc

Lasers

2P Imaging	< > 30	Zero
Mode Default	<input type="button" value="▼"/>	
2P Uncaging	< > 0	Prev
405 nm Uncaging	< > 0	Zero
488 nm Uncaging	< > 0	Zero
573 nm Uncaging	< > 0	Zero

Interlaced scan pattern

Secondary Laser Beam Route

Switch Route

PMTs

PMT Master	Zero	
Ch 1 HV	< > 600	Zero
Ch 2 HV	< > 600	Zero
Ch 3 HV	< > 0	Zero
Dodd HV	< > 0	Zero

DAQs

Max Input Voltage $\pm 1.25\text{V}$

Preamplifier

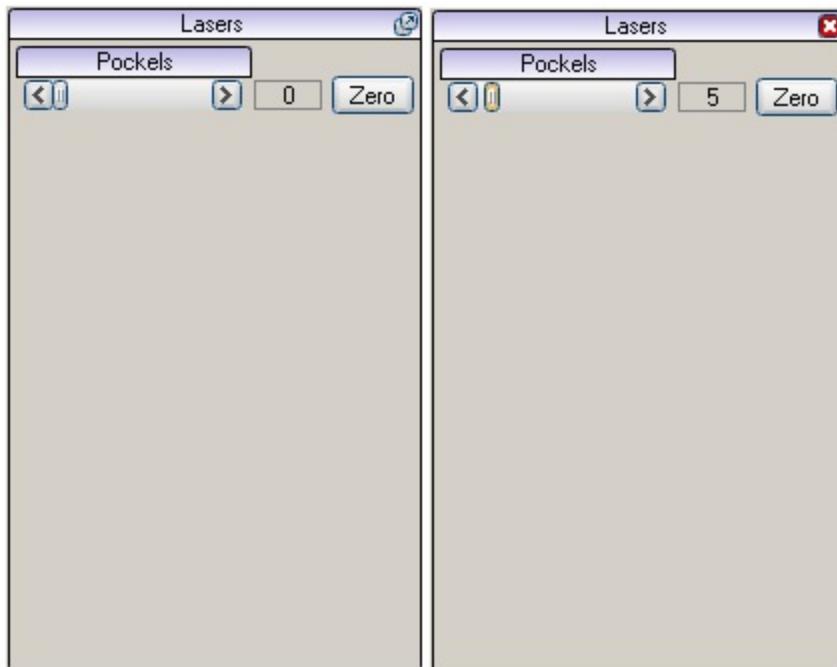
Filter Bank	1.25 MHz	
Ch1 Offset	< > 0.00	Zero
Ch2 Offset	< > 0.00	Zero
Ch 3 Offset	< > 0.00	Zero
Ch 4 Offset	< > 0.00	Zero

10:48:20 AM Successfully connected to Mode Servo Controller
 10:48:43 AM Successfully connected to Preamplifier
 10:51:01 AM Converting RAW Files into useful data.
 10:51:01 AM Finished converting RAW files.
 10:51:01 AM Single Scan: Saved in F:\Save Path\SingleImage-03032016-1048-128

Tear-Off Panels

Certain panels may be "torn off" and placed on the user's desktop.

To tear off a tab, click on the diagonal arrow button located on the right side of the tab label. Prairie View will remember these selections and return the tear-off panels to their last location when Prairie View is restarted. To return a tear-off panel to its original location, click the "X" in the upper-right corner.



Color Control

The title bar of individual panels can be changed. A color wheel will appear in the left corner of the title bar when the mouse cursor is hovered over the title bar. Click on the color wheel and select a new color for the title bar.

Slider Control

Slider controls in the software are configured for both small and large changes. To make a small change in the value, click the arrow at the end of the slider. To make a large change, click in the empty space in the slider between the current position and the arrow. After clicking on a slider, it can also be moved by rotating the mouse wheel, or by using the cursor keys.

Image Resolution

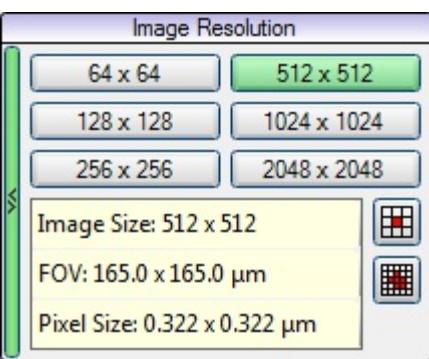
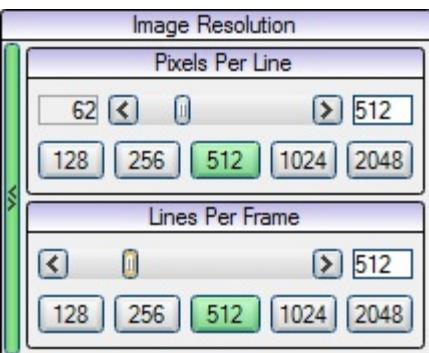


Image resolution is a definition of how collected data is acquired and displayed. In the case of a 512 x 512 acquisition, data is acquired at 512 bins in x by 512 bins in y, with each bin sampled according to the user-specified dwell time.

The information section below the pre-defined options lists the current image size in pixels and microns based on the selected image resolution and objective lens calibration.

Grid buttons to the right of the information section will increase or decrease the image resolution (changing the number of pixels in the scan) by a factor of 2 in both the x and y dimensions.

Note: Clicking the pre-set image resolution buttons while inside a Region Of Interest (ROI) will cause the software to temporarily exit the ROI, change the base image resolution, and then reenter the same ROI scan area with a different pixilation (more/fewer pixels in x and/or y depending on the change).

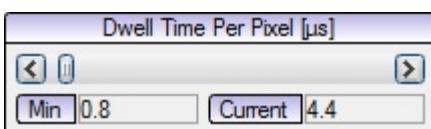


Clicking the green bar to the left of the Image Resolution controls reveals custom control of the Pixels Per Line (x) and Lines Per Frame (y). Use the sliders, pre-set buttons, or text box to enter a custom image size; while making changes, use the green button to flip back to the basic controls to see pixel size information. The text box to the left of the Pixels Per Line slider displays the minimum number of pixels per line allowed for the current scan settings. When imaging in a Region Of Interest (ROI), the Pixels Per Line and Lines Per Frame text boxes will display the dimensions of the current ROI.

Note: Be aware that it is possible to choose configurations in which the pixels are not square. This may affect how the images are displayed by third-party software.

Image Size controls are not available in Camera or SFC modes, and are instead replaced by an information display of image/pixel size.

Dwell Time Per Pixel



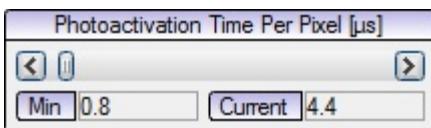
This slider allows the user to control the time that the imaging laser beam is scanning over each pixel location in the image. The minimum value allowed is displayed below and to the left of the slider. Changing pixels per line, optical zoom, and scan rotation may change the minimum allowed dwell time. The current dwell time is shown in the field below the middle of the slider.

When imaging in Galvo or Spiral mode, the underlying sampling rate is 1 sample per $0.4\mu\text{s}$. This means that when scanning with a dwell time of $2.0\mu\text{s}$, each pixel value is made up of 5 samples.

Dwell Time control properties are based on scan mode. When imaging in AOD mode, the underlying sampling rate is $0.1\mu\text{s}$. When imaging in Resonant mode, the dwell time is determined by the hardware and is not user-definable.

Dwell Time controls are not available in Resonant, Camera, or SFC modes.

In Camera and SFC modes, systems configured with a point-scanning system (Ultima, Single Galvo System, or Photoactivation/FRAP module) will replace the Dwell Time Per Pixel slider with a Photoactivation Time Per Pixel slider to control the dwell time of the photoactivation laser(s).



Optical Zoom



This slider controls the size of the area of the specimen that is being scanned by changing the amplitude of the galvanometer movements. An optical zoom of 2 will cause the microscope to scan an area that is $\frac{1}{2}$ the width and $\frac{1}{2}$ the height of a scan at a zoom of 1. Optical Zoom does not affect the number of pixels in the image, but instead changes the size of those pixels. It is possible to increase the true optical resolution of the system by using this function. Selecting **Reset** will cause the zoom to return to the default value of 1. The minimum value allowed is displayed below and to the left of the slider. The current selected zoom is shown to the right of the minimum.

Clicking the green bar to the right of the slider reveals buttons for pre-set zoom factors.

It is not possible to set the optical zoom to less than 1.

Optical Zoom control is not available in Camera or SFC modes.

Pan Control



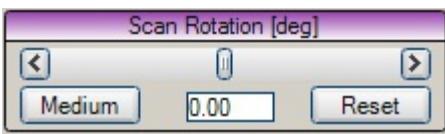
When working at an Optical Zoom of greater than one, Pan Control allows the user to change the position of the scanned area on the sample. On the left is a graphic that shows the relative position and orientation of the scanned area. A red and yellow box indicates the scanned area; the yellow side is by definition the “top” side of the scan. This box will change its size as a result of the Optical Zoom function. Similar changes will be displayed when the image is panned, or shifted laterally, and when the scan is rotated.

The image may be panned left, right, up, or down. To do so, click on the arrow button indicating the direction to pan the image. Clicking the **Reset** button causes the pan to reset to the middle of the field of view.

Click the **Coarse/Medium/Fine** button to change the resolution of the pan arrow buttons.

Pan Control is not available in Camera or SFC modes.

Scan Rotation



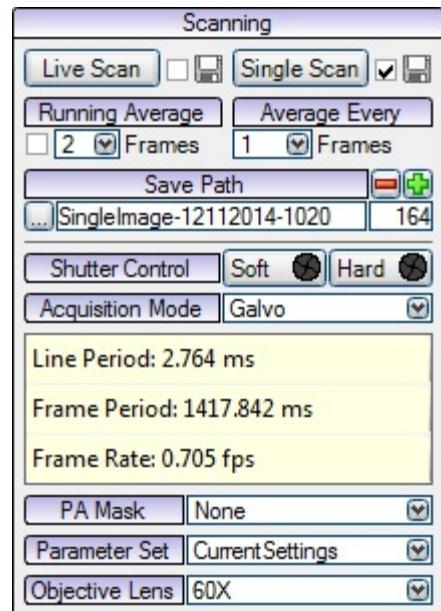
The entire scan can be rotated. Click and/or drag the slider or type a value (in degrees) to change the scan rotation. Rotation is possible from -180° to +180°. Use the **Reset** button to set the angle of rotation back to zero. Use the **Coarse/Medium/Fine** button to change the resolution of the slider.

Rotating the scan while in Galvo mode can increase frame rate by more evenly distributing the work of the X and Y galvanometers.

Scan Rotation controls are not available in Resonant, Camera, or SFC modes.

Scanning Controls

The upper right portion of the Prairie View main control window contains the scanning controls:



Live Scan

Live Scan causes the system to start scanning. During a live scan, this button changes to read **Stop Scan**. Clicking it a second time causes the scan to stop and the laser beam to be shuttered so that it no longer scans across the specimen. If the disk check box next to the **Live Scan** button is checked, then the last frame of every Live Scan is numbered and saved. To save the currently displayed image from a live scan, click the **Snap** button on the left side of the Image window. If **Running Average** is checked, the selected number of the most recent frames acquired will be averaged together to create the displayed image.

Frame Averaging

The **Running Frame Average** affects the **Live Scan** imaging. When this option is checked, the software will average the specified number of the most recent frames acquired during **Live Scan** and update the Image Window accordingly. This is useful for samples with little fluorescence or when using low laser power.

Average Every N Frames is designed to work with the **Single Scan** command, as well as Z-Series and T-Series acquisitions. When set to a number greater than one, it will average the selected number of frames to create each image.

Note that for most acquisition modes, the End of Frame trigger is only generated on the last of the averaged frames making up each image. Thus, if the number of frames to average is set to 4, the End of Frame trigger will be generated at the end of every 4th frame. The frequency of

the End of Frame trigger in SFC mode depends on the firmware in the SFC control box. More information about frame triggers is provided [here](#).

Single Scan

When the **Single Scan** button is pressed, one frame is acquired, with the result displayed in the image window(s). The hard shutter is closed following collection of the image and the image is saved as a temporary file. If the disk check box next to the **Single Scan** button is checked, then every Single Scan is numbered and saved. If this option is not checked, the frame is stored only temporarily unless the **Snap** button  on an Image window is used to preserve it. If **Average Every N Frames** is set to a number greater than one, the selected number of frames will be acquired and averaged to create one image.

Save Path

Each acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the  button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the  button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

By default, Live Scans and Single Scans continuously over-write the data in the folder specified by the Save Path. When the Auto Save option for **Single Scan** is checked, denoted by a disk icon, the file counter will be incremented automatically after a Single Scan so that it will not be overwritten by the next Single Scan or Live Scan. Likewise, when the Auto Save option for **Live Scan** is checked, also denoted by a disk icon, the file counter is incremented automatically after a Live Scan so that it will not be overwritten by the next Single Scan or Live Scan. Note that for **Live scan** Auto Save, the file saved will be the last frame collected by the Live Scan, as displayed in the Image window when the scan was stopped. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data. To manually save a particular single scan after acquisition, use the **Snap** button  on the image window.

Shutter Control

Shutters are automatically opened and closed during imaging. When an acquisition is started, the shutter(s) will open for any laser(s) for which the laser power slider is set to a value greater than zero. If all laser sliders are set to zero, no shutters will open.

The **Hard** and **Soft** shutter buttons allow the user to manually open and close the shutters. Hard Shutters are physical shutters placed in the beam path (on the table or inside a laser launch). Soft Shutters are gates on signals sent to a Pockels cell or AOTF. Although software controlled for routine imaging, it is possible to manually open and close the hard shutter for access to the beam during alignment. A user may also choose to manually open the hard shutter before starting an experiment to avoid any vibration introduced by the mechanical movement. It is also possible to manually open and close the Soft Shutter.

On SFC systems with the Photoactivation/FRAP module, the Hard and Soft shutter buttons allow the user to manually turn on the laser(s) used on the Photoactivation/FRAP module for troubleshooting and alignment purposes. On systems configured with an Ultima for single or multiphoton imaging, or a Single Galvo Scanner used for mulitphoton imaging, the Soft Shutter and Hard Shutter buttons will operate as if Prairie View were running in Galvo Mode. In any configuration, the shutters will open automatically during photoactivation experiments.

Acquisition Modes

The **Acquisition Mode** drop-down menu allows the user to switch between scan modes available on the current system configuration. Some scan controls are only available in particular modes. All Ultima systems offer Galvo and Spiral modes; additional modes are available based on current hardware configurations. Some settings, including laser power and PMT voltages, are recalled individually for each Acquisition Mode. For example, a user is working in Galvo mode with the laser slider set to 100 and the PMT voltages set to 700. When the user switches to Resonant mode for the first time in that session, the laser slider and PMT voltages will be set to 0. The user then sets the laser slider and PMT voltages to values needed to see the sample while scanning in Resonant mode. If the user returns to Galvo mode, the laser slider and PMT voltage settings will be returned to the 100 and 700 values last used in Galvo mode.

Galvo mode is the default mode for scanning with traditional galvanometers. The image is generated from left to right, top to bottom, with lasers blanked during the galvanometer retrace at the end of each line and the bottom of each frame. Unless otherwise noted, all software features described in this manual are available in Galvo mode.

Spiral mode allows faster scanning with the traditional galvanometers. This is accomplished by driving the galvanometers in a continuous pattern and constructing the image from the outside in. The resulting scan is a circular area inside the traditional Galvo scan area. Very large image

sizes are disabled in Spiral mode. Spiral scans at high speeds can cause the center of the image to appear distorted. This can be minimized by adjusting the scan parameters to move the galvanometers more slowly, or by changing the Spiral Scan Duty Cycle.

The Spiral Scan Duty Cycle determines the proportion of the scannable area that will be imaged. This parameter is also set in the scanning controls section. A duty cycle of 1.0 will scan the entire circular area, while smaller duty cycles (for example, 0.95 or 0.90) will leave an unscanned area in the center of the image, generating a “donut scan” area. Lowering the duty cycle can help to reduce the appearance of distortions at the center of the image. It also protects the center of the sample from photo-damage, which can occur because the spiral scan pattern scans the center pixel multiple times when generating a single image.

Certain combinations of Dwell Time and Image Size require some pixels in the scan to be interpolated; in these cases, a warning message will appear at the bottom of the Main Control Window. It is also possible to drive the galvanometers past their limits during a Spiral scan. This will result in the image taking on a “smeared” appearance and the amber warning lights on the Galvanometer Control Box to illuminate. If this occurs, slow down the galvanometers by increasing Dwell Time or Image Size.

Resonant mode generates images at high speeds using a resonant galvanometer for the X dimension and the traditional Y galvanometer for the Y dimension of the image. Frame rate is dependent solely on the number of scan lines in the image. In this mode, dwell time varies across the image and is determined automatically. Because the frame rate in Resonant mode is so much faster than in Galvo mode, image quality can be improved by averaging frames while still collecting images at high speeds. Two scan controls, Resonant Phase Offset and Sampling Method, are unique to Resonant mode and are described later in this manual. Some features are not enabled when imaging in Resonant mode, including Photoactivation Masks and freehand Line Scans.

AOD mode uses the Acousto-Optic Deflector to generate images at high speeds. A plunger on the AOD module allows the user to choose which of four lenses is placed in the beam path, which in turn determines the combinations of scan settings allowed for imaging. In AOD mode, the Optical Zoom, Image Size, and Dwell Time settings are limited to particular combinations. Default AOD settings are 512 x 512 Image Size, 0.1 μ s Dwell Time, 1.0 Optical Zoom, resulting in a frame rate of approximately 25fps. Changes in any one of these three settings will cause Prairie View to automatically compensate by changing the others, to keep the AOD imaging properly. To select one parameter to keep constant, click the checkbox next to the parameter to freeze its value. Prairie View will not change that setting unless the user un-checks the box or restarts Prairie View. If the user changes one of the other two parameters, the software will attempt to automatically adjust the third parameter to provide a proper image. In order to prevent getting lost in settings that do not produce images, keep one of the freeze parameter boxes

checked whenever making changes to another parameter. When re-starting the software, Prairie View will detect the physical plunger position and restore all parameter settings to defaults for that position.

Frame rate is calculated automatically based on the Image Size, Dwell Time, and Optical Zoom settings. For faster frame rates, lower the settings for those parameters. Image Size is the number of pixels in the scan area; higher values produce cleaner images, while lower values allow faster frame rates. Optical Zoom determines the size of the scanned field of view; default settings have the Optical Zoom match the AOD plunger position, but this can be changed to alter the frame rate. Dwell Time is the amount of time spent at each pixel, with a minimum value of $0.1\mu\text{s}$; lower values can help reduce photo-bleaching. Each $0.1\mu\text{s}$ change in Dwell Time changes the Optical Zoom by 1.0 or the Image Size by a factor of 2. Setting the lens plunger to position 4 allows the greatest range of scan parameter combinations.

Increase image resolution by increasing either image size (total number of pixels) or dwell time (amount of time spent imaging each pixel). Note that changing either of these settings will cause the optical zoom and frame rate to change accordingly.

Improve image quality with the Average Every N Frames feature. Because AOD mode scans so quickly, it can average frames much faster than Galvo mode.

FLIM mode enables features to monitor fluorescence lifetime data. This option is only available on systems configured with hardware for lifetime imaging. These features are discussed in more detail elsewhere in this manual.

SFC mode is used to collect images with a Swept-Field Confocal scanner. Due to fundamental differences in scanning hardware, there are many software properties unique to SFC mode. These are discussed in more detail in the Swept-Field Confocal section of this manual.

Camera mode is used to collect images with a camera mounted on the system. This can be used to collect transmitted light or wide-field fluorescence images. Many scan controls are not applicable to Camera mode, but there are camera-specific parameters available in this mode. These parameters become part of the Laser, PMT, DAQ tab, described later in this manual.

Timing Information

This section displayed the line period, frame period and frame rate when applicable to the current acquisition mode. There are mouse over tooltips to display additional details, for example hovering over the line period will display both the line scanning time and the line retrace time, and hovering over the frame period will similarly show the frame scanning time

and the frame retrace time.

Photoactivation Mask

The **PA Mask** drop down allows the user to choose a previously-defined photoactivation mask to be applied to all Live Scans, Single Scans, Z-Series, and W-Series. Only masks valid for the current scan settings (image size and dimensions) will appear in the list of saved masks.

[Photoactivation](#) masks are discussed elsewhere in this manual. The selection does not apply in SFC, Resonant, or AOD mode.

Parameter Set Selection

The **Parameter Set** drop down allows the user to select previously defined and saved settings. These settings can include, but are not limited to: dwell time, laser power, PMT voltages, etc. Parameter Sets are discussed further in the [Parameter Sets Tab](#) section of this manual.

Objective Lens

The **Objective Lens** drop-down menu allows the user to select a previously calibrated objective setting for an objective lens used on the system. Proper objection selection is important as it is used in determining pixel size. Failure to select or use a properly calibrated objective will result in invalid measurements and motor movements. The procedure for calibrating objective lenses can be found in the Calibrate Objective Lens section of this manual. Note that the selection made in this menu will NOT move the objective turret on a microscope with a motorized nosepiece; that must be done in the microscope control window.

Image Windows



New opens a new Image Window.

Smaller decreases the displayed image by ~10%. The image can continue to be reduced by clicking **Smaller** repeatedly.

Fit is the default and the image display is resized to 512 pixels in width, regardless of the Image Size settings for the acquisition. If the image window is increased or decreased in size, the display will be scaled to fit proportionally to fit in the new window.

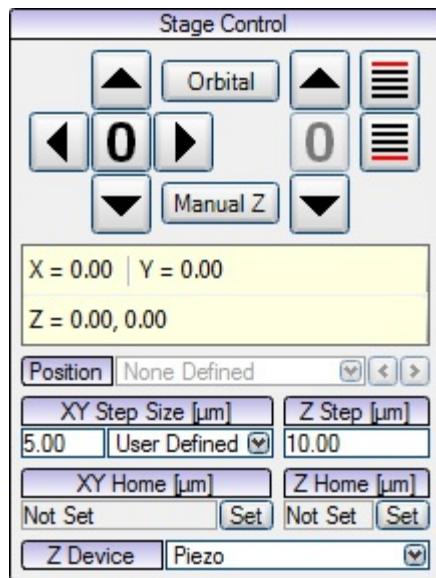
1:1 shows the image at its actual size as defined by the number of pixels in the current Image Size.

Larger increases the displayed image size by ~10%. The image can continue to be increased by clicking **Larger** repeatedly.

Changes made with these buttons are applied to all active Image Windows. Adjusting the Image Window Size alters the display, but does not change scan parameters.

Stage Control

The stage controls allow the user to move the stage in the x and y directions and the objective in the z direction. They also allow the user to recall saved locations, which can be modified on the [XY Stage tab](#).



The **Position** dropdown allows the user to select from a list of previously saved stage/focus positions. Storing and recalling these positions is described in more detail in the [XY Stage tab](#) section.

Stage Controls

The XY stage controls allow the user to scan through the x and y axes of an image in user-defined step sizes. Use the arrow buttons to move the stage by the number of microns indicated in the **XY Step Size** field. To change the step size, click in the field and replace the number with the desired step size, or choose a percentage of the field of view from the dropdown list to the right. Field of view measurements are based on the calibration performed for the current objective lens.

Use the home feature to store an XY location and come back to at a later time. Pressing the **Set** button stores the current XY location as the **XY Home** position and pressing the  button will return to that position. The  button does not appear until an **XY Home** position has been **Set**.

Press the  button to leave the stage where it is and set the current position to be zero. The saved home position will be offset to maintain the same location in the sample. Note that this feature is not available for all stage devices.

Some systems are configured with two XY moving platforms. For example, a moving in-vivo microscope base may be accompanied by a specimen stage. Prairie View only controls one XY platform, configured as the primary X and Y axes in the configuration utility. The secondary X and Y axes (the other platform) can be controlled by the 3-axis knob controller, but will not be taken into account when saving XY positions in the software or when using the stage controls described above.

Focus Controls

The focus controls allow the user to scan through the z-axis of an image in user-defined step sizes.

Use the up and down arrow buttons to move the z-motor by the number of microns indicated in the **Z Step** field. To change the step size, click in the field and replace the number with the desired step size.

Use the home feature to store a Z location to come back to at a later time. Pressing the **Set** button stores the current Z location as the **Z Home** position and pressing the  button will return to that position. The  button does not appear until a **Z Home** positing has been **Set**.

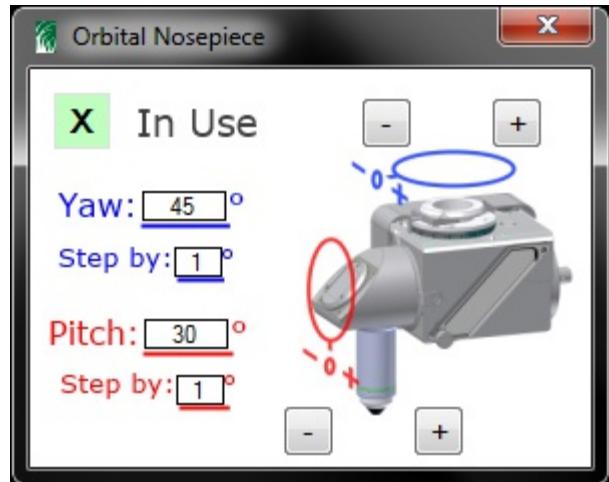
Press the  button to leave the focus where it is and set the current position to be zero. The saved home position will be offset to maintain the same location in the sample. Note that this feature is not available for all focus devices.

Some Z devices can be controlled manually or via an analog output drive signal instead of being controlled by Prairie View. If this is the case the **Manual Z** button will be available to toggle between manual and software control. The button is highlighted green when the current Z device is in manual mode. For most focus devices the software will automatically return to software control when the user attempts to move the device from the software (for example pressing the up/down buttons).

If there is more than one focus device available on the system, such as a high-precision piezo controller, the **Z Device** dropdown will be active to select which device will be controlled. More information about using multiple focus devices can be found in the [Z-Series tab](#) section.

Orbital Nosepiece

On systems configured with an Orbital Nosepiece to adjust the angle of the objective, the X, Y, and Z motors can move in concert to move the perceived X, Y, and Z directions of the image plane. This is done by pressing the **Orbital** button in the **Stage Control** section which will bring up the Orbital Nosepiece dialog, and then selecting the checkbox in the upper left hand corner labeled **In Use**. After checking the **In Use** checkbox the **Orbital** button in the **Stage Control** section will be highlighted green, and will remain highlighted so long as the checkbox remains checked. While the Orbital Nosepiece is **In Use** all stage/focus interaction in the **Stage Control** section will move relative to the imaging plane rather than relative to the table. Unchecking the **In Use** checkbox will restore normal stage and focus behavior.

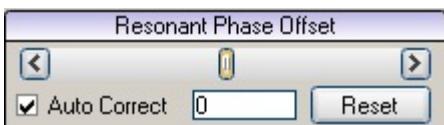


If the Orbital Nosepiece is motorized the **Yaw** and **Pitch** angles will be polled automatically and can be moved through the software either by entering an angle directly or by using the -/+ stepping buttons around the image. When using the stepping buttons the step size can be set underneath the **Pitch** and **Yaw** angle entry controls in the text box labeled **Step by**.

For a manual Orbital Nosepiece the angles must be read off of the dials on the device itself and entered into the software manually. In the case of the Single Axis Rotating Nosepiece, the Yaw controls will be disabled and the Yaw angle will readout "N/A". Either nosepiece, manual or motorized, can be moved into a coarse position by physically moving the device.

When using a Bruker MAMC (Multi-Axis Motor Controller) the 3-axis knob box will also move in the image plane when the Orbital Nosepiece is **In Use** and the software is running. Note that this feature may require a firmware update.

Resonant Phase Offset



Resonant Phase Offset control is available only in Resonant acquisition mode. This feature uses algorithms to measure the offset of alternating lines in images collected with the resonant galvanometer, and provides a corrective offset as feedback to the galvanometer control hardware to adjust its timing. The **Auto Correct** option should be checked during acquisitions. If a situation arises where the scan lines are not aligned properly in the image, the user can uncheck the **Auto Correct** option and use the slider to adjust the offset, and then re-enable auto correction. Based on settings in the Z-Series Preferences dialog, correction can be automatically disabled during acquisition of a Z-Series to minimize artifacts due to the changing features of the image during the acquisition.

The **Reset** button is used to reset the alignment and correction feedback algorithm, and is rarely needed by users.

Sampling Method

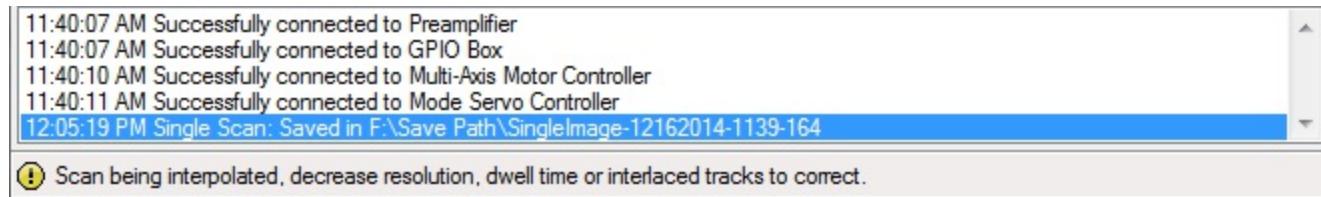


Sampling Method control is available only in Resonant acquisition mode. When the **Enable Multisampling** option is checked, the system collects as many samples as possible at each pixel. This number is indicated in the Samples/Pixel field and is based on the size and number of pixels in the image. The **Averaging/Summing** button allows the user to specify whether the recorded value for each pixel is the average or sum of these samples. When the multisampling is not enabled, the system will collect only one sample to determine the intensity of each pixel.

The **Enable Multisampling** option should remain checked unless the user has a specific reason for reducing the number of samples collected; there are almost no applications in which it is beneficial to reduce the number of samples.

Event Log and Scan Information

At the bottom of the Prairie View Main Window, a list box displays a list of recent events and a status bar below that displays any information, warnings or errors pertaining to the current scan settings or acquisition. In the screenshot below an example of a spiral scan settings warning is shown.



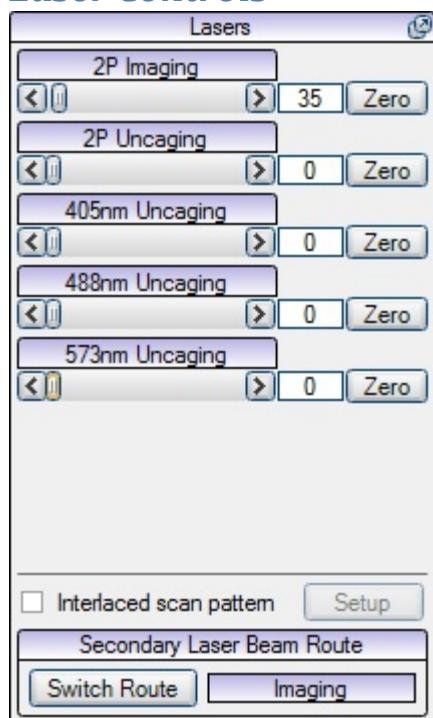
On startup the list of events will start by showing the status of connecting to some of the hardware, and will be added to as acquisitions are performed. In the example the event for a single scan being acquired was added to the list.

At the very bottom of the window, information is displayed by particular operations in the software. This information includes warnings about data integrity and scan settings, and frame counts when acquiring images of averaged frames. Warnings and errors will be prefixed by a colorful icon to draw your attention to important messages, yellow for warnings and red for errors.

Power/Gain Tab

The Laser, PMT, DAQ tab is separated into multiple sections. The sections vary based on system configuration and the current acquisition mode. Green bars on the left edge and the center of the tab allow the user to flip between stacked sections. Individual sections can also be torn out into separate windows via the arrow button on the upper right corner of the section.

Laser Controls



This section allows the user to control the laser power for all lasers configured in the system. Typically, lasers on the imaging light path are listed first, followed by lasers on the uncaging light path. For each laser, the user can drag the slider to change laser power, or enter the desired setting in the field to the right of the slider. Clicking the **Zero/Prev** button will return laser slider to 0 or the previous value, respectively. During a scan, the shutter will open only if one or more lasers associate with that shutter have slider values of greater than zero.

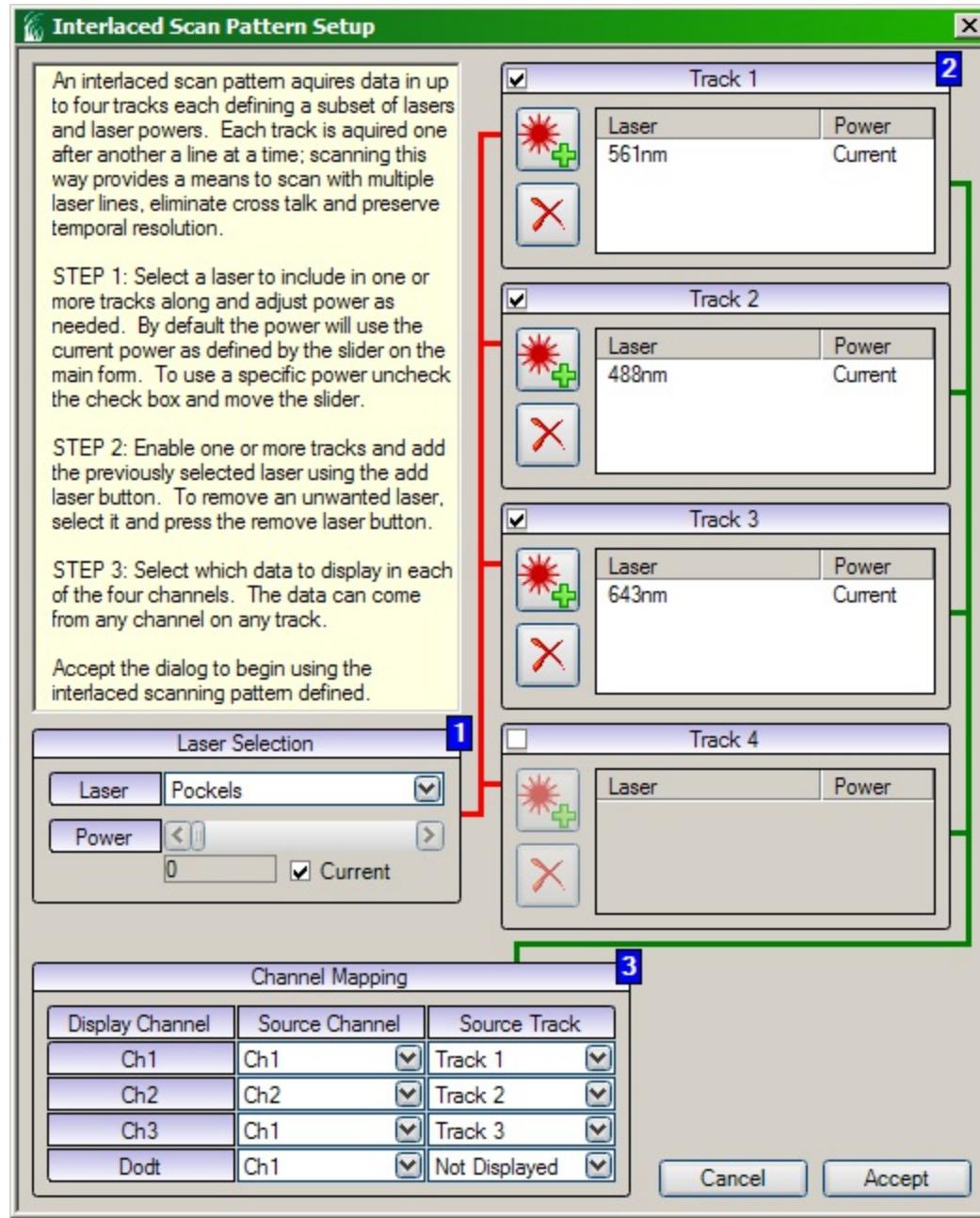
The default system setup has laser power sliders in arbitrary units from 0 to 100 or 0 to 1000, which do not correspond to linear changes in the laser power delivered to the sample. The utility of the laser slider for 2p lasers can be extended to additional modes using the Laser Power Calibration feature, described [here](#). Additional laser slider modes are Attenuation and Calibrated modes. In Attenuation mode, the slider values become percentages of maximum power at the given wavelength. When a full Calibration File has been created for a given set of operating parameters, the laser slider will reflect laser power in milliwatts.

Interlaced Scan Pattern

Interlaced scanning allows the user to scan each line of the image multiple times, using a specific set of lasers and laser powers each time. This feature provides a means to scan with

multiple laser lines while reducing cross talk between excitation channels and preserving temporal resolution.

The checkbox at the bottom of the Laser control section allows the user to enable Interlaced Scanning. Check the box and click the **Setup** button to open the configuration window. The window contains instructions for setting up the interlaced scan.



Interlaced scan patterns allow acquisition of frames using up to four different independent laser settings alternating each line for higher temporal resolution than is possible with using Parameter Sets to alternate settings each frame. One line of each track is scanned to acquire one line of the final image.

While this feature is available for systems with only a single laser, its usefulness with a single laser setup is limited. This feature is primarily useful when a system has more than one laser.

For example, if a system has two imaging lasers, one tuned to 800 nm and one tuned to 900 nm, this feature allows the operator to scan a 512x512 image alternating wavelength with each

line. The data for track one will be saved as the channel one data and the track two data will be saved as channel two.

Whenever the Interlaced scan pattern check box is checked, the defined interlaced settings will be used. This applies to Live Scan, Single Scan, T-Series, Z-Series, etc.

Laser Selection specifies one of the lasers currently configured on the system as well as a power setting. The laser and power here can be added directly to an active track. By default the **Current** power check box is checked which indicates that the laser slider power setting should be used even as the current value changes in the main control window. When the box is unchecked, a static power value can be specified using the slider in the setup window.

A **Track** is a sequence of laser lines and powers to control. Up to four different tracks may be defined, and multiple lasers can be defined in each track. Enable a track by checking the box on the left side of its title bar. Once enabled it is possible to add the selected laser and power to it using the **+** button. The same button can be used to update the power setting from a laser already added to the track. To remove a laser from a track, select the desired line and press the **X** button. Any lasers not included in an active track will be left at zero power for scans. Each track enabled will reduce the frame rate of the acquisition, whether or not the track is mapped to a display channel.

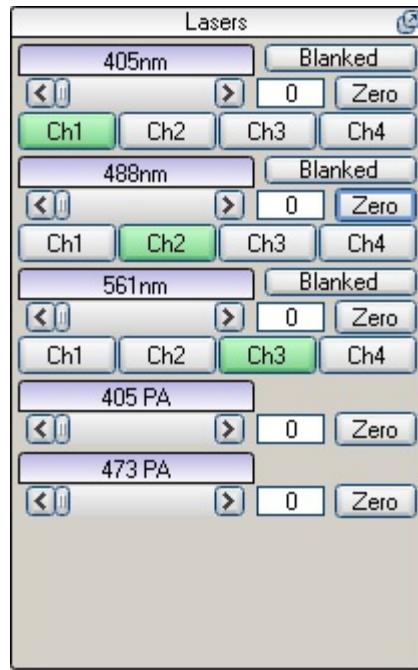
Channel Mapping allows the user to choose the display channel to which each PMT and Track are mapped. An acquisition can generate up to 16 data sources (four source channels times four tracks). Up to four of these combinations can be acquired as image data. Choose a Source Channel (detector) and a Source Track for each Display Channel. In the example above, Display Channel 1 contains data acquired by Source Channel (PMT) 1 during Track 1 (561nm laser), Display Channel 2 contains data acquired by Source Channel (PMT) 2 during Track 2 (488nm laser), and Display Channel 3 contains data acquired by Source Channel (PMT) 1 during Track 3 (643nm laser). Note that the combination of Source Channel and Display Channel used here means that the data stored in Display Channel 3 memory came from Source Channel 1.

Secondary Laser Beam Routing

The **Secondary Laser Beam Route** feature is a TTL level signal that is intended to drive a switch for systems that have two 2-P lasers, where the second laser can be used as an imaging laser or as an uncaging laser. This feature is enabled/disabled and configured during installation within the Prairie Configuration Utility. This signal may be configured to use a digital output line from any of the National Instruments DAQ boards. This parameter can be included in Parameter Sets, allowing the operator to create Parameter Sets that use either just one laser or potentially both lasers. This also allows the operator to control the routing of the second laser for use in Imaging/Photoactivation masks and Mark Points experiments (e.g. First image using both lasers, then via a Parameter Set, change the routing of the second laser, then use Mark Points, and so on).

Laser Controls in SFC Mode

While operating in SFC mode, the Laser tab differs slightly.

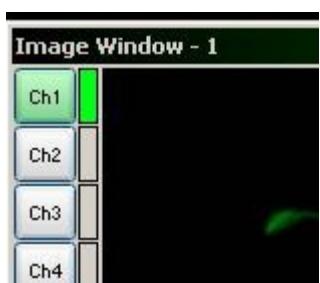


Lasers used by a secondary scanning unit operate as described in the previous section. Lasers routed through the SFC scanner have additional controls, described below.

The **Blanked** button can be used to turn on (unblank) an imaging laser when not scanning. This can be useful when performing power measurements or when troubleshooting to make sure no system optics are blocking the light path to the sample.

Each imaging laser can be mapped to one or more acquisition channels by activating the **Ch1** through **Ch4** buttons below the slider. Each laser can be assigned (mapped) to one or more channels, or can be left unassigned. Conversely, a given channel can have more than one laser assigned to it. Depending on the experiment, this may lead to detection of multiple fluorophores in one channel, which cannot be separated for measurement. Lasers are mapped to channels by clicking the channel buttons below each laser slider. In the example above, the 405nm, 488nm, and 561nm lasers are mapped to channels 1, 2, and 3, respectively. This means that for each scan, three exposures will be acquired - one with the 405nm laser, the second with the 488nm laser, and the third with the 561nm laser.

For a channel to be acquired, it must also be activated in an Image window. To do this, click one or more of the channel buttons on the left side of the Image window. In the example below, channel 1 is active in Image window 1, and is pseudocolored green. The pseudocolor is chosen by clicking the narrow vertical bar to the right of the channel button. An Image window may have more than one active channel, and the same channel may be activated in multiple Image windows.

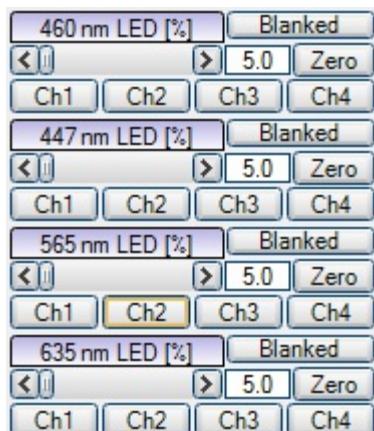


The mapping of lasers to channels and the combination of active channels can differ between Parameter Sets (see further discussion in the [Parameter Sets](#) Tab section of this manual).

Each frame acquisition is made up of scans for all the active channels. Thus, an acquisition with three active channels will have a lower frame rate than an acquisition with one active channel.

LED Controls when using the XLED

If the system is configured to use the XLED, a few additional controls are added. These controls correspond to each LED module installed in the XLED housing and their behavior is described below.



When not in either the Camera acquisition mode or the Opterra Widefield imaging mode, only the intensity and Blanking controls are active. The **Blank** button is used similar to its corollary in the SFC laser controls. That is, it is used to turn on or off an LED when not scanning.

Additionally, the intensity controls can be used to set the intensity of each individual LED on a scale from 5% to 100%.

On the other hand, when in either Camera mode or the Opterra Widefield imaging mode, the Channel Assignment buttons also become active. These controls work exactly the same as those for an SFC laser control. To use an LED in any channel, assign the LED to that channel by clicking the corresponding channel until the button is highlighted in green. As is the case in SFC mode, channels will only be acquired if they are active in at least one image window, and will go in order from 1 through 4. The channel assignments also mimic the SFC controls in that they can be included in Parameter Sets if it is desired to change the assignments during an acquisition, and that a frame acquisition is considered to be the sum of each individual channel acquisition.

PMT Controls



The PMT sliders control the voltage which amplifies the photon-electron signal-to-current pulse for each detector. Increasing the voltage will increase the output current pulse and thus increase the brightness of image. Multialkali PMTs run at voltages up to 1250V, while GaAsP detectors have a maximum voltage of 900V.

To adjust individual PMT voltage, drag or click the slider bar or arrows or type in the text field to the right of the slider. Clicking **Zero/Prev** at the end of a PMT slider sets that PMT to zero or the previous value, respectively.

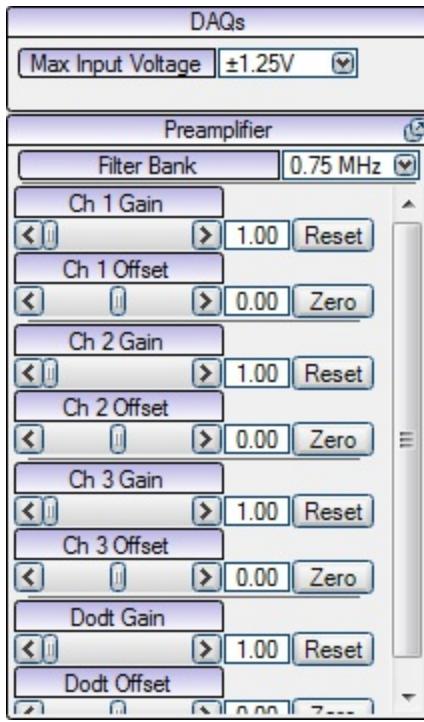
To reset all PMT voltages to zero, click **Zero** next to PMT Master at the top of the section. The button will then change to read **Previous**; clicking **Previous** will restore each PMT slider to its position when the master **Zero** button was clicked.

DAQ and Preamplifier Controls

The DAQs and Preamplifier sections of the tab are stacked behind the PMT controls section; click the green bar to the right of the PMT control section to reveal the DAQs and Preamplifier controls. This section is not relevant to Camera and SFC acquisition modes.

Emission photons from the sample are detected by the PMTs, which send current to the preamplifier. The preamplifier integrates and scales the input current, and sends voltage signals to the acquisition card in the GPIO box or computer.

When used together with the PMT voltage and laser power settings, the controls in this section allow the user to maximize the range of data collection for the sample.



The DAQs section allows the user to control the range of the data acquisition board. Systems equipped with a resonant scanner use a General Standards card for data acquisition. The maximum input voltage of the card is directly selected from a drop-down menu; options are 0.625V, 1.25V, and 2.5V. Systems without a resonant scanner use a National Instruments 6110 card for acquisition (or 6115 for AOD systems). On the NI cards, the user can select a different input range for each of the 4 input channels. This table shows the input voltages realized by each DAQ Gain setting:

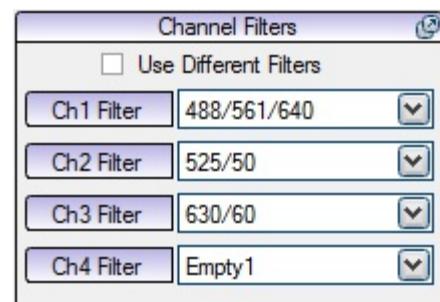
DAQ Gain	Input Voltage Range
0.2x	0 – 42V
0.5x	0 – 20V
1x	0 – 10V
2x	0 – 5V
5x	0 – 2V
10x	0 – 1.0V
20x	0 – 0.5V
50x	0 – 0.2V

The Preamplifier controls allow the user to choose additional settings to adjust the signals coming out of the preamplifier, which is read by the acquisition card. A low-pass filter is applied to the signal and affects the collection rate of the channel. This filter is selected from a drop-down menu to the right of each preamplifier channel. In the example above, the filter is

0.75MHz. All channels must use the same filter; changing the filter for one channel will apply that filter to all channels. Each channel also has a **Gain/Filter** slider and an **Offset** slider to scale and translate the voltage signals coming to the acquisition card. While scanning a sample in Range Check display mode (described later in this manual), adjust the **Offset** slider until just a few blue pixels appear; this translates the incoming voltages so that few or none have zero or negative voltage values. Adjust the **Gain/Filter** slider so that just a few red pixels appear; this scales the signal to use the full range of the acquisition card (based on the DAQ Gain selected for that channel), with few or no saturated pixels.

SFC Channel Filter Controls when using the FLI Filter Wheel

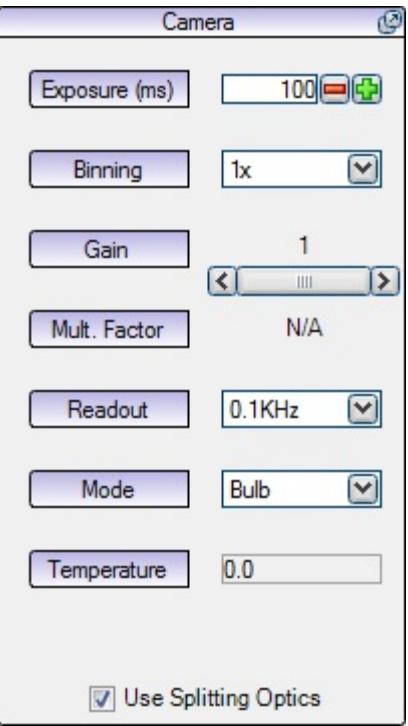
If the system is equipped with the FLI filter wheel, an additional control panel becomes visible in SFC mode. These controls are used to assign different filters to each channel.



If it is desired to change filters between channels, ensure that the **Use Different Filters** checkbox is checked and select the appropriate filter for each channel. Then, when live scanning or acquiring data, the filter wheel rotates into the appropriate position immediately before acquiring the image for the corresponding channel. After the channel is acquired, the filter wheel will rotate into the position for the next channel and acquire that channel. This process repeats for every channel that is enabled in at least one image window, and then is looped back to the first channel along with the data acquisition.

Camera Controls

When operating in the Camera acquisition mode, the Camera controls panel becomes active. Note that control of a camera while operating in SFC mode is handled in the SFC settings window, not the Laser, PMT, DAQ tab.



The parameters and values available in Camera controls vary based on the camera that has been configured in the Prairie Configuration Utility.

Exposure time is the amount of time the camera will collect photons from the sample, entered in milliseconds.

The **Binning** menu allows the user to combine charge from adjacent camera pixels. Increasing the binning factor will increase speed and signal-to-noise, but will decrease spatial resolution.

Many cameras supported by Prairie View have two types of gain control, which amplify signal after it is acquired by the camera. Options in the first **Gain** slider or menu determine the way signal is digitized – they describe the number of photoelectrons that make up one unit of digital signal. The second gain control slider or menu allows the user to set the multiplication gain that is applied on the camera's chip; this is called **Mult. Factor** or **EM Gain**.

The **Port** or **Readout Mode** menu offers choices for the speed at which the camera transmits data to the computer, with or without multiplication gain, denoted by "(M)". Faster readout rates allow faster frame rates, but may affect the dynamic range and read noise of the signal.

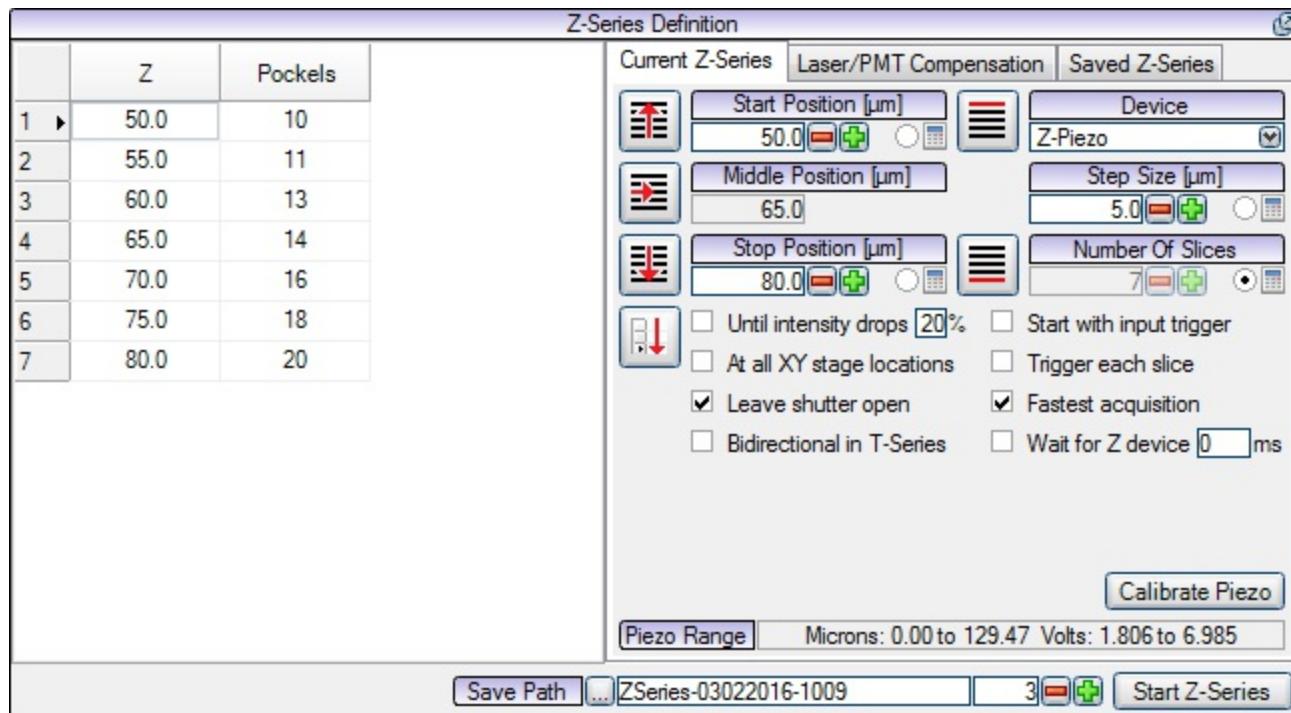
The **Mode** describes the communication between the computer and the camera. In **Timed** mode, the exposure time specified in Prairie View software is used by the camera to generate frames. In **Bulb** mode, each of the camera's exposures is controlled by a signal from an external source; unless the user connects and sends a trigger to end each frame, the camera will not read out data to the computer. In general, **Timed** mode is the preferred mode of operation, but some camera models may require the use of Bulb mode for certain acquisitions.

Temperature displays the temperature of the camera's internal sensor, if supported by the camera.

Use Splitting Optics is an option available on systems configured with splitting optics for simultaneous collection of multiple channels of data using a single CCD or CMOS image sensor. When the box is checked to enable this option, the software will separate a single camera image into multiple channels of data (either 2 or 4, depending on the splitting hardware). More information is available in the [Image Splitting Optics](#) section of this manual.

Z-Series Tab

Use the Z-Series controls to define, save, and recall Z-Series definitions, and to acquire image stacks. The left portion of the window is a table displaying the z device positions defined in the current Z-Series. Controls in the window vary based on system configuration.



Position Controls

The set start and set stop buttons set the current z device position as the start or stop of the Z-Series, respectively. Use the + and – buttons to increase/decrease the set position by the amount displayed in the Step Size field. Use the buttons to the left of the start, middle and stop position fields to move to specific slices (including changing laser powers and PMT gains if those columns are enabled, see the compensation section below for details). The goto middle slice button will move to the center slice rather than the center of the focus travel range, rounding toward the stop position when there is an even number of slices.

The user may also enter z device positions by typing in the Start Position and Stop Position text boxes. Note that the set start and set stop buttons always use the current motor positions, so the user should *not* click these buttons after typing values into the text boxes, unless the user wants to overwrite the value typed previously.

Defining and acquiring a basic Z-Series

A Z-Series is defined by four parameters: a start position, a stop position, a step size, and a number of slices. Defining any three of these parameters allows the fourth to be calculated. Click the **Calculate** radio button (next to the calculator icon) in the Z-Series tab next to the one

parameter to have calculated. The following steps describe setting the start and stop positions of the image stack and choosing the step size.

1. Use **Live Scan** and/or **Single Scan** (see the [Scanning Controls](#) section of this manual) to locate the sample
2. Use the up/down software buttons to navigate to the top or bottom of the desired Z-Series
3. Set the current position as the start of the Z-Series by clicking the set start button  on either the Z-Series tab or in the Stage Control section of the main control window; the + and – buttons will increment the start position by the value displayed in the Step Size field
4. Use the up/down software buttons to navigate to the desired stop position of the Z-Series
5. Set the current position as the stop of the Z-Series by clicking the set stop button  on either the Z-Series tab or in the Stage Control section of the main control window; the + and – buttons will increment the stop position by the value displayed in the Step Size field
6. Enter the desired step size into the Step Size field in the Z-Series tab; the + and – buttons will increment the step size by $0.1\mu\text{m}$
7. Confirm that the **Save Path** and file name point to the intended location of the images
8. Click the **Start Z-Series** button

The user can move to a previously set or calculated Start, Middle, or End position by clicking the goto button to the left of the corresponding text box in the Z-Series tab.

To change the Start or Stop position after defining a Z-Series, either type the new position into the text box or use the + and – buttons to increment the position by the amount specified in the Step Size field.

Note that Prairie View will not allow a Z-Series definition of greater than 10000 slices. If the user attempts to define a stack of more than 10000 slices, the Step Size and Number of Slices will be adjusted to cover the range between the Start and Stop location without exceeding 10000 slices.

Saving Z-Series Images

Each Z-Series acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data.

Laser Power and PMT Gain Compensation

It is sometimes desirable to change laser and/or PMT power during a Z-Series, as signal intensity is often a function of depth in thick samples. There are a few different options to do this on the **Laser/PMT Compensation** tab. The relative compensation options allow laser powers and PMT gains to be set just for the defined Z-Series, while the absolute compensation option uses a global table of power and gains which are applied based on where the Z-Series is actually run, and selecting no compensation will leave the laser power and PMT gains at their current levels. These settings are displayed in the table on the left side of the Z-Series tab.

	Z	Pockels
1	50.0	10
2	55.0	11
3	60.0	13
4	65.0	14
5	70.0	16
6	75.0	18
7	80.0	20

Z-Series Definition

Current Z-Series Laser/PMT Compensation Saved Z-Series

None
Laser power and PMT gain will remain constant for every image

Relative (Exponential Gradient)
Laser power and PMT gain will be interpolated based on the values recorded with the start and stop positions

Laser Selection: Pockels Exponential Gradient Value: Default
 Applies to all lasers Custom

Relative (Manual)
Laser power and PMT gain values for each slice will be those manually entered in the Z-Series grid

Absolute (Not Yet Implemented)
Laser power and PMT gain will be interpolated based on an absolute table of values recorded at different focus locations

... ZSeries-03022016-1009

The default relative laser compensation uses an exponential gradient to increase laser power faster when deeper in the sample, but using a custom gradient value of 0 will achieve a linear change in power; it is also possible to change the custom gradient value to control the rate at which power increases further into the sample. To see a graph of laser power vs depth while configuring the gradient value press the graph button next to the text box. It is possible to use a different gradient for each individual laser by unchecking **Applies to all lasers** and using the

Laser Selection dropdown to configure each laser individually. PMT gains are always incremented in a linear fashion. All these interpolation methods use the laser powers and PMT gains recorded with the start and stop locations (which can be edited by typing new values in the top and bottom rows of the grid).

The relative manual compensation option will keep whatever powers/gains were already in the table, but allow each individual cell to be edited manually. Normally only the top and bottom rows are able to be edited. Switching back and forth between other compensation modes will clear out any modifications made in manual mode.

The absolute compensation option will interpolate, or extrapolate, laser powers and PMT gains linearly based on a global table accessed by pressing the **Edit Values** button. The absolute table can control any subset of lasers/PMTs on the system and those which are not selected will retain their current values. Adding rows to the table is a lot like setting the start/stop limits on a Z-Series, but there is no limit to the number of rows that can be added. When adding a row the current laser powers and PMT gains will be recorded along with the current position in Z. If there are multiple Z devices the absolute Z location is calculated using the sum of the locations for all devices. Like the manual compensation option it is possible to edit the values in any of the cells, with the exception of the focus position.

Note that using a compensation mode other than None will change laser powers and PMT gains for each slice overriding values set in the Laser and PMT sections of the main form, as well as laser powers defined for [Photoactivation Masks](#).

Hard Shutter Control

The **Leave shutter open** option allows the user to determine the behavior of the hard shutter during acquisition of a Z-Series. When checked, the hard shutter opens at the start of the Z-Series and remains open until acquisition is complete. When unchecked, the hard shutter closes between slices of the Z-Series. The option is not available when performing a Z-Series in Fastest Acquisition mode.

Bidirectional Z-Series in a T-Series

When multiple repetitions of a Z-Series will be performed as a [T-Series](#) cycle, the user can choose to perform the Z-Series bidirectionally. If the **Bidirectional in T-Series** option is enabled as part of the Z-Series definition, consecutive Z-Series repetitions will be acquired in opposite directions, eliminating the time needed to move back to the start position between each repetition. The first Z-Series will go from the Start position and go towards the Stop position and the second Z-Series will go from the Stop position towards the Start position. Playback controls will adjust for the opposing orientations of consecutive Z-Series. Note that this bidirectional acquisition applies only in the T-Series tab.

Multiple X,Y Stage Locations

A Z-Series can be performed at multiple pre-defined X,Y stage locations (see [XY-Stage Tab](#)) by checking the **At all XY stage locations** checkbox in the Z-Series tab. The saved X,Y stage locations include coordinates for the X and Y axes as well as the position of the Z device (focal plane). A setting in the [Z-Series Preferences](#) dialog (Preferences > Z-Series) allows the user to choose whether the saved stage location becomes the center or the start of the Z-Series.

Automatically Truncating a Z-Series

The **Until intensity drops** feature allows the user to set up a large Z-Series, but to end the acquisition before the Stop position if the image intensity drops below a user-defined level. This is useful when defining a Z-Series that will be used at multiple time points for a sample that may move or shrink during the experiment. Acquisition will begin at the Start position and will continue until the overall intensity of the image drops below the some fraction of the overall intensity of the previous image; the user determines the fraction by entering the value in the % field. If the image does not reach that intensity cutoff during the Z-Series, the acquisition will continue until the originally defined Stop position is reached.

If a Z-Series with this option enabled is being executed from within a T-Series, BOT data cannot be collected simultaneously and the BOT check box will be disabled.

Fastest Acquisition Mode

Fastest Acquisition mode is available when using a Z-piezo device, or when using the MAMC in a triggered or pulsed mode to move the Z motor. The Z-piezo or MAMC must be cabled to receive a frame trigger from Prairie View software (usually an End of Frame trigger). Z-Series can be performed in **Fastest Acquisition** mode by checking the associated box in the Z-Series tab. In this mode, the scanner does not wait for the Z device to finish moving before beginning to acquire the next frame. Instead, the Z device moves on receipt of a frame trigger signal, and the next scan begins immediately. When using a fast Z device in Galvo scan mode (for example, with an Ultima system), the user can define a wait time to add additional time between frames to give the Z device time to move and any defined changes in PMT and Laser power to occur before the next frame is acquired. This wait time is not implemented in SFC mode.

When using a Bruker piezo device there is an additional button displayed labeled **Calibrate** which will run the piezo through the z-stack very quickly and synchronize that motion with the acquisition. This option will be used by default whenever possible as it is the fastest and most accurate option available, however the piezo is moving at a constant speed during each frame which means that the frames acquired are actually titled by the step size. To bypass this mode it is possible to check the **Wait for Z device** option, even if the time to wait is zero it will revert to the standard triggered motion instead of the calibrated constant motion. If the calibration is not done before an acquisition, it will be done automatically before the acquisition, but will take up to 30 seconds; if timing is a concern after hitting the start button it is recommended

that the calibration be done ahead of time. Once calibrated, the calibration process will not need to be repeated until either the frame period or the z-series definition change. Additionally the calibrated mode is limited how long of a waveform it can store, the limit is configuration dependent and will be displayed at the [bottom of the main form](#) if exceeded; when this happens the z-series will revert back to a normal triggered acquisition. This isn't really a limitation since the triggered mode and the calibrated mode are very close in performance for stacks which take a longer time to acquire.

Z-piezo devices

If Prairie View is configured to control a Z-piezo device, additional options and controls are present in the Z-Series tab. Capabilities of Fastest Acquisition Mode are described above.

If the Z-piezo is a Bruker Fluorescence Microscopy device (rather than a device manufactured by a third party), the **Software Control** checkbox allows the user to dictate whether the device is controlled by Prairie View software or by the manual knobs on the Piezo Amplifier/Driver box.

If the piezo is a Bruker Fluorescence Microscopy piezo device (rather than a third-party device), the range of the piezo is reported in the Z-Series tab. Range and drive voltage can vary based on parameters such as the mass of the objective in use. The **Calibrate Piezo** button will drive the piezo through the currently defined Z-Series and use a feedback algorithm to improve positioning accuracy during the acquisition. If any parameters have changed since the previous calibrated acquisition, the software will automatically calibrate the piezo before acquiring the next Z-Series.

Using multiple Z devices

Many systems are equipped with more than one Z device that can be controlled by Prairie View software. For example, a system may have a focus device on the microscope's objective nosepiece as well as a Z-piezo.

The active Z device is chosen from the drop-down menu in the Stage Control section of the Prairie View main control window; this is the device that will move when the user clicks the software's up and down buttons for Z position.

A second drop-down menu for choosing the Z device can be found in the Z-Series tab; this is the Z device that will be moved during acquisition of the Z-series.

Ensure that the Z device used when defining the top and bottom of the Z-Series is the device used when acquiring the Z-Series. If the top and bottom of the stack is set using the nosepiece focus device but the acquisition device is the piezo, neither Z device will move during the acquisition.

Users may choose to link these two drop-down menus by choosing **Z Device controls linked** in the [Z-Series Preferences](#) dialog (Preferences > Z-Series). If the option set to not link the

controls, the user may independently select which Z device to be used with the Z-Series definition and which to be used with the Stage Control panel Up/Down arrows. If the option is set to link the controls, selecting a Z device with one of these drop-down menus will cause the other selection to automatically change to match.

Additionally, an option in the **Display** menu allows the user to choose whether the focus position information displayed in the lower portion of the Prairie View main control window is the position of the currently active device, all positions separated by commas, or the sum of the positions of all devices.

Triggered Z-Series

A Z-Series can be started on its own, or set to respond to input triggers from other equipment. Triggering options are selected via a drop-down menu in the Z-Series tab.

If neither **Start with input trigger** nor **Trigger Each Slice** is checked, then the Z-Series will start immediately when the Start Z-Series button is pressed.

If **Start with input trigger** is checked and **Trigger Each Slice** is not, then after Start Z-Series is pressed, the z device will move to the position of the first slice/frame, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first slice/frame. All subsequent slices/frames are acquired automatically.

If **Trigger Each Slice** is checked (**Start with input trigger** is forced to be checked), then after Start Z-Series is pressed, the z device will move to the position of the first slice/frame, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first slice/frame. This process is then repeated for each subsequent slice/frame in the Z-Series.

These controls only affect Z-Series executed as stand-alone experiments. They do not affect a Z-Series executed as a cycle in a T-Series.

Triggers sent to the electronics rack should be 5V TTL-style signals on a BNC cable. If the system is configured with a GPIO box, input triggers are received by the Trig 1 In through Trig 8 In connections in the middle row on the front on that box. Input triggers can also be received by configuring one of the numbered Aux connections on the SFC electronics box. These connections are described in the SFC section of this manual. If an SFC Aux line is configured to receive incoming triggers, then Prairie View software will look to that line for signals (rather than to the GPIO box).

Saving Z-Series Definitions

Multiple Z-Series definitions can be set up and saved for use during a Prairie View session; definitions are not preserved when Prairie View is closed. To save a Z-Series definition that has

just been set up, click the  button next to the list of Saved Z-Series. Give a custom name to the definition by clicking once on the name of the Z-Series in the list and typing a new name. To delete a definition from the list, select it and click the  button. These definitions can be recalled to run as individual Z-Series in this tab, or referenced within a T-Series (as described in the [T-Series Tab](#) section of this manual). Note that the Z-Series definition includes all options selected in the Z-Series tab, including the state of the **Bidirectional in T-Series** checkbox.

Focus Lock

Some microscope manufacturers offer a feature where an offset can be recorded and maintained between the objective and the coverslip (for example Nikon's Perfect Focus System); this offset is referred to the Focus Lock Offset in Prairie View. If a Focus Lock is present when a starting position is set for a Z-Series, the Focus Lock Offset will be recorded with that position and used whenever the Z-Series is acquired to ensure the sample does not drift out of focus over the course of a prolonged experiment, for example repeating the same Z-Series many times within a T-Series waiting multiple minutes between Z-Series. If the starting position is edited manually the Focus Lock Offset will remain the point of reference when acquiring the Z-Series. The stop position does not keep track of a Focus Lock Offset.

The Focus Lock state after a Z-Series depends on the **Z Motor Position After Z-Series** setting in the [Z-Series Preferences](#) dialog accessed through the [Preferences menu](#).

More information about Focus Lock and XYZ stage locations is available in the [XY Stage](#) section.

The following is a simple example of how a user would commonly use the focus lock feature with a Z-Series definition to execute a Z-Series over time and correct the focal position before each repetition:

1. Enable the focus lock accessory
2. Adjust the focus lock offset to position the Z motor at the start of the stack
3. Click the Set Start button the Z-Series tab (or use the equivalent button next to the Z motor controls)
4. Disable the focus lock feature
5. Move the Z motor to the end of the stack
6. Click the Set Stop button on the Z-Series tab (or use the equivalent button next to the Z motor controls)
7. Place a Z-Series cycle into the T-Series definition
8. Change the Z-Series cycle's repetition count and period to the desired values
9. Make sure Max Speed is not checked on the Z-Series cycle and Run at all XYZ stage locations is not checked for the T-Series options
10. Run the T-Series

This will execute the Z-Series the requested number of times, enabling focus lock and applying

the offset before each execution. The Z-Series will begin at the focus locked position and each slice will occur relative to that starting position.

If the above example changed to have the repetitions run at max speed, the focus lock correction would only be applied before the first repetition.

If a piezo Z device is present and being used to perform the Z-Series, be sure to have the piezo at its starting position before adjusting the focus lock offset and recording the start position.

T-Series Tab

The T-Series tab gives users the ability to generate a series of images and data using different Parameter Sets, regions of interest (ROIs), XY stage locations, Z-Series, Photoactivation masks, external triggers, etc. The variety of options allows the user to optimize a complex set of experimental parameters to the acquisition of key data.

The screenshot shows the 'T-Series' dialog box. On the left is a table titled 'T-Series Cycles' with columns for Cycle Type, # Reps, Period [s], Max Speed, Duration [s], Resource Selection, Photo-activation, BOT, External Trigger, Triggers Before Starting, Synchronize with Voltage Recording, Synchronize with Mark Points, and Synchronize with Voltage Output. Rows include Images, Z-Series, PA, BOT, XZ/YZ, ROI, Paramet, Script, Wait, Mark, VOut, VRec, XY, and Image. A triangle icon is visible next to the 'Image' row. On the right side of the dialog are several buttons: Move Up, Move Down, Clear All, Remove Selected, Add / Insert Cycle, Image Sequence, Region of Interest, Z-Series, Parameter Set, Line Scan, Mark Points, Photoactivation, Voltage Output, BOT, Voltage Recording, XZ/YZ Image, Stage Position, Script, Wait, Export..., Import..., Total Images (60), Estimated Time Left (0 s), Iterations (1), Total Duration (N/A), Iteration Period (0.00), Save Path (TSeries-12162014-1139), and Start T-Series.

The T-Series Cycles table displays cycles in the current T-Series and allows the user to specify characteristics of each cycle. The selected T-Series cycle is denoted by a triangle on that line to the left of the **Cycle Type** column.

There are a number of buttons on the right side of the T-Series grid:

- **Move Up/Down** will move the selected row/cycle up or down in the grid to allow an experiment to be reordered without having to remove and recreate rows
- **Clear All** will remove all rows/cycles
- **Remove Selected** will remove just the selected row/cycle
- **Export** will save an [Environment file](#) containing the current T-Series definition so it can be imported back in later
- **Import** will open a load file dialog where an [Environment file](#) can be selected, opening the file will load just the T-Series definition portion of the Environment file

Additionally there are a bunch of buttons to **Add** or **Insert** various types of cycles into the T-

Series grid base on the radio button selection above them. Adding a cycle will place it at the end of the T-Series whereas inserting a cycle will place it above the currently selected row/cycle. More details on the types of cycles available can be found [below](#).

When changing acquisition modes, the contents of the T-Series Cycles table may change. The types of cycles available for various modes may differ slightly, so some modes maintain their own T-Series settings from the last time that mode was in use. For example, if the user defines a T-Series in Galvo mode and then switches to SFC mode, the contents of the T-Series Cycles table will change to reflect the last set of cycles defined in SFC mode.

Columns in the T-Series Cycles Table

The left section of the T-Series Cycles Table is made up of columns to define parameters. Some columns/parameters apply to only a subset of cycle types (described below), and are therefore unavailable in some rows.

- **Cycle Type** describes the function performed in the cycle
- **# Reps** shows the number of times the cycle is to be repeated
- The user can set the **Period** as the amount of time between the start of consecutive repetitions. This time does not include motor movements in a Z-Series. When **Max Speed** is checked, the period is automatically set to the minimum time needed to complete one repetition of the cycle.
- When **Max Speed** is checked, images are acquired continuously. Max Speed is available for Z-series only when piezo control is available.
- **Duration** is the minimum amount of time necessary to acquire the image(s) in all repetitions of the cycle as calculated by Prairie View. The actual time required may increase based on a number of factors, including motor movements in a Z-Series, readout and transfer time of a camera image, etc.
- The **Resource Selection** column is enabled for cycles requiring the user to choose a previously-defined definition (Z-Series, ROI, Parameter Set, Script, etc.)
- An imaging cycle can be configured to use a previously-defined Photoactivation Mask via the **Photoactivation** column. Only masks valid for the current scan settings (image size and dimensions) will appear in the list of saved masks. More information can be found in the [Photoactivation](#) section of this manual.
- Check the box in the **BOT** column to perform a previously-defined brightness-over-time measurement during an imaging cycle. More information about BOT features is provided in the [BOT](#) section of this manual. Note that a BOT cannot be performed while collecting a Z-series using the **Until intensity drops ____%** feature.
- The **External Trigger** column allows the user to trigger acquisitions within a cycle. More

information is provided in the Triggering Acquisitions within a T-Series section of this page.

The remaining columns are described later in this section of the manual.

Types of T-Series Cycles

Buttons in the center of the T-Series tab allow the user to add various types of cycles to the T-Series. The **Add** and **Insert** radio buttons indicate whether the new cycle will be placed at the end of the series or inserted previous to the selected line, respectively.

- **Image Sequence** performs the current single image scan, including applying the current ROI, frame averaging, and Parameter Set settings.
- **Z-Series** performs a Z-Series; select the Current or saved Z-Series definition in the Resource Selection column. The current ROI, frame averaging, and Parameter Set settings are used. When performing multiple repetitions of a Z-Series in one cycle, the state of the checking the **Bidirectional** in T-Series option on the Z-Series tab will apply. More information is available in the discussion of the [Z-Series tab](#).
- **Line Scan** performs a Line Scan using whatever is currently defined on the [Line Scan](#) dialog, as if the **Start Linescan(s)** button was pressed; note that repetitions on the Line Scan dialog are combined with the number of repetitions of the cycle, so entering 5 in both places would result in 25 line scans, the same goes for triggering options, both the T-Series settings and Line Scan settings are taken into account.
- **Photoactivation** or **PA** applies a previously-defined Photoactivation mask to a single Image Sequence; select the desired mask from the Photoactivation column drop-down menu. Only masks valid for the current scan settings (image size and dimensions) will appear in the list of saved masks. To apply a 3D Photoactivation mask to a Z-Series, add a Z-Series cycle and select the desired mask in the Photoactivation column. The current ROI, frame averaging, and Parameter Set settings are used. [Photoactivation](#) masks are described elsewhere in this manual.
- **BOT** performs the currently defined Brightness Over Time acquisition within the T-Series. The current ROI, frame averaging, and Parameter Set settings are used. More information about [BOT](#) features is provided elsewhere in this manual.
- **XZ/YZ Image** performs an XZ/YZ scan; select the Current or saved XZ/YZ scan definition in the Resource Selection column. The current ROI, frame averaging, and Parameter Set settings are used. When performing multiple repetitions of an XZ/YZ scan in one cycle, the state of the checking the **Bidirectional** option in the XZ/YZ Scan Definition dialog will apply. More information is available in the discussion of the [XZ/YZ Scanning](#) dialog.
- **Region of Interest** is a modifier cycle that will apply to all subsequent lines in the T-Series. Select the previously-saved ROI from the Resource Selection drop-down list. No scans are performed in this cycle; follow it with an Image Sequence or other cycle to acquire images.

See more information discussed with [Regions of Interest](#) section of this manual.

- **Parameter Set** is a modifier cycle that will apply to all subsequent lines in the T-Series. Select the previously-defined Parameter Set from the Resource Selection drop down; the settings of that Parameter Set will be applied to subsequent cycles. No scans are performed in this cycle; follow it with an Image Sequence or other cycle to acquire images. See more information discussed with [Parameter Sets Tab](#) section of this manual.
- **Script** allows the user to execute a previously written script within the T-Series. More information is provided in the [Scripts](#) section of this manual.
- **Wait** allows the user to specify a delay before the next cycle. Enter the number of seconds to wait in the Period column. If the very last cycle to be executed in a T-Series is a Wait cycle, that cycle will not be executed; this avoids unnecessary delay at the end of the T-Series.
- **Mark Points** inserts a cycle which executes an experiment defined in the Mark Points dialog. Choose the Current or saved definition from the drop-down list in the Resource Selection column. The Mark Points experiment is run to completion before the T-Series moves to the next cycle. Note that if the first point of the first repetition of the first line of the Mark Points experiment is configured to start on receipt of an input trigger, that first trigger will be provided by the T-Series. Subsequent triggers within Mark Points must be provided by the user to avoid locking up the T-Series; this is especially problematic if the Mark Points experiment is waiting for PFI0 or PFI8 triggers, which are not being generated while the T-Series is waiting for the Mark Points experiment to finish. [Mark Points](#) experiments are described elsewhere in this manual.
- **Voltage Output** inserts a cycle which executes an experiment defined in the Voltage Outputs dialog. Choose the Current or saved definition from the drop-down list in the Resource Selection column. The Voltage Outputs experiment is run to completion before the T-Series moves to the next cycle. Note that if the Voltage Output experiment was configured to start on receipt of an input trigger, the T-Series will provide that trigger and the Voltage Output cycle will begin as soon as it is reached within the T-Series. [Voltage Output](#) experiments are described elsewhere in this manual.
- **Voltage Recording** inserts a cycle which executes an experiment defined in the Voltage Recording dialog. Choose the Current or saved definition from the drop-down list in the Resource Selection column. The Voltage Recording experiment is run to completion before the T-Series moves to the next cycle. Note that if the Voltage Recording experiment was configured to start on receipt of an input trigger, the T-Series will provide that trigger and the Voltage Recording cycle will begin as soon as it is reached within the T-Series. [Voltage Recording](#) experiments are described elsewhere in this manual.
- **Stage Position** is a modifier cycle that will move the XY stage and/or Z device(s) to previously-saved coordinates. Select the previously-saved position from the Resource Selection drop-down list. No scans are performed in this cycle; follow it with an Image

Sequence or other cycle to acquire images. See more information discussed with [XY-Stage Tab](#) section of this manual.

Note that the T-Series will start with the current scan settings, including the currently applied ROIs and Parameter Sets (see further description of [ROIs](#) and [Parameter Sets](#) in other sections of this manual). If multiple ROIs and Parameter Sets have been applied in the current Prairie View session, the user must ensure that all settings have been returned to the desired settings of the first T-Series cycle before starting the T-Series. One way to do this is to include a T-Series cycle to select a saved ROI (including “No ROI”) and/or the desired Parameter Set. Also, frame averaging defined in the main form Average Every N Frames setting will be applied to each imaging in the T-Series.

If the T-Series definition includes any positions for the XY or Z devices, and the user changes the position of a device manually while the T-Series is running, the system will attempt to move the device(s) back to the position defined in the T-Series. If the T-Series definition does not include any XY or Z device positions, the user can move the devices without interfering with or being over-ridden by the T-Series.

The **Leave Shutter Open** option allows the user to determine the behavior of the hard shutter(s) during acquisition of a T-Series. When checked, the hard shutters for all lasers used during the T-Series open at the start of the T-Series and remain open until acquisition is complete. When unchecked, the hard shutters close between cycles of the T-Series.

Synchronizing Functions Within a T-Series Cycle

There are many ways to include Voltage Recording, Mark Points, and Voltage Output experiments within a T-Series. These options fall into two general categories: an experiment run as a separate cycle (described above and summarized below), and an experiment embedded within a cycle (described below).

Summary of Experiments Run as Separate Cycles

The Voltage Recording, Mark Points, or Voltage Output experiment can be run as its own cycle, as described in the Types of T-Series Cycles section of this manual. Add the desired cycle type to the T-Series and choose the Current or saved experiment definition from the Resource Selection drop-down menu. The entire cycle will be run to completion before the T-Series moves to the next cycle.

Embedding Experiments Within a Cycle

The Voltage Recording, Mark Points, and/or Voltage Output experiment can be embedded within some other types of cycles, synchronized with each other and/or with imaging sequences. This is done using columns in the right side of the T-Series Cycles table.

Triggers Before Starting	Synchronize with Voltage Recording	Synchronize with Mark Points	Synchronize with Voltage Output
0	None	None	None

The user can choose from the Current or saved definitions for Voltage Recording, Mark Points, and/or Voltage Output via drop-down menus for each type of experiment.

Synchronizing with Frame Triggers

If the number in the **Triggers Before Starting** column is 0, the embedded experiments will start at the beginning of the cycle. By entering a non-zero value in the **Triggers Before Starting** column, the user can determine a number of frame triggers to count before starting the embedded experiment(s). For example, the user can create an Images cycle with 100 repetitions, set **Triggers Before Starting** to 10, and synchronize with a Mark Points experiment. This allows the user to collect a few images as a baseline before starting the Mark Points experiment.

Frame triggers can be generated at the start of frame or end of frame. In the **Preferences** menu > **Frame Trigger Output Selection**, select either **Start of Frame Trigger** or **End of Frame Trigger**. In order for the **Triggers Before Starting** feature to be used, the **Trigger Logic** must be set to **Generate/Count Start of Frame OR End of Frame Triggers**. More information about frame trigger generation is provided [here](#).

Frame triggers are received on the PFI8 line of the National Instruments 6052E card, via connections inside the GPIO box. Some system configurations, including those with Resonant Scanner, SFC, and some Camera configurations, require frame triggers to be routed through external connections on the GPIO box. These connections will be made and described during system installation. Contact Bruker Fluorescence Microscopy personnel with questions about these systems.

Including Multiple Synchronized Experiments Within a T-Series

It is recommended that a user make a global decision on where to configure Voltage Recording and Voltage Output experiments for a given T-Series. It is possible to configure a Mark Points experiment that calls a Voltage Recording or Voltage Output experiment from inside the Mark Points configuration. If using this option, it is not advised to also call Voltage Recording or Voltage Output from the same cycle within the T-Series Cycles table. For example, do not configure the cycle shown below if the current Mark Points experiment also contains Voltage Output experiments:

Cycle Type	# Reps	Period [s]	Max Speed	Duration [s]	Resource Selection	Photo-activation	BOT	External Trigger	Triggers Before Starting	Synchronize with Voltage Recording	Synchronize with Mark Points	Synchronize with Voltage Output
Images	10	0.31133	<input checked="" type="checkbox"/>	3.11333	...	None	<input type="button" value="▼"/>	<input type="checkbox"/> No Trigger	<input type="button" value="▼"/>	0	None	<input type="button" value="▼"/>

It is not possible to start a Mark Points, Voltage Recording, and/or Voltage Output experiment while another Mark Points, Voltage Recording, and/or Voltage Output experiment is running. Consider the example shown below. A Voltage Output is embedded in an Images cycle. The Images in this cycle make up the 4.26 second Duration. The length of the Voltage Output experiment is not displayed. The second cycle is a single repetition of a Z-Series, and the third cycle is a Voltage Recording. The Voltage Recording cycle cannot begin until the Voltage Output experiment is completed. If the Voltage Output lasted only 4 seconds, it would be finished during the Images cycle; the Voltage Recording cycle would start immediately when it is reached in the T-Series Cycles list. However, if the Voltage Output experiment lasted 60 seconds and was still running after the Z-Series cycle finished, the system would wait for the Voltage Output to finish before starting the Voltage Recording cycle.

Cycle Type	# Reps	Period [s]	Max Speed	Duration [s]	Resource Selection	Photo-activation	BOT	External Trigger	Triggers Before Starting	Synchronize with Voltage Recording	Synchronize with Mark Points	Synchronize with Voltage Output
Images	10	0.42603	<input checked="" type="checkbox"/>	4.26033	---	None	<input type="button" value="▼"/>	<input type="checkbox"/> No Trigger <input type="button" value="▼"/>	0	None	<input type="checkbox"/> None <input type="button" value="▼"/>	<input type="checkbox"/> Current <input type="button" value="▼"/>
Z-Series	1	4.68749	<input type="checkbox"/>	4.68749	Current	<input type="button" value="▼"/> None	<input type="checkbox"/> <input type="button" value="▼"/>	<input type="checkbox"/> No Trigger <input type="button" value="▼"/>	0	None	<input type="checkbox"/> None <input type="button" value="▼"/>	<input type="checkbox"/> None <input type="button" value="▼"/>
V Rec	---	---	---	4	Current	<input type="button" value="▼"/> None	<input type="checkbox"/> <input type="button" value="▼"/>	---	---	---	<input type="checkbox"/> None <input type="button" value="▼"/>	<input type="checkbox"/> None <input type="button" value="▼"/>

Imaging With Simultaneous Mark Points Requires Two Scanners

In order to embed a Mark Points experiment in a cycle which scans the sample (Image Sequence, Z-Series, Photoactivation, BOT), an Ultima system must have two sets of galvanometers. Systems with only Imaging galvanometers can perform Mark Points experiments as separate cycles between images (using the single set of galvanometers for both functions); the imaging and Mark Points components are performed sequentially.

Simultaneous imaging with Mark Points requires two scanners with independent lasers. SFC systems configured with an Ultima or Photoactivation/FRAP module can carry out Mark Points with the Ultima or Photoactivation/FRAP module while simultaneously imaging with the SFC.

Embedded Experiments Using Start Triggers

Embedded Voltage Recording and Voltage Output experiments will start as defined in the T-Series Cycles table and not wait for any trigger defined in the Voltage Recording or Voltage Output windows. Embedded Mark Points experiments will override a trigger on the very first point of the experiment (first point of first repetition of first line), if defined, but will respect all other triggers defined in the Mark Points Series.

More information about [Mark Points](#), [Voltage Recording](#), and [Voltage Output](#) can be found in other sections of this manual.

Iterations of a T-Series

The user can specify a number of times to perform the entire T-Series by increasing the value in

the **Iterations** field. A period between the start of consecutive iterations can be defined in the **Period** field below the **Iterations** field. If the user enters a period that is shorter than the amount of time needed for one iteration to take place, the next iteration will begin immediately after conclusion of the preceding iteration.

Time

Total Images displays the total number of images in the current T-Series definition.

The **Total Duration** is the total scanning time for all images defined in the T-Series, as well as any Mark Points, Voltage Recording, and Voltage Output experiment configured as its own cycle. Embedded Mark Points, Voltage Recording, and Voltage Output experiments are not included in the reported time, though they may influence the duration of the experiment. Motor movement times are also not included. Note that time estimates are not valid when any cycle is waiting for a trigger, so the Total Duration field will display N/A.

The **Estimated Time Left** is designed to include time that the system is not scanning. This value will update during the experiment as the system gets more information about inter-cycle and inter-image timing.

Waiting For displays a count-down when the T-Series is waiting for a Wait cycle or pre-defined period between acquisitions.

Saving T-Series Images

Each T-Series acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data.

Triggering Acquisitions within a T-Series

There are several options available for triggering acquisitions in a T-Series. Triggers sent to the

system should be 5V TTL-style signals on a BNC cable. If the system is configured with a GPIO box, input triggers are received by the Trig 1 In through Trig 8 In connections in the middle row on the front on that box. Input triggers can also be received by configuring one of the numbered Aux connections on the SFC electronics box. These connections are described in the SFC [Triggers](#) section of this manual. If an SFC Aux line is configured to receive incoming triggers, then Prairie View software will look to that line for signals (rather than to the GPIO box).

- When **Trigger First Repetition** is selected from the External Trigger column for a given T-Series cycle, Prairie View will wait for an input trigger before processing the selected line.
- When **Trigger Every Repetition** is selected from the External Trigger column for a given T-Series cycle, Prairie View will wait for a trigger before acquiring each repetition in the selected cycle.
- When **Start And Stop Trigger** is selected from the External Trigger column for a given T-Series cycle, Prairie View will wait for a trigger before acquiring each repetition in the selected cycle, and stop acquiring images for that cycle if another trigger is received before it finishes naturally. This option will not work with inputs on the SFC electronics box.
- When the **Start with input trigger** option at the bottom of the T-Series tab is checked, Prairie View will wait for an input trigger before beginning the entire T-Series. Click **Start T-Series** and the system will prepare the acquisition, but will not actually begin collecting data until a trigger is received.

[*Collecting a Basic T-Series*](#)

A Basic T-series is one in which an image or Z-series is collected at a desired interval.

This example shows how to set up a T-Series to take 10 repetitions of a Z-Series, with 120 seconds between the start of consecutive stacks.

1. Click **Z-Series**. A row with default settings will be created.
2. To select the number of images or Z-series to acquire, click on the **# Reps** field, type “10”, and press **<Enter>**.
3. Click on the **Period** field, type “120”, and press **<Enter>**.
4. Select a **Z-series** from the Resource Selection drop down menu. This will cause the Z-series to be collected as defined on the Z-Series Tab.
5. Verify the **Save Path**.
6. Press **Start T-Series**.

[*Collecting a Multi-Cycle T-Series*](#)

A more complex T-series is one in which numerous steps defining different operations are strung together into a single experiment. It is possible to collect a Z-series at a set interval or to collect individual images and Z-series alternately.

This example shows a T-Series in which there are three steps; 1) collection of 10 images with 30 seconds between each image, 2) a waiting period of 4 minutes, and 3) the collection of a Z-series:

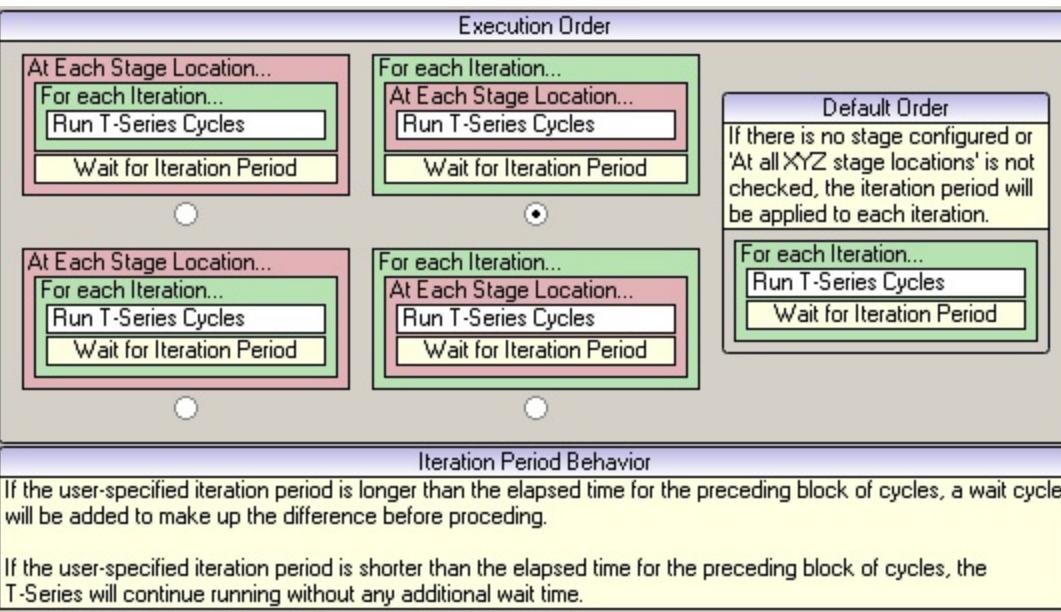
1. Click **Image Sequence**. A row with default settings will be created.
2. To select the number of images or acquire, click on the **# Reps** field, type "10", and press **<Enter>**.
3. Click on the **Period** field and type "30", the total time for the system to wait between start of one repetition and the start of the next repetition. Press **<Enter>**.
4. Click **Wait**. A second line appears.
5. Click on the **Period** field and type "240", the total time for the system to wait between the preceding and following acquisitions. Press **<Enter>**.
6. Click **Z-Series**. A third line appears.
7. Select a **Z-Series** from the Resource Selection drop down menu. This will cause the Z-series to be collected as defined on the Z-Series Tab.
8. Verify the **Save Path**.
9. Press **Start T-Series**.

Saving and Loading T-Series Settings

A T-Series setup can be saved for later use via the File Menu > Save T-Series Settings. It can then be recalled via File > Load T-Series Settings. Note that when a saved T-Series definition is loaded, any cycles calling saved components (ROIs, Photoactivation Masks, Voltage Recording experiments, etc.) will be set back to default selections; new selections must be made by the user for these resources.

T-Series Execution Order

In cases where the number of T-Series **Iterations** is greater than 1 AND the **Run at all XYZ stage locations** box is checked, the order of operations in a T-Series can be chosen by the user. The [T-Series Preferences](#) dialog (Preferences > T-series) contains graphical representations of the T-Series **Execution Order**. The **Iteration Period** designation in the execution order options indicates at which layer of operations the **Period** for iterations is applied.



Consider as an example a T-Series which contains 5 cycles totaling 3 minutes, 2 iterations, the **Run at all XYZ stage locations** box is checked, 4 stage locations are saved, and the Period is set to 3600 seconds. The option selected in the screen shot above is “For each Iteration, At Each Stage Location, Run T-Series Cycles”, with “Wait for Iteration Period” at the Iteration level. This means that the 5 cycles would be performed at the first stage location, then the second stage location, etc, and then the system would drive back to the first stage location. The 3600 second period would be applied at the iteration level, so after a total of 3600 seconds (including acquisition time), the whole cycles/stage locations acquisition would be repeated as the second iteration.

If the user had chosen the upper left order instead (“At Each Stage Location, For Each Iteration, Run T-Series Cycles”, with “Wait for Iteration Period” at the Stage Location level), all 5 cycles would be performed twice (1,2,3,4,5,1,2,3,4,5) at the first stage location, then the system would move to the second stage location and wait, then all 5 cycles would be performed twice at that location, and then again at the third and fourth stage locations. The first image at location 2 would occur 3600 seconds after the first image at location 1, because the period was applied at the level of the stage location.

Additional Preferences

Additional selections in the [Preferences](#) menu and the [T-Series Preferences](#) dialog pertain to T-Series acquisitions.

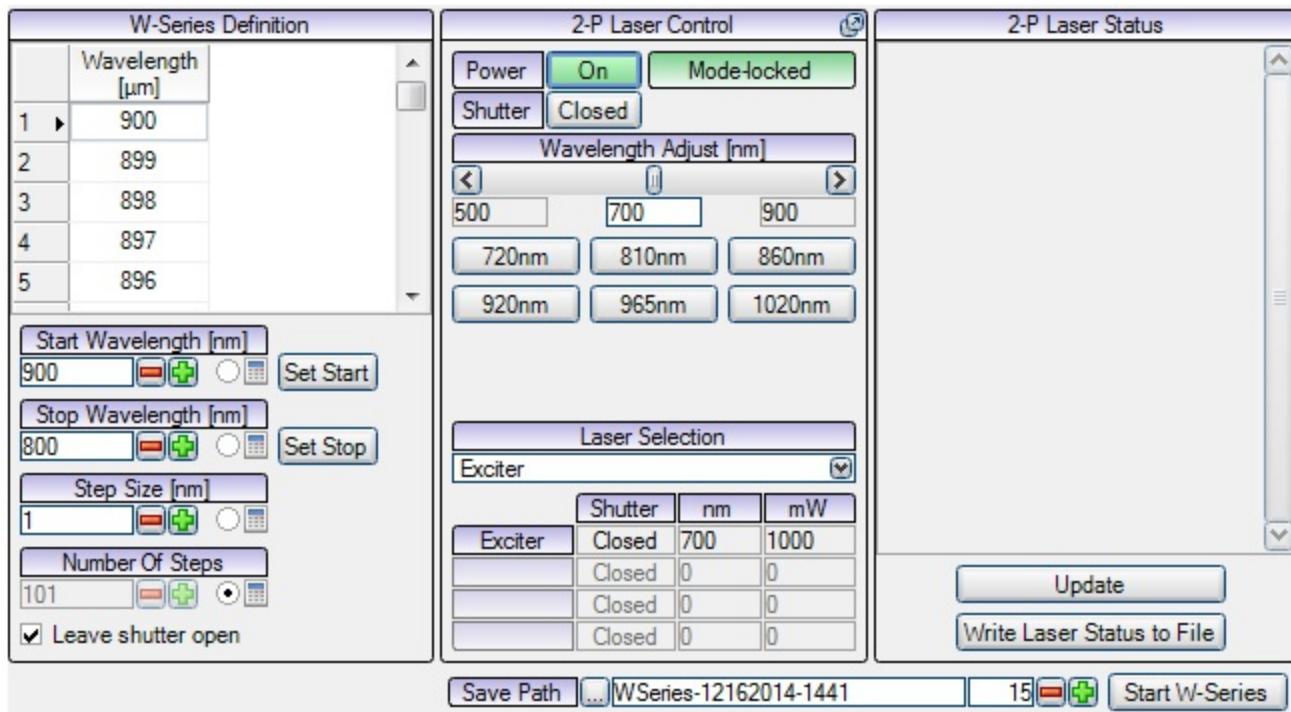
The user can choose whether or not data is automatically written to TIFFs after a T-Series with the Preferences menu options to [Automatically Convert Raw Files](#). If images are not automatically converted, there is less processing overhead at the end of the T-Series before another image can be acquired. The images can be converted later using the stand-alone [Image-Block Ripping Utility](#). Users of systems with solid state drives (SSDs) are advised to have their Save Path set to the drive letter of the solid state drive (typically the E: drive) and to have the automatic conversion turned off. The data should be moved onto the system (C:) drive and

then converted to TIFF images there using the Image-Block Ripping Utility. This reduces the amount of data being written to the SSD, prolonging its useful lifetime.

When files are automatically converted after a T-Series, the user can also choose whether to review T-Series data immediately after conversion. This is accomplished using the **Automatically Start Playback After Acquisition** option in the Preferences menu.

2-P Laser Tab

The 2-P Laser tab provides option for the control of up to four ultra-fast pulsed laser(s) used by the system. Prairie View can control up to four 2-P lasers. This tab is visible only when the system has been configured for Prairie View software control of one or more ultra-fasted pulsed lasers. Note that when a laser is shared between multiple systems, it is common to disable Prairie View control of the laser.



Wavelength Series

The left portion of the tab allows the user to define a Wavelength Series to perform a sequence of images using different excitation wavelengths. The table displays one line for each image in the defined series, and the wavelength used for each slice. Define the acquisition with the controls below the table. There are four variables that must be set; three by the user and one to be calculated by the software. Click the calculator radio button next to the parameter to be calculated. Then enter the desired values for the remaining parameters. To set the current wavelength as the start or stop of the Wavelength Series, click the **Set Start** or **Set Stop** button. Otherwise, type an integer wavelength value into the appropriate text field or use the + and - buttons to change the current value by the number of nanometers defined in the Step Size field. Step Size is the number of nanometers between consecutive images in the series. Number of Steps is the number of images in the acquisition. The **Leave shutter open** checkbox allows the user to determine whether the hard shutter will be closed or left open between images (while the laser is tuning to the next wavelength). Once the variables have been entered, the experiment can be started by pressing **Start W-Series**. At the end of the acquisition, the laser will return to the wavelength in use before the W-Series was started.

Each W-Series acquisition is saved in its own folder. The location of the folder is determined by

the **Save Path**, which can be viewed or changed by clicking the button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and – buttons . Note that decreasing the counter value can result in overwriting data.

2-P Laser Control

The right section of the tab contains controls for the ultra-fast laser(s) controlled by the software. If there are multiple lasers controlled through Prairie View, each can be adjusted separately by choosing the desired laser from the **Laser Selection** pull-down menu in the middle of the section. Laser names are set during installation in the Prairie Configuration Utility; contact Bruker Fluorescence Microscopy personnel before making any changes in the Prairie Configuration Utility.

The **Power On/Off** button displays the current status of the laser's main power control. Depending on the laser manufacturer, the button may allow the user to control the power; some lasers must be turned on/off via a key on the laser itself. The **Shutter Open/Closed** button displays the status of the laser cavity shutter and, depending on laser manufacturer, may be used to open and close the laser cavity shutter.

The **Mode-locked** label (hidden when not applicable) indicates whether or not the 2P Laser is currently mode-locked. Its main purpose is to provide an easy way to check the mode-locked status of the laser when the laser control box's display isn't easily viewed, or isn't available. Lasers in need of service may fail to mode-lock, or fall out of mode-lock, unexpectedly making it important to check their status occasionally.

Change the laser wavelength by clicking one of the pre-set wavelength buttons or typing in the text box below the **Wavelength Adjust** slider. The text field to the left of the slider displays the minimum allowed wavelength for the laser, while the text field to the right of the slider displays the maximum allowed wavelength for the laser. These limits are properties of the laser and are defined in the Prairie Configuration Utility; contact Bruker Fluorescence Microscopy personnel before making any changes in the Prairie Configuration Utility. The wavelengths included in the preset buttons are also configured in the Prairie Configuration Utility. After selecting a new wavelength, there will be a delay for the tuning to take place and

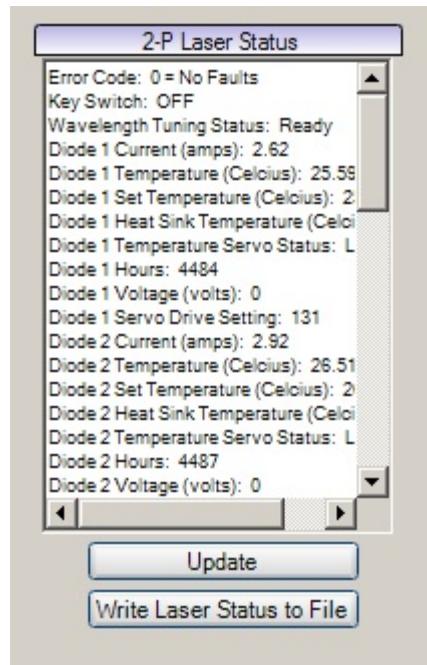
for communication between the software and the laser's control unit. The user can also adjust the wavelength by clicking in the slider or on the arrows at the end of the slider. However, due to the delay mentioned above, the slider may have a sluggish feel.

The status of up to four lasers is shown in a table at the bottom of the section. Note that the power displayed in this table is the power reported by the laser's internal sensors. Table optics on most systems send only a portion of this power to the microscope.

Some ultra-fast lasers configured with a wavelength-extending OPO include an option to bypass the OPO and operate as a traditional ultra-fast laser. For systems configured with these lasers, an additional checkbox appears in the 2-P Laser Control section to **Bypass OPO**. Checking this option will stop the OPO output and pass through 100% of the driving laser's output; when the option is not checked, 80% of the driving laser's power is sent to the OPO and 20% is used as a traditional Ti:Sapphire laser output.

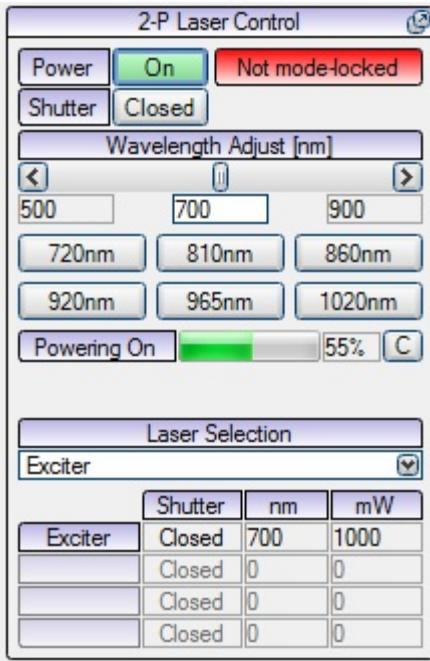
2-P Laser Status

By clicking on the green bar in the center of the tab, the right-hand side of the tab will be replaced with the 2-P Laser Status section.

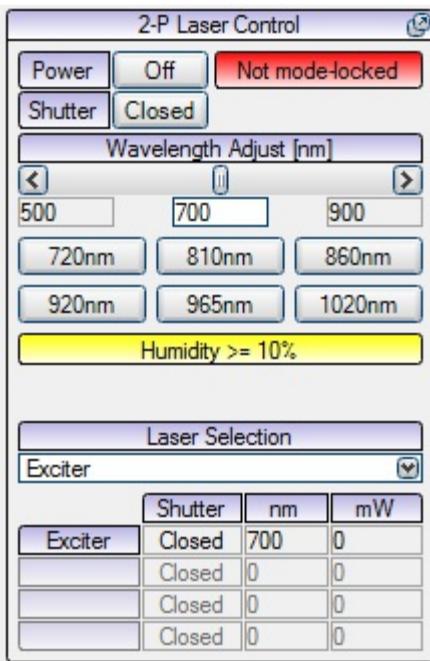


This section displays a text readout of the commands and information received from the laser itself. It is useful for diagnosing errors and checking the status. The **Update** button will query the laser for the most recent information.

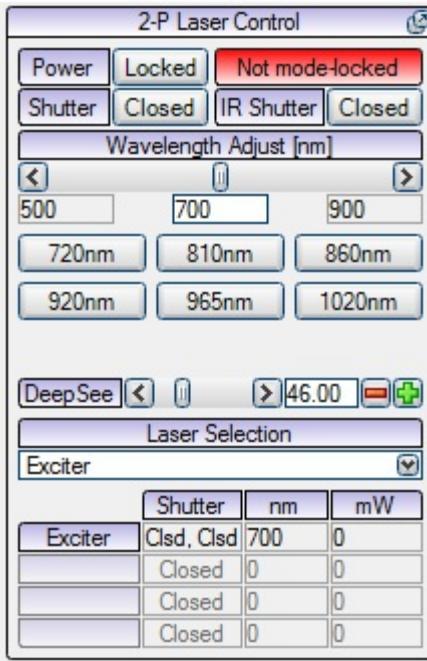
The information displayed in the window can be sent to a text file for printing by clicking **Write Laser Status to File**.



All Spectra Physics lasers will display a **Warm-Up Progress Bar** after the On button was pressed. The warm-up takes between 2 minutes after the laser was keyed-off, or up to an hour if the laser was completely powered down. After warm-up is complete the software will automatically turn on the laser, to prevent this last step from occurring the **C(ancel) Button** next to the progress bar can be clicked. This will not interrupt the warm-up itself however.



If during power-on the humidity sensor reads above 10% a yellow **Humidity Warning** label is displayed underneath the wavelength section. The laser should be serviced according to the directions in the laser manual prior to operation.



The **DeepSee Scrollbar and Textbox** are shown for Spectra Physics Lasers with a DeepSee dispersion controller. This will override the laser's built-in tables for dispersion control until the next wavelength change. For the Insight alone, an **IR Shutter Button** to control the 1040nm shutter is provided next to the main shutter button.

XY Stage Tab

The XY Stage tab allows the user to select and save specific coordinates in the x, y, and z dimensions. This makes it possible to return to locations of interest in the sample and is especially useful in setting up complex experiments.

Some systems are configured with two X,Y moving platforms. For example, a Moving In Vivo microscope may be accompanied by a Specimen Stage. Prairie View software controls only one X,Y platform, configured as the primary X and Y axes in the Prairie Configuration Utility. The secondary X and Y axes (the other platform) can be controlled by the 3-axis knob controller, but will not be taken into account when saving X,Y positions in Prairie View software or using the Stage Control section on the main control window.

The screenshot shows the 'Add/Modify Locations' tab of the XY Stage Tab interface. On the left, there's a vertical stack of buttons: 'Add Current', 'Shift Selected', 'Shift All', 'Remove Selected', 'Remove All', 'Move Motors To', 'Selected Location', 'Next Location', 'Previous Location', 'Grid Display...', 'Import Locations...', 'Export Locations...', and a checked 'Save Optical Zoom' checkbox. The central part is a table with columns: X [μm], Y [μm], Z [μm], Optical Zoom, Focus Lock Offset, and a small icon. Two rows are shown: Row 1 has X=0.00, Y=0.00, Z=-46.90, 5.00, Focus Lock Offset=1.00, and a small arrow icon; Row 2 has X=-292.19, Y=324.43, Z=-92.22, 5.00, Focus Lock Offset=1.00, and a small arrow icon. To the right are two sections: 'Mark Stage' with checkboxes for Invert X and Invert Y, and dropdowns for Point Display (Red/Crosshairs) and Show Point Indices; and 'Plate Definition' with sections for Corner Location (None Set), X (Horiz.) Offset (None Set), Y (Vert.) Offset (None Set), and buttons for Set and Goto, along with X and Y position inputs (12 and 8) with increment/decrement buttons (+/-).

	X [μm]	Y [μm]	Z [μm]	Optical Zoom	Focus Lock Offset
1	0.00	0.00	-46.90, 5.00	1.00	---
2	-292.19	324.43	-92.22, 5.00	1.00	---

Saved positions appear in the list in the upper portion in this tab and are also available from the drop-down menu in the Scan Control section of the Prairie View main control window. The selected location is indicated by a small arrow in the left column.

Add Current adds the current stage and focus position to the list of locations in the table. Positions can also be added in the Image window by activating the **Mark Stage** button and using the right mouse button to click at the position the user wants to add to the list, or by using [Atlas Imaging](#).

Shift Selected updates the selected stage location based on the current stage location. When clicked, the selected stage location will be modified to have its coordinates match the current position of the stage and Z device(s).

Shift All updates the selected stage location based on current stage location, as described above. In addition, it updates all other saved stage locations by the difference between the old and new position for the current stage location.

Remove Selected erases the currently selected location from the table.

Remove All clears the entire table.

In the Move Motors To section there are a number of buttons which will move to previously saved locations:

- **Selected Location** will move the stage and focus devices to the selected location in the table.
- **Next Location** will move the stage and focus devices to the location after the selected location in the table, selecting the next row in the table at the same time. At the last location the first location will be used.
- **Previous Location** will move the stage and focus devices to the location before the selected location in the table, selecting the previous row in the table at the same time. At the first location the last location will be used.

Import Locations loads a previously saved list of stage locations; this can also be accomplished via File > Load XY Stage Locations.

Export Locations saves the current list of XY stage locations for later use; this can also be accomplished via File > Save XY Stage Locations

The **Save Optical Zoom** option allows the user to record the current Optical Zoom value at the time when the stage location is added to the table. The user can then employ different Optical Zooms for different locations. When an XY Stage location that includes an Optical Zoom value is referenced (in a T-Series, Z-Series, etc.), the Optical Zoom will be set accordingly. If a stage location does not include the Optical Zoom value, then the Optical Zoom will not change. Record Optical Zoom does not apply to Camera or SFC imaging modes.

The checkboxes for **Invert X** and **Invert Y** allow the user to change the direction moved in X or Y when using the **Mark Stage** button and mouse clicks on the Image window to move the stage. When the **Mark Stage** button is active, clicking with the left mouse button will move the stage to put the clicked position in the center of the scan. If the stage moves in the wrong direction for one or both axes, change the checked/unchecked status for that axis. See further description in the discussion of the **Mark Stage** button in this manual.

Options in the Point Display section allow the user to determine whether and how saved locations are marked in the Image window. When the overlay is enabled, a marker will appear in the image when the user has navigated to a field of view which includes a saved stage location.

The Z-Series and T-Series tabs have checkboxes to perform the given Z- or T-Series at all saved XYZ locations. See more information about these options on in the discussions of the [Z-Series](#) and [T-Series](#) tabs in this manual.

Grid Definition

To define a grid of locations use the [Atlas Imaging](#) feature to specify some bounding locations by moving around and using the using the **Add Current** button (or the **Add Location/Update Z** button on the Altas Overview window) and then press the **Generate Montage** button on the Altas Overview window to generate a grid of locations covering the area bounded by the bounding locations. Before pressing the **Generate Montage** button the **% Overlap** between stage locations can also be set on the Altas Overview window. See the [Atlas Imaging](#) documentation for more details.

After a grid has been defined, the **Grid Display...** button becomes active. When clicked, this button opens a dialog to allow the user to navigate to positions in the defined grid.

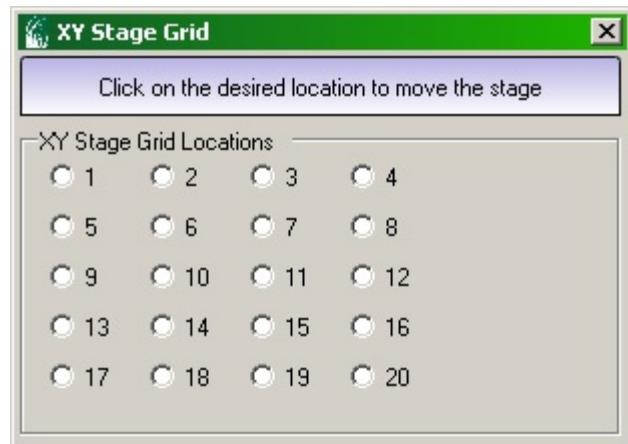
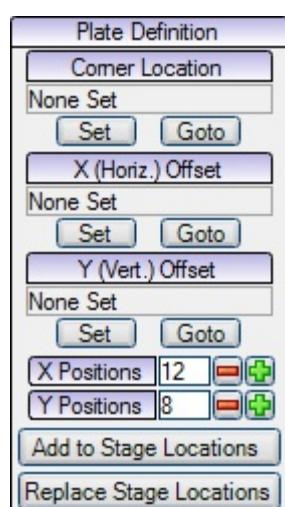


Plate Definition

By clicking the vertical green bar next to the Grid Definition section, the user reveals the **Plate Definition** setup pane. These controls allow the user to generate a list of stage positions that correspond to the wells of a multi-well plate.



Set the position of one corner well by driving to that location and clicking the Corner Location Set button. The current location will be displayed.

1. Move to the next well along the horizontal axis and click the X (Horiz.) Offset **Set** button. This move can include a shift in Y and Z, if needed. The current location will be displayed.

2. Move back to the Corner Location by clicking the Corner Location **Goto** button.
3. Move to the center of the adjacent well along the vertical axis and click the Y (Vert.) Offset **Set** button. This move can include a shift in X and Z, if needed. The current location will be displayed.
4. Define the number of locations in the X and Y directions using the X Positions and Y Positions text boxes or + and – buttons. For example, a 96-well plat would have 12 X Positions and 8 Y Positions.
5. Use the **Add to Stage Locations** button to add the plate definition to the existing list of stage locations, or the **Replace Stage Locations** button to delete the currently saved locations and replace the list with the plate definition.

If the user can mount the sample plate on the stage so that it is consistently oriented, then it may only be necessary to define the plate locations once. The definition can then be saved to a file and re-loaded when needed. This requires that the stage can be reliably set to a defined position for the corner location. When initially setting the Corner Location for the plate definition, before clicking the **Set** button, click the **0** button in the Stage Control section of the main control window. Define the X and Y offsets and positions as above, and replace the list of stage locations with the plate definition. Then export the list of locations to a file. To perform an experiment on the same type of plate in the future, first place the plate on the stage and then position the stage in the same corner that was used when defining the saved plate definition. Then press the **0** button in the Stage Control section of the main control window. Finally, import the saved plate definition.

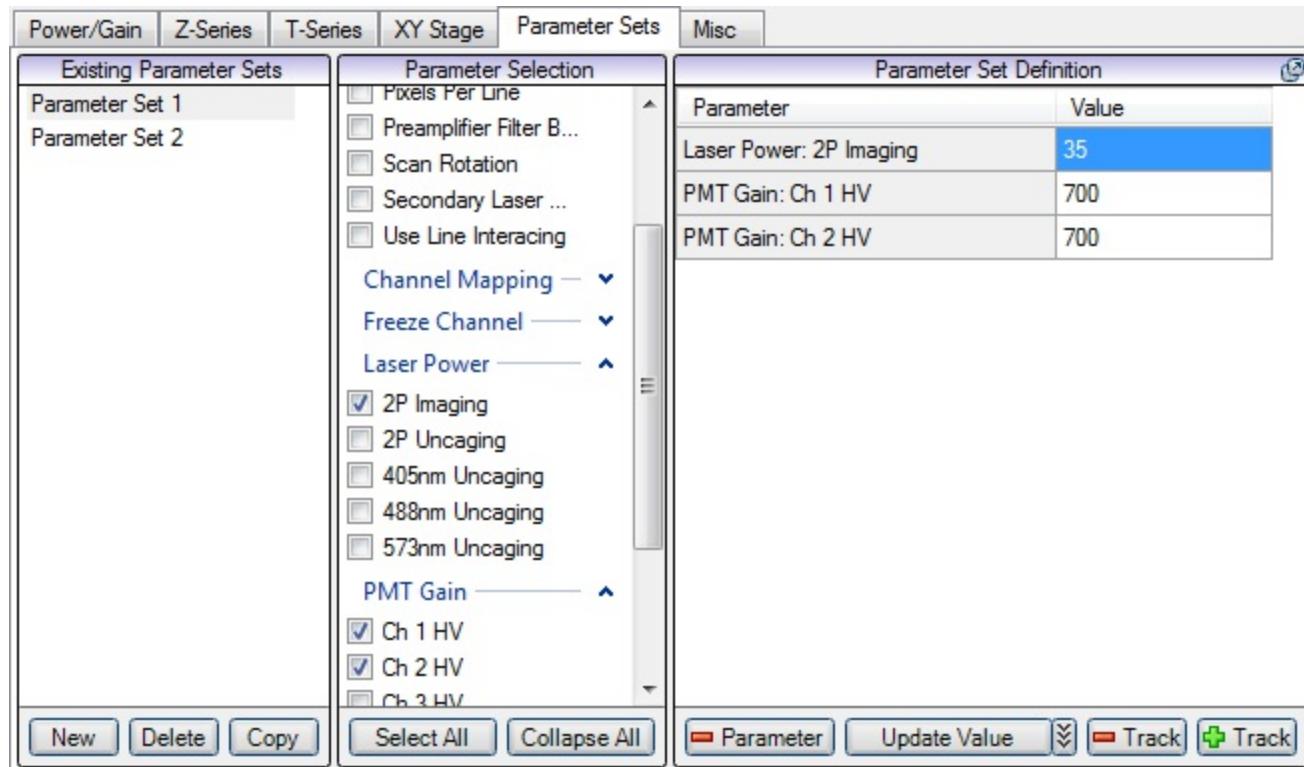
Focus Lock

Some microscope manufacturers offer a feature where an offset can be recorded and maintained between the objective and the coverslip (for example Nikon's Perfect Focus System); this offset is referred to the Focus Lock Offset in Prairie View. If a Focus Lock is present when a recording an XYZ stage position, the Focus Lock Offset will be recorded with that position and used whenever moving to that position to ensure the sample does not drift out of focus over the course of a prolonged experiment, for example repeating the same Z-Series many times within a T-Series waiting multiple minutes between Z-Series. When a Focus Lock state is recorded for a given position the offset will be displayed in the **Focus Lock Offset** column, otherwise the column will display ---. When moving to an XYZ stage location without a Focus Lock Offset recorded, Focus Lock will be disabled. For more information about this feature see the Focus Lock section of the [Z-Series](#) documentation.

Note that [Atlas Imaging](#), which also records XYZ stage locations, does not use Focus Lock.

Parameter Sets Tab

The Parameter Sets tab gives the user the flexibility to define and save a set of operational parameters for imaging control including, but not limited to: laser power, PMT settings, channels, and pixel dwell time; available parameters vary based on system configuration and acquisition mode. Each Parameter Set is essentially a snapshot of a subset of acquisition parameters, which the user can recall at a later time. It is also possible to set up multi-track Parameter Sets for more complicated or multi-laser applications, where there are multiple values for each parameter.



Starting on the left side there is a list of all of the Parameter Sets for the current acquisition mode, this list will change when the acquisition mode changes. The list will most likely start empty, but if upgrading from a previous version of the software any Labels defined (a legacy feature replaced by Parameter Sets) will be imported as Parameter Sets. Buttons underneath this list allow new Parameter Sets to be created, or existing Parameter Sets to be deleted or copied to create a new duplicate Parameter Set.

Once a Parameter Set is created and/or selected the list of parameters included in the Parameter set will be displayed in the center list of all available parameters for the current acquisition mode. Checking/unchecking parameters will add/remove them from the table on the right side which lists all the selected parameters and their values. The buttons below this list allow all parameters to be selected, or to collapse an sections that have been expanded with one click to make it easier to find a parameter without scrolling.

The right side consists of a table containing all the selected parameters and their values. As new parameters are added their values default to the current value, but can be changed by editing the cell, or by changing the current value and pressing the **Update Value** button below.

By using the dropdown button next to the update button, or by right clicking either button, it is possible to update an entire row or column, or even change the default behavior of the button to always update a row (parameter) or column (track) instead of a single value. There are also buttons to remove a parameter as well as add or remove additional tracks which will be described in more detail below with multi-track parameter sets.

Creating a Parameter Set

1. Press the **New** button in the lower left corner of the tab
2. Type in a name that will describe the new Parameter Set or what it will be used for
3. Select one or more parameters from the center list by checking parameters individually or pressing the **Select All** button at the bottom
4. After the desired parameters are selected their values can be changed by changing the current value and pressing the **Update Value** button, or manually entering the value in the cell

Using a Parameter Set

To apply the settings of a Parameter Set, select it from the **Parameter Set** drop down in the [Scanning](#) section of the Prairie View main control window. Any changes made to the selected Parameter Set from the Parameter Sets tab will be applied to the current scan settings.

To use a Parameter Set during a T-Series, use the **Parameter Set** button in the T-Series tab to add a Parameter Set cycle to the T-Series. Select the desired Parameter Set from the drop down list in the Resource Selection column. The chosen Parameter Set will be used for all subsequent cycles, until another Parameter Set cycle is encountered.

Updating or Changing a Parameter Set

1. Select the Parameter to update/change on the left side of the tab
2. To update a value or set of values, simply change the current settings to the desired values and press the **Update Value** button (right clicking the button will provide more options)
3. To change a value simply type a new value into the table on the right side, or use the drop down provided for parameters with a defined set of potential values (Note that if the parameter set is currently active the changes made will also be applied)

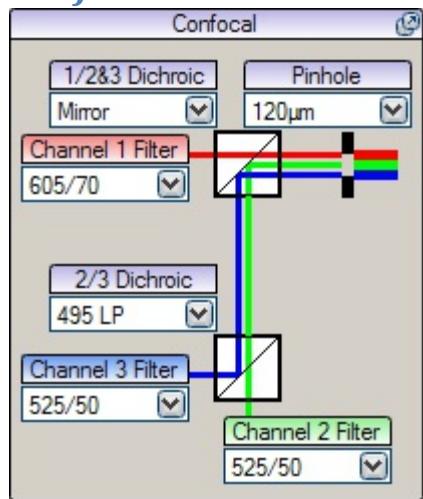
Multi-Track Parameter Sets

Multi-Track Parameter Sets allow the user to sequentially apply anywhere from two to four sets of values during an acquisition (one for each track). To add additional tracks to a Parameter Set simply press the **+ Track** button which will add another set of values to the Parameter Set and default their values to whatever they are currently set to.

When using a Multi-Track Parameter Set, each acquisition (Single Scan, Z-Series, T-Series repetition, etc.) will be performed sequentially with all the tracks defined in the Parameter Set. For example, in a Z-Series with a multi-track Parameter Set, the first slice will be acquired with all tracks, followed by the second slice for all tracks, etc., until the last slice is completed. A multi-track Parameter Set is not used when live scanning or in a T-Series cycle in which the **Max Speed** option is enabled (a warning is displayed in the T-Series grid when this is the case), in both of these cases just the settings from the first track will be used.

The Misc tab contains controls for several different features of the Prairie View software. Many of the controls are used to control optional features or components of the system. The controls displayed on this tab will vary depending on the configuration of the system.

Confocal Controls



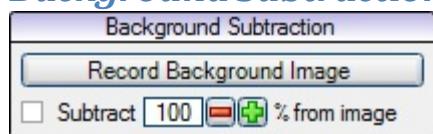
For systems configured with a 3-channel descanned confocal module, this section allows the user to select the dichroic mirrors, band pass filters, and confocal pinhole in the emission/detection light path. The controls are located behind the Laser control section of the Laser, PMT, DAQ tab; click the green bar on the right side of the Laser control section to reveal Confocal controls. Filter positions and names are configured by Bruker Fluorescence Microscopy personnel while building and installing the system. Pinhole positions are set during installation and can be updated by the user in a dialog accessed via Tools -> Calibration/Alignment/1-P Pinhole Alignment; more information is provided laser in this manual. The red, blue, and green lines are for general illustration only, and do not update based on the filter combinations selected.

In the example above, all light passing through the 120um pinhole will be reflected toward the Channel 2/3 Dichroic. The 495 long pass dichroic in that position will transmit light above 495nm toward the Channel 2 band pass filter, which will allow light between 500 and 550nm to reach the PMT. Wavelengths shorter than 495nm will be reflected toward the Channel 3 band pass filter, which will block all that light, as the filter transmits only wavelengths between 500nm and 550nm. Thus, only Channel 2 will detect useful emissions in this example.

Position	Function	Examples (differing from diagram)
1/2&3 Dichroic	Transmit light toward Channel 1 Reflect light toward Channels 2 & 3	565LP transmits red light toward Ch1 and shorter wavelengths toward Ch2/Ch3
Channel 1 Filter	Band pass filter to set bounds on Channel 1	605/70 transmits 570-640nm to Ch1

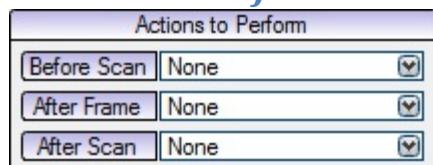
2/3 Dichroic	Transmit light toward Channel 2 Reflect light toward Channel 3	495LP transmits green light toward Ch2 and reflects blue light toward Ch3
Channel 2 Filter	Band pass filter to set bounds on Channel 2	525/50 transmits 500-550nm to Ch2
Channel 3 Filter	Band pass filter to set bounds on Channel 3	460/50 transmits 410-510nm to Ch 3

Background Subtraction



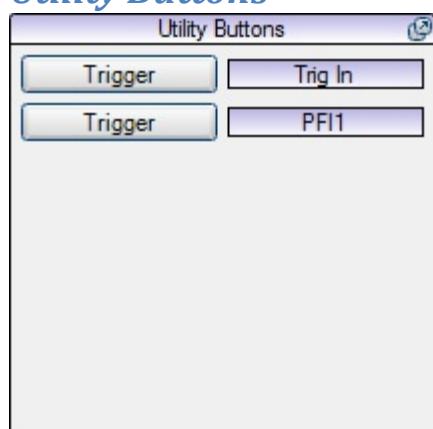
A **Background Image** can be saved to use as a subtraction mask from the signal generated in a scan. The intensities of the background image will be subtracted from the intensities of any new images when enabled. A background image is stored by pressing the **Record Background Image** button and will be used until replaced or the program terminates. To use background subtraction, check the **Subtract** box and specify the percentage of the background image to subtract.

Actions to Perform



Actions previously defined in the Actions dialog can be selected for use as before scan, after frame, and after scan events. For information about defining Actions, refer to the [Actions](#) section of this manual.

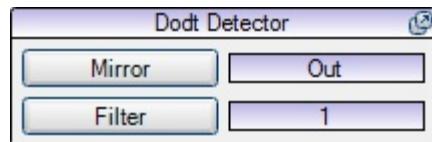
Utility Buttons



Utility Buttons can be configured in the Misc tab of the Prairie Configuration Utility and

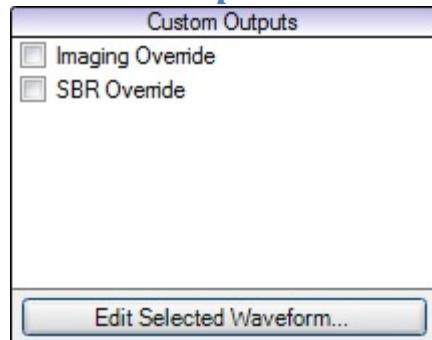
determine the state of up to 8 digital output switches within the GPIO box. The states of these switches can be sensed by external equipment. The name of the button and the text that appears when the button is on or off can be defined in the Prairie Configuration Utility. Only those utility buttons which have been enabled in the Prairie Configuration Utility will be displayed. Additionally, cabling must be present inside the GPIO box to route these signals out to the user.

Dodt Detector



The **Dodt Detector** section controls the Dodt gradient contrast detector, if one is configured. When the Dodt mirror is **In**, the lamp house will automatically turn off to protect the Dodt PMT from being saturated. Filters can be placed before the mirror to filter light being sent to the PMT. There are two filter positions. The Dodt detector has no acquisition capabilities in SFC mode, but the controls remain active in this mode to allow the user to move the mirror and filters out of the way when using the transmitted light lamp house.

Custom Outputs

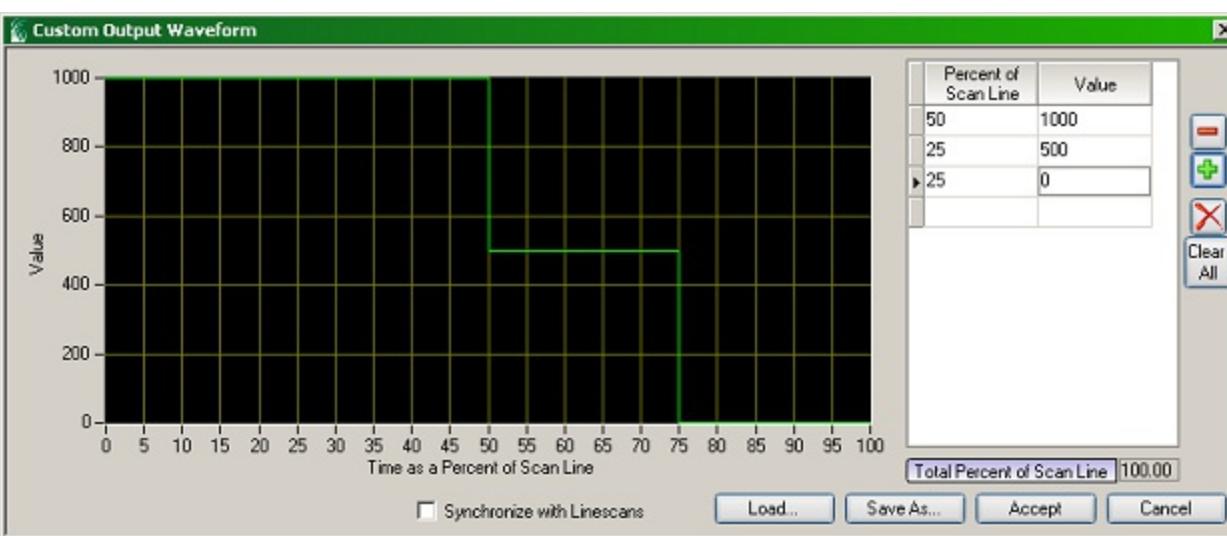


The **Custom Outputs** section specifies custom waveforms which will be synchronized with each line of an acquisition. When enabled, outputs are applied during all scans.

To be available in Prairie View software, Custom Outputs must be enabled in the Prairie Configuration Utility. Additional cabling must be put inside the GPIO box. Contact Bruker Fluorescence Microscopy support personnel for assistance with this feature. Depending on hardware and system configuration, up to eight custom outputs can be defined.

Once enabled, a Custom Output will appear in the Prairie View Misc tab. Turn on the Custom Output by checking the box next to its name.

To define a waveform for a Custom Output, select it by clicking the name of the output in the list, and click **Edit Selected Waveform...** to open the Custom Output Waveform editor dialog.



Fill in the table on the right side of the window to define the output. Entries in the Value column are scaled based on the parameters defined in the Prairie Configuration Utility. Buttons to the right of the table allow the user to move a selected row up or down in the table, delete the row, and delete all rows.

The custom outputs are synchronized with each acquisition and are adapted to the current line period. Waveforms are defined as a value for a certain percentage of the line period. A waveform defined as 100% of the line period would occur once every line. One defined as 50% would occur twice every line, while one defined as 200% would occur once every two lines. Limitations are put on the total percent such that it divides evenly into 100 or is a multiple of 100 so that all custom outputs can share a common clock. Therefore, it is not possible to define a waveform that occupies 75% of the line period, but it is possible to define a waveform that occupies 300% of the line period and simply repeats the 75% waveform four times.

Note that the scanline period (on which the custom output is based) includes the retrace time of the galvanometers, when the move from the end of one line to the start of the next line. This means that an output defined to use 50% of the scan line at one value and 50% of the scan line at another value will not change states in the center of the image, but instead will change closer to the left side of the image. Retrace time can be calculated by multiplying the number of pixels per line by the dwell time per pixel, and subtracting that value from the scanline period; the retrace time occurs at the start of the line, before the scan reaches the first pixel of the line.

The **Save As...** and **Load...** buttons below the table allow the user to save and load waveform definitions. Click **Accept** to accept the current definition and close the dialog, or **Cancel** to close the dialog without saving changes.

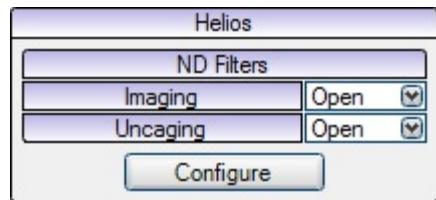
The **Synchronize with Linescans** checkbox is used to automatically program a custom output for use with the 3-D Linescan feature. This feature is described in the Line Scan section of this manual.

Custom outputs can also share the same physical output line as a laser. In such cases when the custom output is enabled it overrides the laser control. Disabling the custom output will return control to the laser slider.

It is also possible to configure a Custom Output to produce a waveform based on a drive signal from a BOT (Brightness Over Time) region, to turn intensity data into an analog signal. This option is set up in the Prairie Configuration Utility.

In Spiral acquisition mode, the entire frame is acquired in one line. Thus, the line period is the same as the frame period, and Custom Output definitions are based on a percentage of that period. Custom outputs are not applicable in SFC mode.

Helios ND Filters



On systems configured with a Helios Laser Launch, a drop-down menu allows the user to choose a neutral density filter inside the launch. These filters reduce the amount of laser power from the launch and allow finer control of power using the laser power sliders in the Laser, PMT, DAQ tab. Use the **Configure** button to open a dialog which allows the user to define a name for the launch.

SFC Basic Acquisition Mode

When using the SFC, Prairie View can operate in a “Basic Acquisition” mode, which allows for a more straightforward way to set up and run common experiments. This mode is entered by clicking on the **Basic Acquisition** item in the **Display** menu. Entering the basic mode replaces the controls on the Main Form with new controls that can be used to perform most types of acquisitions. If it is desired to go back to the advanced Main Form, simply click the **Advanced Acquisition** item in the **Display** menu. Going back to the advanced form will translate the settings chosen from the basic form to the advanced form to show how the same experiment would be set up using the advanced controls.

Click on the image for more information on a specific topic

Collapsible Groups

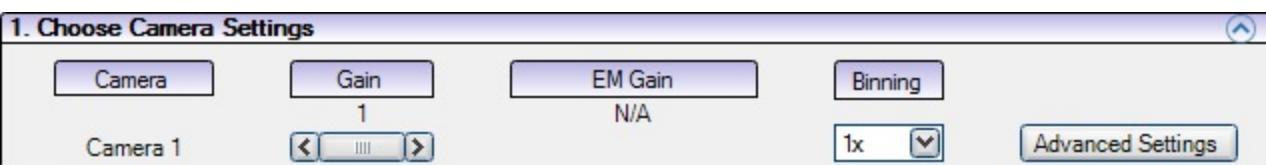
Each of the six groups in the interface can be collapsed to save space and hide parameters which won't change during the acquisition setup process.

To collapse a group, click on the title bar of that group or the red “-“ symbol on the right side of the title bar. Collapsing a group will resize the form to fit the new group sizes. To expand a collapsed group, simply click the title bar again or the green plus sign on the right side of the group's title bar.

Color Control

The color of the title bar of each group can be changed. A color wheel will appear in the left corner of the title bar when the mouse cursor is hovered over the title bar. Click on the color wheel and select a new color for the title bar.

Camera Settings



The camera settings group is used to set the parameters that relate to the camera acquisition. On a system that is configured for more than one camera, the group is resized to allow for controls for a second camera.

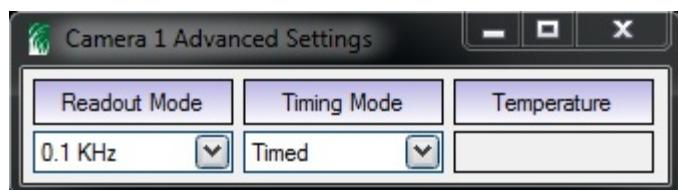
The **Camera** label specifies which camera is used for the acquisition.

The **Gain** is used to select between the available amplification settings of the camera.

The **EM Gain** sets the Electron Multiplication Gain of the camera, if the camera supports this feature.

Binning is used to bin together neighboring pixels to increase signal levels at the cost of lower resolution.

Clicking on the **Advanced Settings** button displays the following dialog, in which uncommonly changed parameters can be set:



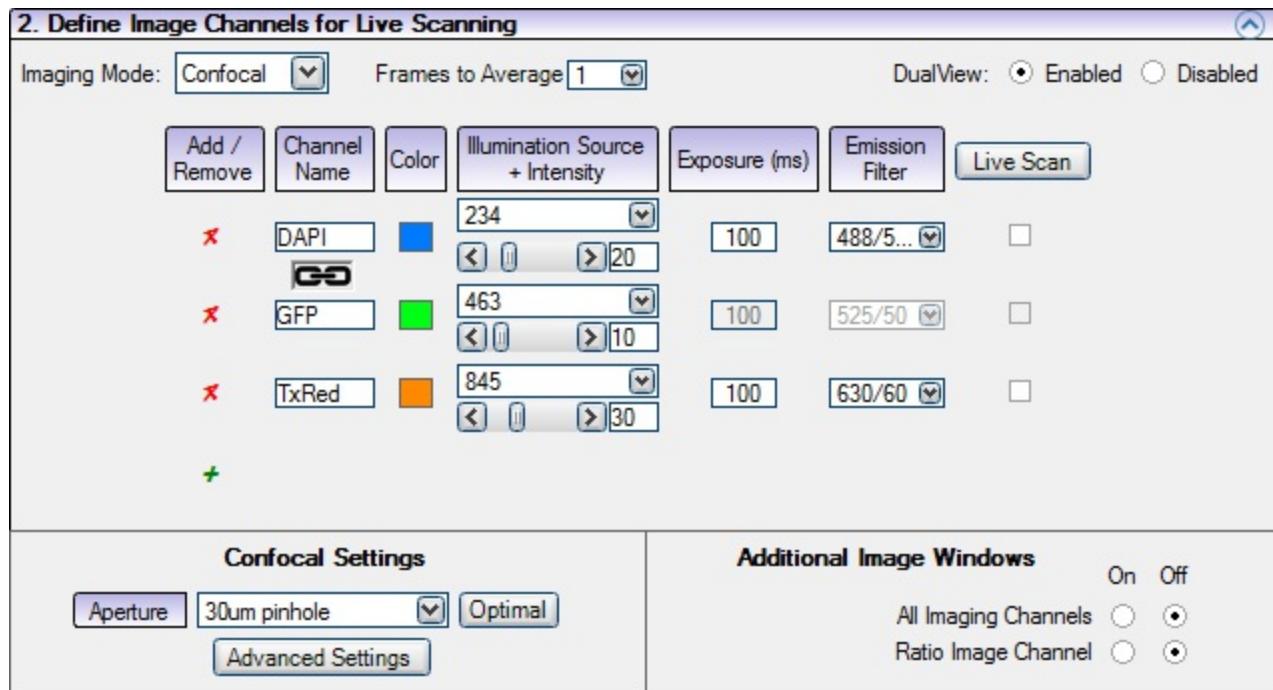
The **Readout Mode** dropdown is used to select which output port is used from the camera.

Timing Mode toggles the camera between master (Timed) and slave (Bulb) mode. Not all cameras support both modes, so use caution when changing this value.

The current **Temperature** of the camera is also displayed here.

Channel Definition

In the Basic Acquisition mode, the Channels group is used for defining which channels will be used for the experiment. In addition, some settings can be adjusted relating to the confocal module, and additional image windows can be toggled on and off.



At the top of the window, the **Imaging Mode** drop down can be used to switch between Confocal, Widefield (if equipped), and Spectral (if equipped).

Multiple images can be averaged into the single image by selecting a number other than 1 from the **Average Every** dropdown menu.

If the microscope is equipped with image splitting optics, the option to enable the split will be shown in the top right corner.

To add an imaging channel, click on the green +. Conversely, to remove a channel, click on the red X. Each channel corresponds to one image window with only that channel enabled. Adding a channel opens a new window, and removing a channel removes the corresponding window.

Each channel can be assigned a unique name by typing in the **Channel Name** textbox.

To set the color associated with the channel, click on the **Color** box and select the desired color in the context menu.

The **Illumination Source + Intensity** column is used to choose an illumination source (a laser in confocal mode), and set its intensity. Select the appropriate light source from the dropdown menu and set its intensity by either dragging the slider or typing into the textbox beneath the source selection.

The channel's exposure time is set using the **Exposure (ms)** textbox.

The emission filter associated with the channel can be selected from the **Emission Filter** dropdown.

If in Widefield mode, the **Filter Block** dropdown is used to choose which turret cube to use in the microscope.

If equipped with more than one camera, the **Camera** column indicates which camera will be used for this channel.

The **Live Scan** checkbox toggles whether or not to acquire this channel when the **Live Scan** button is pressed.

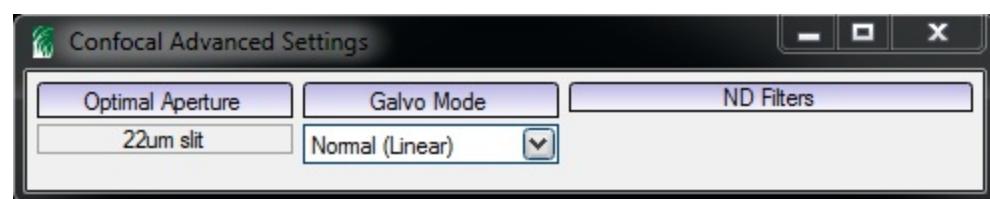
If using splitting optics, the link icon can be clicked to link multiple channels together. Linked channels will acquire simultaneously, whereas unlinked channels acquire sequentially.

Although it is possible to set all of these values uniquely for every channel, doing so will cause the acquisition to pause in between channels to load in the new settings. The parameters for which differing values cause this pause are: Exposure Time, Emission Filter, and Filter Block. In addition, if two channels use the same illumination source and differing intensities, this pause will be added between the channels.

In the Confocal Settings, the **Aperture** can be selected from the dropdown menu. To open a dialog with more advanced confocal module settings, click the **Advanced Settings** button (see below for a description of the window opened).

In the Additional Image Windows section, two other image windows can be added. The **All Imaging Channels** window contains all enabled channels in one overlaid image. The **Ratio Image Channel** opens a window in which two channels can be selected and a ratio of the two channels is displayed.

Advanced Confocal Settings



The Optimal Aperture is calculated and displayed based on the objective being used and the illumination sources chosen in the channel definitions

The **Galvo Mode** can be switched between Normal, Sinusoidal, and Harmonic as desired. As the mode is switched from Normal to Sinusoidal to Harmonic, the image acquisition gets faster, but at the cost of decreased image intensity uniformity.

If equipped with one or more Helios laser launches, the **ND Filters** dropdowns can be used to rotate a neutral density optical filter in front of the laser to decrease the intensity of the laser reaching the sample.

Single Scan



The Single Scan group is used to acquire a single image from every channel defined in the Channel Definition group.

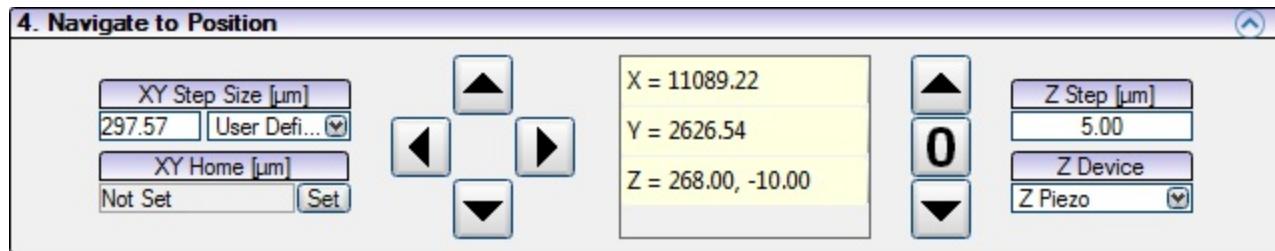
The **Save Path** sets the directory into which the resulting images will be saved. The path consists of a base directory, which can be set using the ... button, a subdirectory which is set by typing into the leftmost textbox, and an iteration number which is set by typing into the rightmost textbox or pressing the green + or red – buttons.

The checkbox with the save icon toggles the auto-increment behavior of the save path. With the checkbox checked, each single scan will increment the iteration number and therefore create a new subdirectory. With this checkbox left unchecked, the iteration number is not incremented each time, and therefore subsequent single scans will overwrite the previous single scan data.

To acquire the single scan, click the **Single Scan** button. Once the scan is started, the button text will change to **Abort**, which can be clicked to stop the single scan immediately.

Stage Control

The Stage Control group is used to move the sample into the appropriate position for the upcoming acquisition. The group is divided into three sections. On the left side are controls relating to the X and Y positions of the current field of view. In the middle of the group, the current position of all motors is displayed. Finally, on the right side are controls for changing the focal plane for the experiment.



The **XY Step Size** defines the distance the stage moves when one of the XY arrows is pressed. This distance can be set by typing into the textbox or by choosing a percentage of the current field of view from the dropdown menu on the right. Ensure that the current objective has been calibrated prior to setting the step size.

The **XY Home** position provides a way to store a stage location that will not be used during the acquisition. By clicking the **Set** button, the current position of the X and Y axes is stored and can be returned to at any time by pressing the XY Home button. The Home button is not visible until a home position has been set.

Pressing any of the arrows on the left side of the group moves the stage in X or Y by the current **XY Step Size**.

The center listbox displays the current positions of all three axes. In the case that multiple devices exist for changing the focal plane, the Z readout can be toggled to display all Z positions, the position of the active Z device, or the sum of all Z device positions. This preference is located under the **Display -> Z-Position Display** menu.

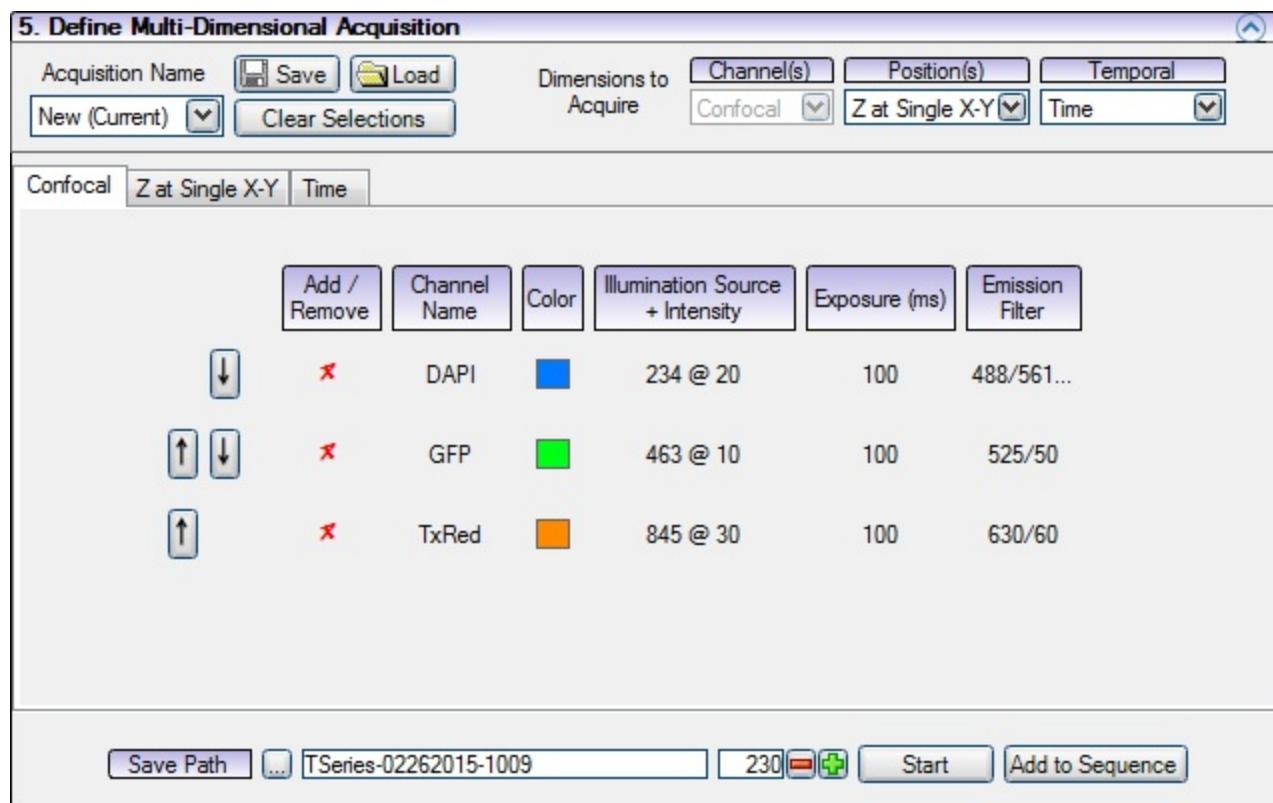
Pressing one of the arrows on the right side moves the active Z device (chosen from the **Z Device** dropdown) by the Z step size (set in the **Z Step** textbox). Pressing the **0** button in the center of these arrows re-orients the Z axis so that the current position is 0.

Entering a value into the **Z Step** textbox defines how much to move the active Z device (chosen from the **Z Device** dropdown) when one of the Z arrows is pressed.

Choosing one of the devices in the **Z Device** dropdown menu selects that device as the active Z device. This is the device that will be moved when one of the arrows is pressed and will be used during the acquisition.

Acquisition Definition

The Acquisition Definition group of the Basic Acquisition interface is used to define and run most common experiments. The general process is to define a subset (and order) of the channels from the Channel Group, a positional aspect, and a temporal attribute of the experiment. Each of these selections shows the appropriate tab that can be used to set up the details of that type of experiment. The possible selections are explained in detail below. Once a selection has been made for all three of these categories, the **Start** button is enabled, which can be used to run the newly-defined experiment. If the defined acquisition is going to be part of a larger sequence of acquisitions, then the **Add to Sequence** button can be clicked to add this acquisition to the upcoming sequence. In the simplest case, a single image can be acquired by selecting “Current” for the position and “None” for the temporal menus.



Prior to selecting anything in the **Position(s)** or **Temporal** dropdown menus, the group will display the channels over which to acquire. From this tab, the order of channel acquisition can be changed using the up and down arrows on the left side of the table. Pressing the up button will move that channel definition upward, causing it to acquire earlier in the sequence, and vice versa. Additionally, if only a subset of the defined channels is needed for the acquisition, clicking the red X will remove that channel definition from the upcoming experiment. After a channel has been removed, a green + will be displayed to add that channel definition back into the experiment.

Current Position

5. Define Multi-Dimensional Acquisition

Acquisition Name
New (Current)

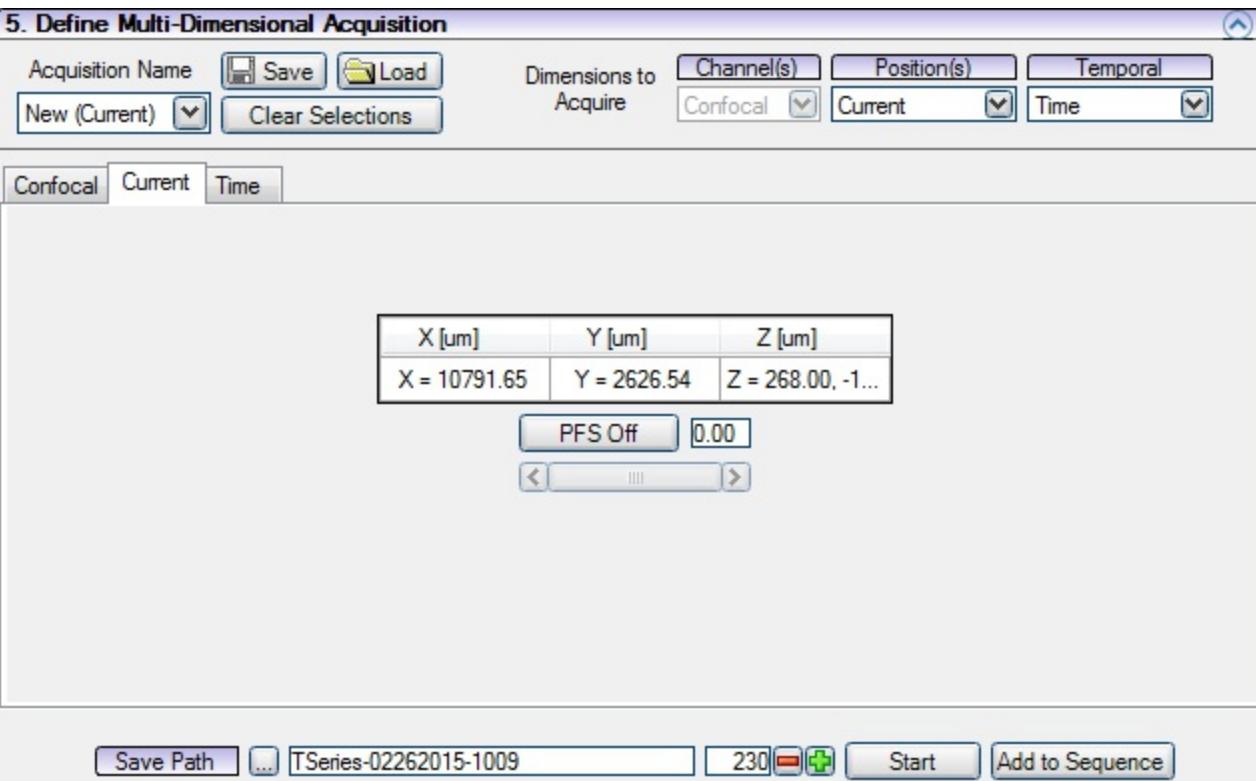
Dimensions to Acquire Channel(s) Position(s) Temporal
Confocal Current Time

Confocal Current Time

X [um]	Y [um]	Z [um]
X = 10791.65	Y = 2626.54	Z = 268.00, -1...

PFS Off 0.00

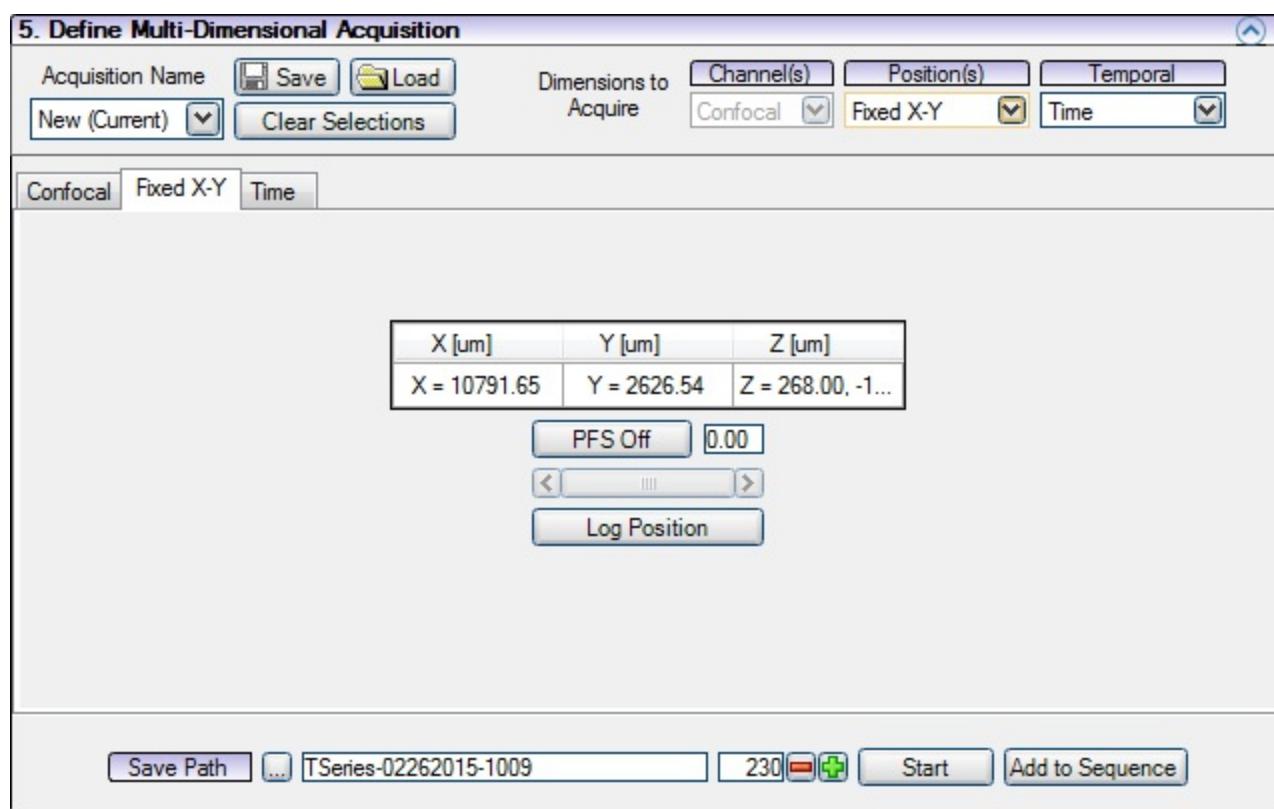
Save Path TSeries-02262015-1009 230 Add to Sequence



The Current position selection is used to perform experiments at whichever location the stage is currently at. When this is selected, the above table is shown and populated with the current positions of each stage motor on the system.

Perfect Focus can be toggled on or off from here and the offset adjusted accordingly.

Fixed Position

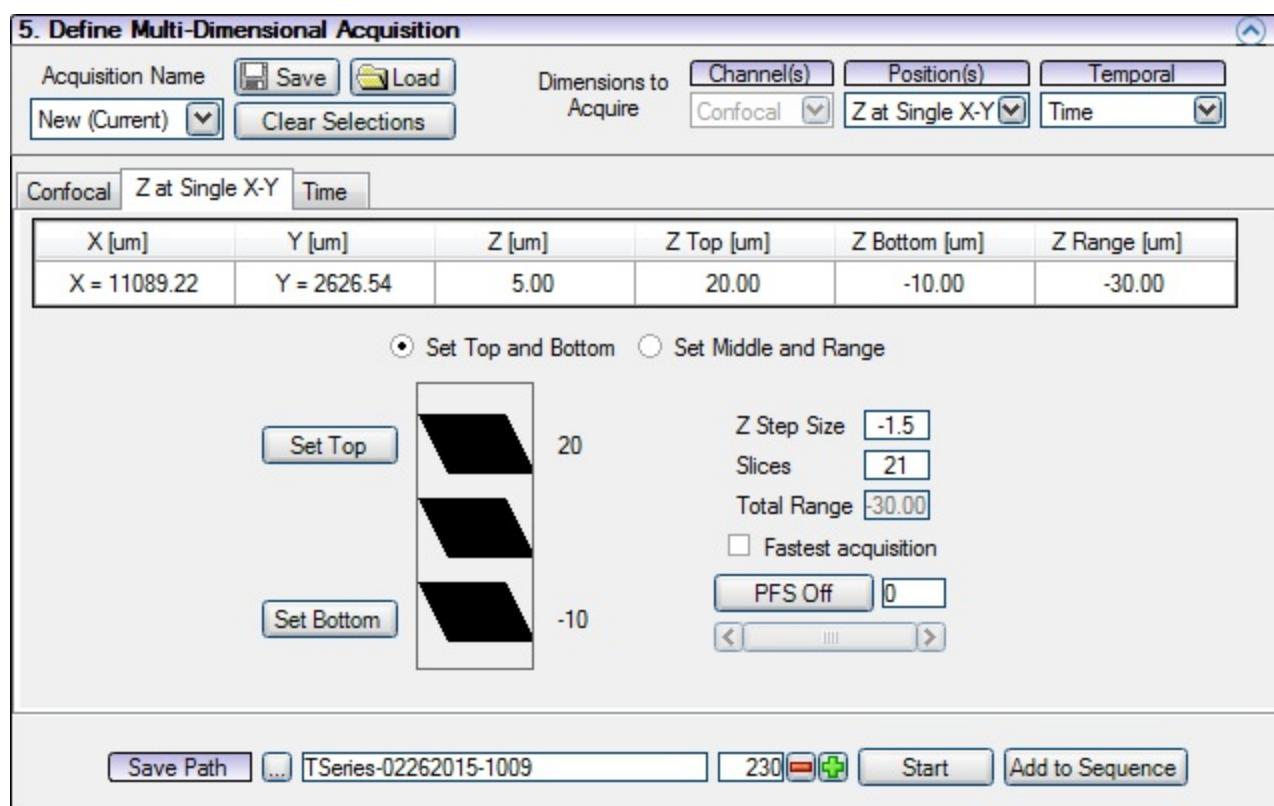


The Fixed X-Y position selection is used to perform an experiment at the same location every time. If this is selected, the table above will display the stored position at which to acquire image data.

The **Log Position** button is used to set the location at which the experiment is run. In this case, when the experiment is started, the stage will drive to the logged position and then perform the defined experiment.

Perfect Focus is available, in which case the stored Z position is replaced with the Perfect Focus offset set using the controls in this tab.

Z at Single XY



The Z at Single X-Y position selection is used to perform a standard Z series.

The table shows the parameters of the Z series related to each axis used for acquisition.

The radio button immediately below the table is used to toggle between two methods of defining the Z series. In the **Set Top and Bottom** case, the position of the top and bottom slice, and either the z step size or the number of slices must be set. In the case of **Set Middle and Range**, the position of the middle slice, the total range, and either the z step size or the number of slices must be set.

On the left side of the Z series parameter controls is a visual representation of the Z series, with the top, bottom, and current Z slice displayed. Clicking the mouse in this area will cause the Z motor to drive to the appropriate position and the thumbnail to display in the new position.

The **Set Top**, **Set Bottom**, and **Set Middle** (visible when **Set Middle and Range** is selected) are used to set the position of the corresponding slice of the Z series.

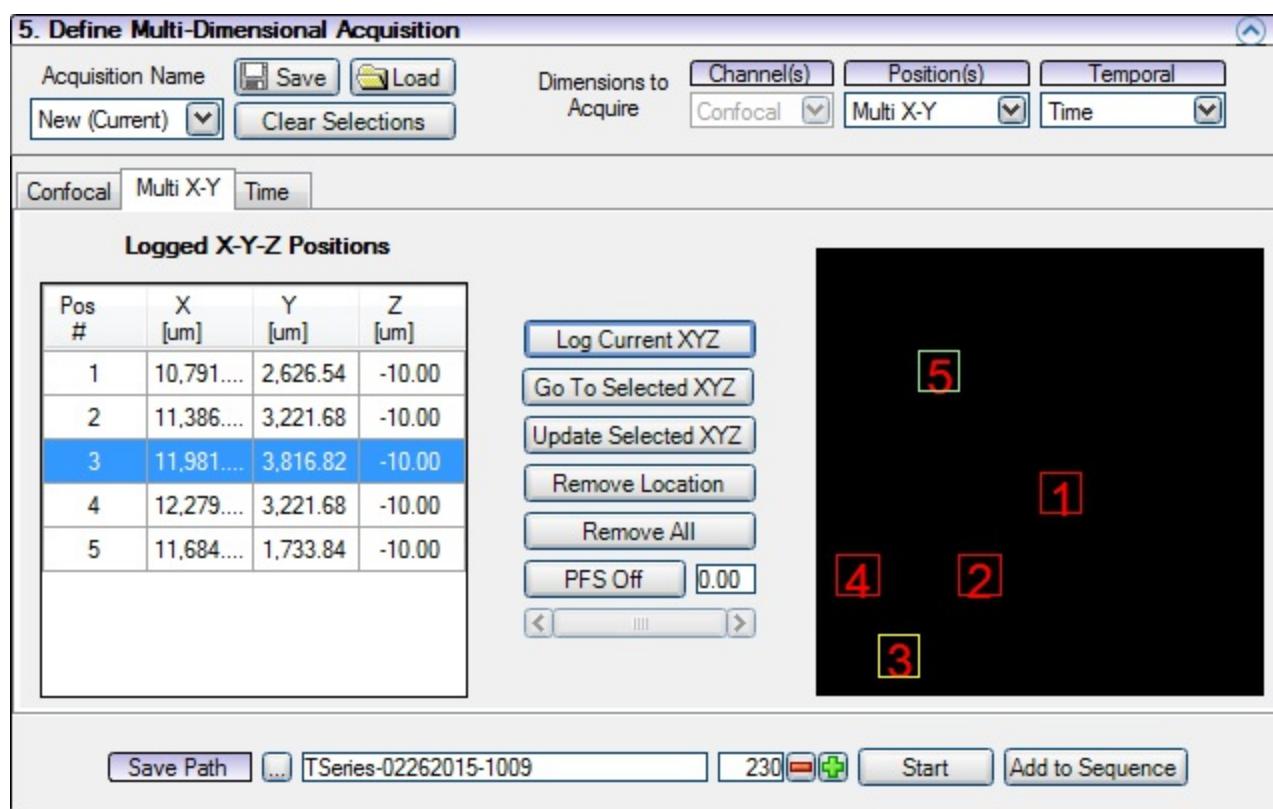
The **Z Step Size**, **Slices**, and **Total Range** (enabled when **Set Middle and Range** is selected) can be used to set the corresponding values for the Z series.

The **Fastest Acquisition** checkbox is used to toggle between acquiring each slice individually and acquiring the entire Z series in one acquisition. This option is not available for all Z devices, and will not be visible if using a Z device that does not support this method of operation.

The Perfect Focus controls are available here to allow for use of perfect focus during the Z series. If set to use perfect focus, the acquisition will begin by moving to the start of the Z series, use perfect focus to regain the focus initially set during acquisition definition, and the

perform the Z series offset from that location.

Multiple XY Locations



The Multi X-Y position selection is used to define an experiment which takes a single image at multiple stage locations.

In this tab, the stage locations are represented both by a table on the left side and in an image on the right side. The image shows stage locations with a red square, the current stage position with a green square, and the highlighted location in the table with a yellow square.

Stage locations can be added to the experiment definition using the **Log Current XYZ** button.

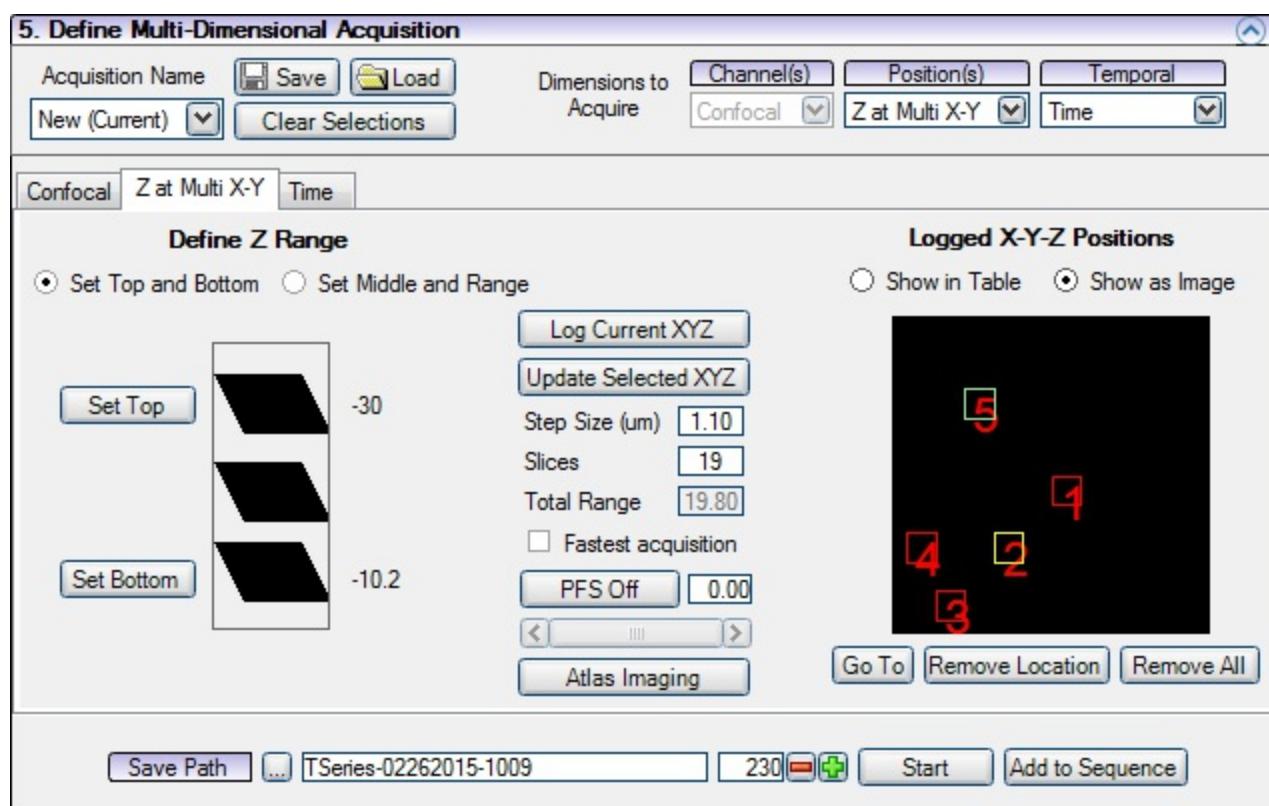
To drive to a previously defined stage location, highlight the location in either the table or the image and click the **Go To Selected XYZ** button.

To change an existing location definition, highlight it in the table and click **Update Selected XYZ** to replace the existing location with the current position of the stage.

The **Remove Location** button is used to remove the highlighted stage location in the table from the upcoming acquisition, whereas the **Remove All** button will clear all stage locations from the upcoming acquisition.

The Perfect Focus controls will store and use perfect focus offsets with each stage location, instead of their absolute Z positions. This allows for images to stay at the same focal planes over lengthy experiments.

Z at Multiple XY Locations



The Z at Multi X-Y selection combines Z series and multiple XY locations to acquire 3D spatial datasets.

The radio button immediately on the left is used to toggle between two methods of defining the Z series. In the **Set Top and Bottom** case, the position of the top and bottom slice, and either the z step size or the number of slices must be set. In the case of **Set Middle and Range**, the position of the middle slice, the total range, and either the z step size or the number of slices must be set.

On the left side of the tab is a visual representation of the Z series, with the top, bottom, and current Z slice displayed. Clicking the mouse in this area will cause the Z motor to drive to the appropriate position and the thumbnail to display in the new position.

The **Set Top**, **Set Bottom**, and **Set Middle** (visible when **Set Middle and Range** is selected) are used to set the position of the corresponding slice of the Z series.

The **Z Step Size**, **Slices**, and **Total Range** (enabled when **Set Middle and Range** is selected) can be used to set the corresponding values for the Z series.

The **Fastest Acquisition** checkbox is used to toggle between acquiring each slice individually and acquiring the entire Z series in one acquisition. This option is not available for all Z devices, and will not be visible if using a Z device that does not support this method of operation.

The stage locations can be represented by either a table or an image, toggled using the **Show in Table** and **Show as Image** radio buttons.

Stage locations can be added to the experiment definition using the **Log Current XYZ** button.

Adding a location also associates the current Z series definition with the location.

To drive the stage to a previously defined location, highlight the location in either the image or the table and click the **Go To** button.

To change an existing location definition or the Z series associated with that location, highlight it in the table and click **Update Selected XYZ** to replace the existing location with the current position of the stage and the current Z series definition.

The **Remove Location** button is used to remove the highlighted stage location in the table from the upcoming acquisition, whereas the **Remove All** button will clear all stage locations from the upcoming acquisition.

The Perfect Focus controls are available here to allow for use of perfect focus during the Z series. If set to use perfect focus, the acquisition will begin by moving to the start of the Z series, use perfect focus to regain the focus initially set during acquisition definition, and then perform the Z series offset from that location.

Time Series

5. Define Multi-Dimensional Acquisition

Acquisition Name	Save	Load	Dimensions to	Channel(s)	Position(s)	Temporal	
New (Current)	▼	Clear Selections	Acquire	Confocal	Current	Time	
Confocal Current Time							
Add/Remove	Sequence	Repetitions	Triggering	Interval	Units	Duration	Units
	1	10	None	100.00	ms	1.00	s
	2	1	Trigger First ...	100.00	ms	100.00	ms
	3	50	None	200.00	ms	10.00	s

Loop Table
Repetitions: 5
Interval (s): 600
Duration (s): 3000

Save Path ... TSeries-02262015-1106 230 Start Add to Sequence

The Time selection is used to set up repetitions of the defined experiment over time.

In the table, multiple timing sequences can be entered using the green +, and removed using the red X.

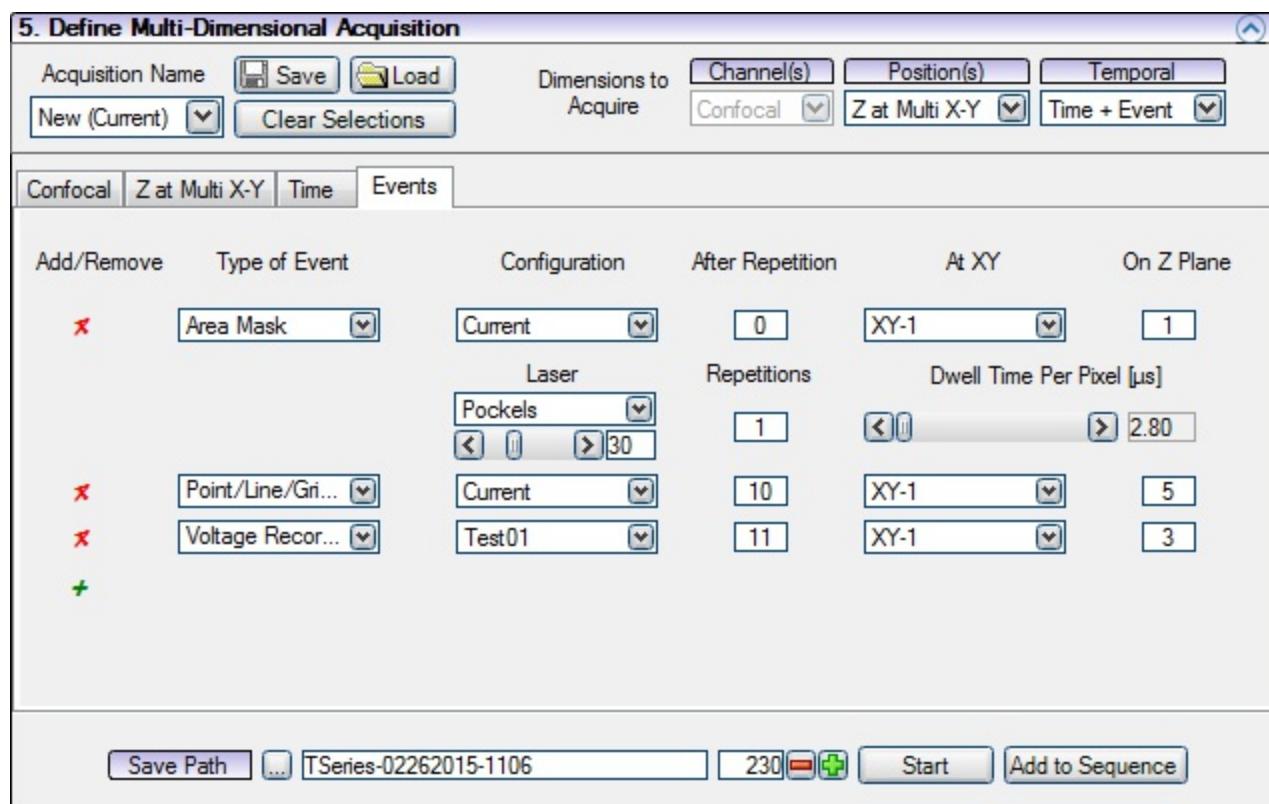
Each sequence can be set up to use **Triggering** using the column. The options are to trigger the first repetition, trigger every repetition, or use triggers to both start and stop the sequence.

For each sequence, entering a value into any two of the **Repetitions**, **Interval**, and **Duration** columns will automatically calculate the value of the third.

The units of both the **Interval** and **Duration** can be chosen from their respective columns. Changing the selected value will recalculate the appropriate value to match the new selection.

Checking the **Loop Table** checkbox allows for repetitions of the entire table. Again, entering values into two of the **Repetitions**, **Interval (s)**, and **Duration (s)** will automatically calculate the third.

Synchronization



In addition to imaging, various experiments can be synchronized with individual images for the upcoming experiment. These experiments include: Photoactivation by area mask, Photoactivation of points, lines or grids, Voltage outputs, and Voltage recordings.

To add a synchronized event, click the green +. Conversely, to remove an event, click the red X.

The type of synchronized event is chosen from the **Type of Event** drop down menu.

After choosing the type of event, a saved definition of the event can be loaded from the **Configuration** drop down menu. If the current definition is desired instead of a saved definition, the "Current" selection can be chosen.

If the acquisition definition includes a time series, the **After Repetition** textbox can be used to specify after which repetition of the acquisition the synchronized event should start.

If the acquisition definition includes more than one stage location, the **At XY** dropdown can be used to choose at which stage location the synchronized event should be performed.

If the acquisition definition includes at least one Z series, the **On Z Plane** textbox can be used to specify on which slice the synchronized event should start.

If the event type is chosen to be an **Area Mask**, a few extra controls are added that can modify the event definition. The **Laser** can be selected and have its power set appropriately, multiple **Repetitions** can be performed, and the **Dwell Time Per Pixel [μs]** can be set to specify the length of time each pixel is activated.

Acquisition Sequencer

Note: This section of the Basic Acquisition Interface has been removed, as it did not provide functionality that fit within the basic workflow methodology the interface provides.

If multiple acquisition definitions are desired to run sequentially, the Sequencer group can be used to define the sequence.

6. Sequence Acquisitions and Acquire Images

Add / Remove	Acquisition Name	Grouping	Repetitions	Interval	Duration
X	Acquisition 1		5	10	s
X	Images		12	500	ms
X	Z Series		1	2	s
X	3D Montage		5	300	s
+					50
					s
					6
					s
					2
					s
					1500
					s

Remove All Update Acquisition Group Selected Total 1558

Save Path ... TSeries-02262015-1118 230 Start

Click the green + to add the current acquisition definition to the sequencer. Conversely, clicking the red X will remove a previously defined acquisition. When an acquisition is added, it is assigned a default name that can be changed by clicking in the **Acquisition Name** column. This name is then added to the dropdown menu in the Acquisition Definition group so that it can be viewed and/or modified from there.

Click on the up and down arrows on the left to change the order of acquisitions. Clicking the up arrow will swap the acquisition with the one defined above it, whereas clicking the down arrow will swap with the one below it.

If a subset of the acquisitions is desired to be run together, multiple names can be highlighted by control-clicking and then the **Group Selected** button will denote them as grouped. Grouped acquisitions cannot be moved or deleted. After grouping acquisitions, the word “Grouped” will appear in the **Grouping** column. Highlight this word and click **Ungroup Selected** to split these back into single acquisitions.

Typing into any two of **Repetitions**, **Interval (s)**, and **Duration (s)** automatically calculates the third parameter. Acquisitions will then be run following the defined timing.

The units of both the **Interval** and **Duration** can be chosen from their respective columns. Changing the selected value will recalculate the appropriate value to match the new selection.

Clicking the **Update Acquisition** button will overwrite the highlighted acquisition’s definition

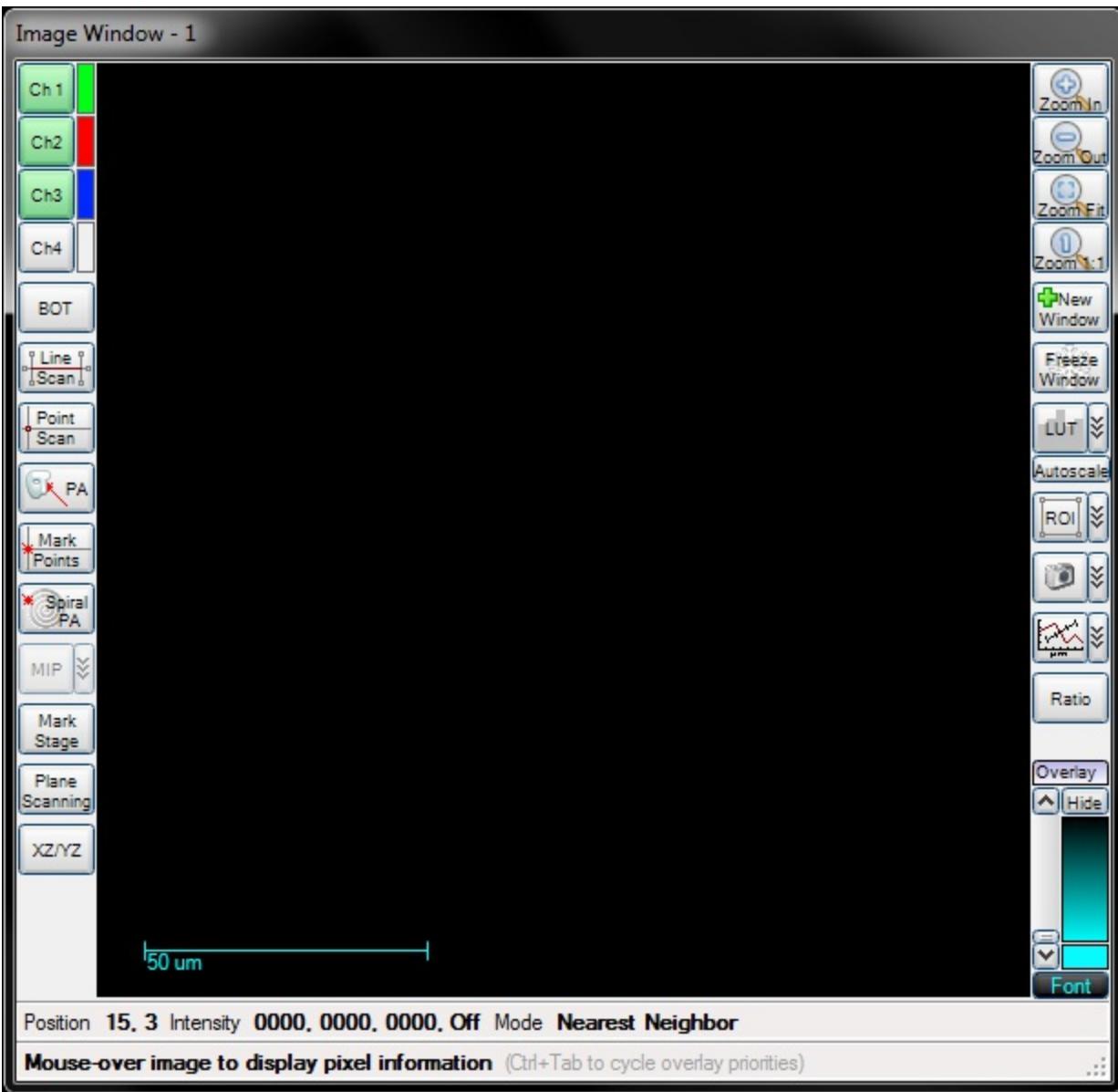
with the currently defined acquisition.

The **Remove All** button can be used to clear out the sequencer and start from scratch.

Double-clicking on any acquisition name will load that acquisition into the form above, where it can be viewed and/or modified. Be sure to **Update Acquisition** if any changes are made.

Once the sequence is defined, click the **Start** button to acquire all acquisition definitions in the Sequencer, with the specified timing.

Image Window Overview



Images acquired are displayed in Prairie View Image Windows. Up to 8 Image windows may be open at one time. Each Image Window is identified by a label in the banner section with a number e.g., "Image Window – 1". All open windows will be updated when **Live Image** or **Single Scan** is clicked.

Some consideration should be given to the number and size of active Image Windows, as the system may become sluggish when attempting to update too many windows simultaneously.

Located on the left and right sides of the Image Window are buttons for many controls and applications, described in detail in subsequent sections of this manual (note that not all controls are available in all acquisition modes). Additional applications and controls can be accessed via the [Applications](#) menu.

- **Ch1/Ch2/Ch3/Ch4:** The [channel buttons](#) indicate which input channel is being used to collect data for that Image window; button names may be configured differently on some systems

- **BOT (Brightness Over Time)**: Used for measurement of average pixel intensity of user-defined regions to be monitored over multiple images
- **Line Scan**: Used for definition and acquisition of a Line Scan
- **Point Scan**: Used for definition and acquisition of one or more Point Scans
- **PA (PhotoActivation)**: Used to define masks to limit laser exposure to certain areas
- **Mark Points**: Opens a dialog to set up a Mark Points experiment
- **Spiral PA (Spiral PhotoActivation)**: Opens a dialog to set up a [Spiral Activation](#) experiment
- **MIP (Maximum Intensity Projection)** : Displays the maximum intensity profile for a stack of images; the button is only active when playing back a Z-Series
- **Mark Stage**: Allows the user to add stage locations (x,y,z coordinates) to the list of saved positions
- **Plane Scanning**: Define and scan a cut-plane from a Z-Series
- **XZ/YZ**: Scan in a plane or curtain vertically through the sample
- **LUT (Look Up Table)**: Used to adjust the display intensity scale for an image
- **Autoscale (Look Up Table)**: Used to continuously adjust the lookup table limits as new image data is acquired
- **ROI (Region of Interest)**: Used to define a region of interest to acquire a portion of the full field of view
-  ([Snap](#)): Saves the image currently displayed in the image window
-  ([Line Profile](#)): Used to display intensity values and distance measurements over a line
- **Ratio**: Displays an image as the ratio of intensities between a pair of channels

Additional buttons on the right side of the Image window allow the user to adjust the displayed image. Zoom buttons scale the contents of the window and to open additional Image windows. Unlike the buttons in the Image Window Size section of the main control window, scaling changes applied in an Image Window do not affect the display in other Image Windows.

- **Zoom In** increases the image size by ~10%; the image can continue to be increased by clicking the button repeatedly
- **Zoom Out** decreases the image by ~10%; the image can continue to be reduced by

clicking the button repeatedly

- **Zoom Fit** is re-sizes the image to 512 pixels in width; if the image window is increased or decreased in size, the display will be scaled proportionally to fit in the new window
- **Zoom 1:1** shows the image at its actual size as defined by the acquisition settings
- **New Window** opens an additional Image window
- **Freeze Window** freezes the current contents of that Image window. When activated, the background of the button will change from grey to green. Data acquisition continues, but new images are not displayed in the frozen window. All channels in all other Image windows will continue to be updated and displayed. To unfreeze the window, click again on the **Freeze Window** button to deactivate it.
- **Overlay** controls allow the user to adjust any overlays drawn on the image window. These include scale bars, masks, lines, and other objects. Use the **Show/Hide** button to make all overlays appear or disappear. Adjust the transparency of all overlays using the vertical slider. Clicking the colored rectangle and/or **Font** buttons below the slider allows the user to change the font and color used in overlays.

Along the bottom of the Image Window are two status bars, the first displays the following information:

- **Pixel Position** of the cursor within the frame
- **Intensity** value (grey level) of the pixel in each of the four acquisition channels
- Interpolation **Mode** used to render the image (only applicable if the zoom is not 1:1)

The second status bar displays a caption describing the interaction with the overlays and/or image window at the current pixel. When applicable the activity being interacted with (Brightness Over Time, Mark Points, Line Scan, etc.) will be displayed in grey text at the far left while what actions are currently available will be displayed in black bold text either alone or to the right of the activity name. If there is room off to the right side a note will be displayed mentioning the Ctrl-Tab keyboard shortcut to cycle through the active activities/overlays to interact with a different activity/overlay when more than one is active. The bottom status bar will toggle to display the active activities/overlays when Ctrl+Tab is pressed, the active activity/overlay will be black while the other activities/overlays will be grey. Pressing Tab while holding the Ctrl key will continue to cycle the active overlay (also holding down the Shift key will go backwards), when the Ctrl key is released the display will toggle back to the actions available for the current pixel.

In addition to the Ctrl-Tab keyboard shortcut to select which overlays is currently active when multiple activities are being used at the same time, it is also possible to click the window/dialog

associated with a particular activity/overlay to make it active. For example, when using Brightness Over Time overlays at the same time as Mark Points overlays it is possible to click anywhere in the Brightness Over Time dialog to force the Brightness Over Time overlays to get priority over the Mark Points overlays. Similarly it is possible to click anywhere in the Mark Points dialog to make it so that the Mark Points overlays are given priority over Brightness Over Time overlays, or any other overlays currently displayed.

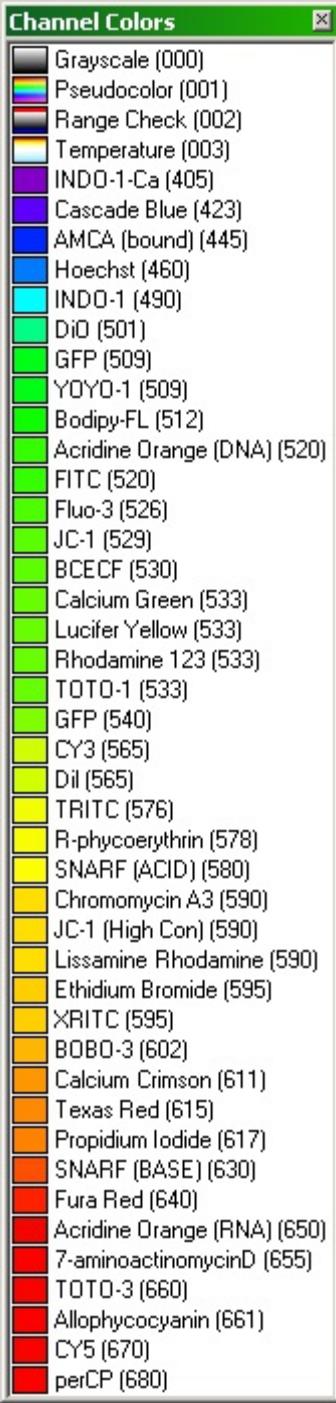
Input Channel Buttons

The top four buttons on the left-hand side of the Image Window indicate which input channels are to be displayed in that window. Up to four input channels are available at one time. To activate a channel, click on the corresponding channel button. The background of the button will change from grey to green.

For most systems, these channels generally correspond to PMTs. By convention, Ch 1 represents PMT 1 on the Prairie View main control window, Ch 2 represents PMT 2, etc. Substage PMTs may share an acquisition channel with the upper PMT corresponding to the same detection color. In SFC mode, these channels correspond to the channels to which lasers are assigned in the Laser,PMT,DAQ tab of the Prairie View main control window.

Channel Display Color

To select a display color for a channel, click the color label to the right of the channel button and a dialog with color choices will appear. Multiple channels can be displayed in the same image window with different colors. The Pseudocolor option applies colors based on pixel intensity. The Range Check option displays the image in grayscale, with any saturated pixels in red and any pixels of less than or equal to zero intensity in blue.



Channel Menu

Right clicking on a channel button will bring up a context menu with several options specific to that channel.

- **Set Channel Color** brings up the Channel Colors dialog described above.
- **Freeze Channel** stops update on that channel in all Image windows. When a channel is frozen a snowflake icon appears on the channel button and the channel will no longer be updated in any Image windows during any acquisitions. The frozen channel data will continue to be displayed. To unfreeze a channel, simply right click on the frozen channel and select Unfreeze Channel.
- **Automatically Adjust Lookup Table Levels** adjusts the display parameters for the

channel to maximize the contrast of the current image in the display. This is not a continuous adjustment, but rather an instantaneous one using the current frame's channel data.

- **Reset LookUp Table Levels** moves the display cutoffs back their minimum and maximum values.
- **Set Channel Source** allows the user to choose the source of data for the channel on systems configured with more than 4 input channels. These systems have multiple Preamplifiers.
- **Cancel** closes the context menu without making a selection.

Brightness Over Time

BOT is used to measure image areas for pixel intensity over time. This feature can be used on active image collections and previously collected datasets in Playback Mode.

Brightness Over Time

X



Graph Options

Horizontal Scale

- Best Fit
 - Fixed
- Maximum [s]
50.000
- Minimum [s]
0.000

Horizontal Units

- Seconds
- Frames

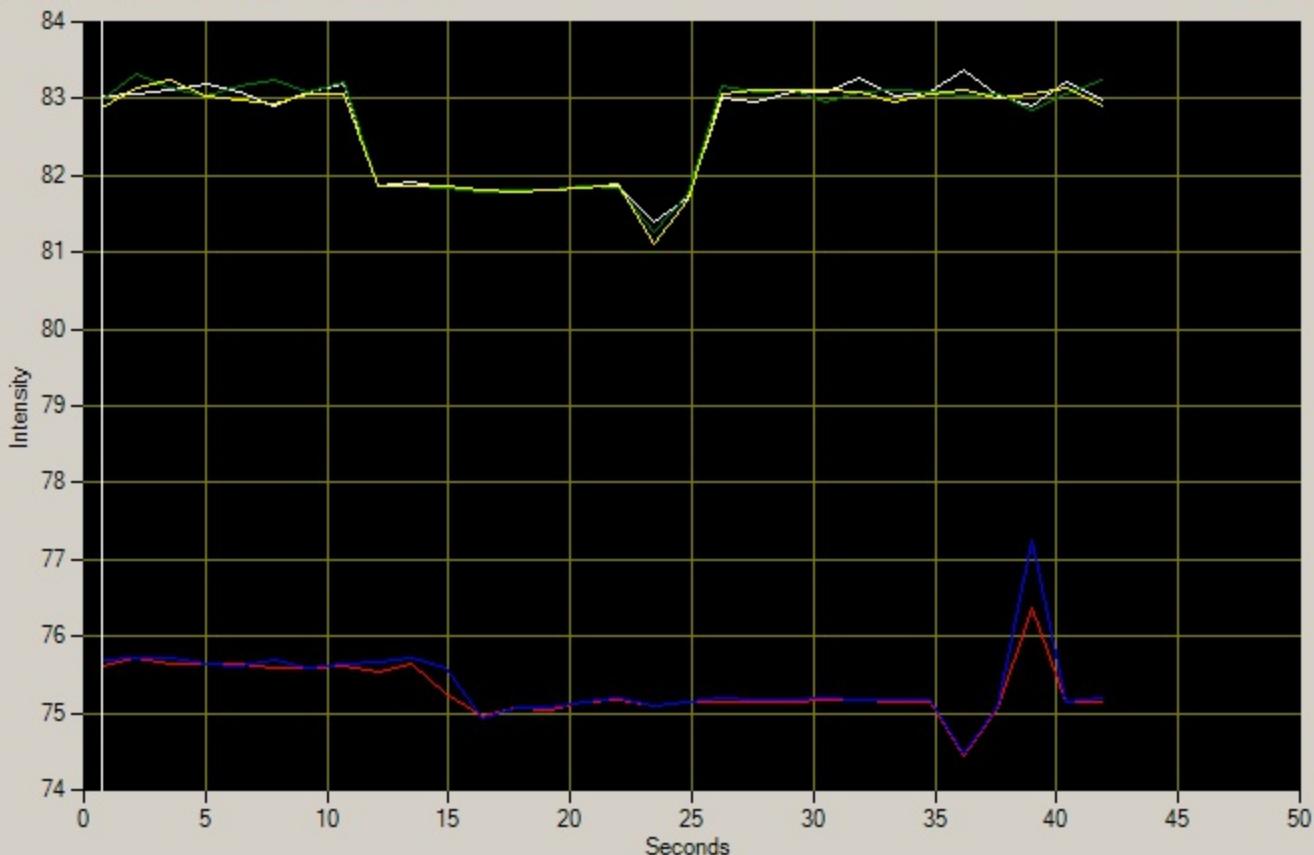
Vertical Scale

- Best Fit
 - Fixed
- Maximum
84.000
- Minimum
74.000

Show Average For

1 Frames Update Display

Save Graph...



Brightness Over Time Regions

Units pixels µm

Index	Display?	Channel	Measurement	Position	Width	Height	Area	Rotation	Shape
1	<input checked="" type="checkbox"/>	Ch1	Mean	{98,125}	147	78	9005	0°	Ellipse
2	<input checked="" type="checkbox"/>	Ch2	Mean	{282,276}	69	133	9177	0°	Rectangle
3	<input checked="" type="checkbox"/>	Ch1	Mean	{256,54}	124	114	6537	0°	Polygon
4	<input checked="" type="checkbox"/>	Ch2	Mean	{256,54}	124	114	6537	0°	Polygon
5	<input checked="" type="checkbox"/>	Ch1	Mean	{156,310}	132	107	7204	0°	Brush

Region Options

- Create Regions As
- Ellipses
 - Rectangles
 - Polygons
 - Brush
- Medium 4.00
- Don't Create

Move All

- Right Click To
- Resize
 - Rotate
 - Clone

Region Groups

Region Functions

Voltage Recording Experiment

None

Mark Points Experiment

None

Voltage Output Experiment

None Start with Input Trigger ... BrightnessOverTime-03202013-1239156 Select a Category Select a Category

Start

1.

Graph Options

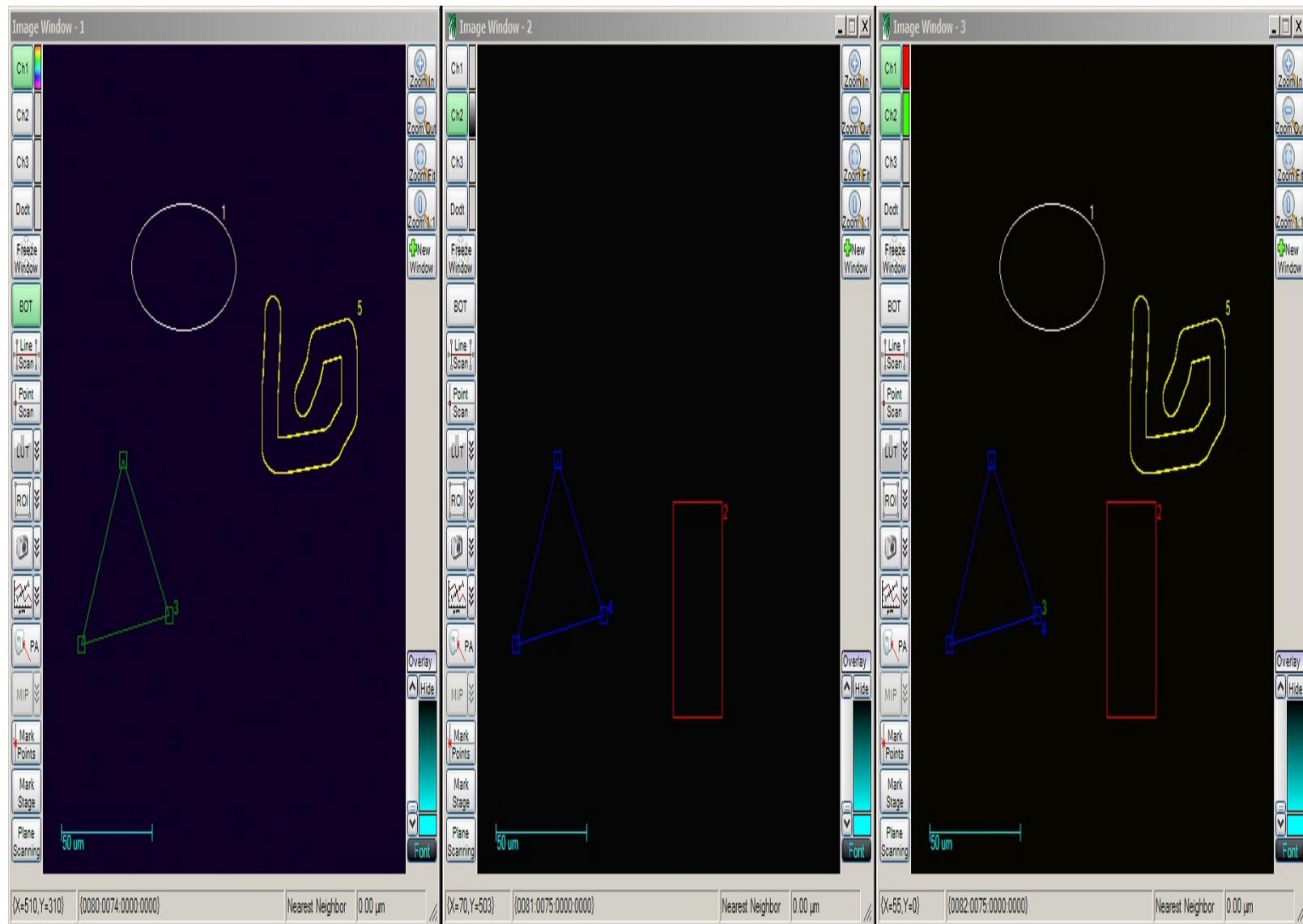
Along the right hand side of the graph window are controls for the graph display. There are separate controls for both the horizontal (x) and vertical (y) axes. Both axes can either be scaled as **Best Fit** (an automatic scale) or **Fixed**. **Best Fit** will adjust the visible values as necessary according to the data being displayed, while **Fixed** will be limited to the values entered in the **Maximum** and **Minimum** fields for each axis.

For the horizontal scale units, the operator may select from **Seconds** or **Frames**. The vertical axis is set to display intensity values.

Located below the horizontal and vertical scale controls, **Show Average For** allows the operator to choose how many frames to average for each data point.

Save Graph will save a TIFF of the current graph.

Region Options



There are four different shapes that can be used to create BOT regions: **Ellipse**, **Rectangle**, **Polygon** and **Brush**. Using the mouse, the operator can sequentially add regions for analysis. To add a region, choose the desired shape and use the mouse to draw the region on the Image window. To finish a polygon, use the right mouse button to connect the clicked coordinate to

the first segment of the polygon. If there is more than one channel active on the image window when a region is created, an identical region is created for each active channel.

The **Brush** option offers six different sizes for free-hand region creation: **Tiny**, **Small**, **Medium**, **Large**, **Huge** and **Custom**. When **Custom** is selected, the user can define the size of the brush .

Once a region is created, there are several options for manipulating the position:

When **Move All** is selected, all of the regions will be shown in the selected highlight color of cyan. Place the cursor over any region number in the Image window so the hand cursor is shown, click and hold the left mouse button, and move the mouse to move all of the regions. Release the left mouse button to fix the regions in the desired locations. As soon as the mouse is released, **Move All** is turned off.

Right Click To offers three different manipulations. The user can choose to **Resize**, **Rotate** or **Clone** a region by selecting the desired change, hovering over the desired region, and right-clicking. For **Resize** and **Rotate**, the user must right-click and hold down the mouse button until the region is the desired size or orientation. If **Clone** is selected, then by right-clicking on a region number, an exact copy of the region can be made, including size, location, and associated channel.

A single region can be moved only when **Move All** is off (not highlighted). The cursor will automatically turn into a hand cursor when hovered over a region number. This indicates that particular region can be selected. Click and hold the left mouse button to select the region and move it to the desired location.

Brightness Over Time Regions

Each region created on the Image is described in a table below the graph. There are several columns of information specific to each region:

- **Display?**: The user can choose which region's information to show on the graph by checking or un-checking the box to the left of the region number. Each region number is shown with a background color representative of the graph line created for that region. This option can be toggled both before and after data is acquired.
- **Channel**: Denotes the channel to which the region pertains
- **Measurement**: Allows the user to choose whether the mean or standard deviation of pixel values is collected for each region
- **Position**: This number is representative of the top left-hand corner of the region. For ellipses, polygons and brush shapes, this is the top left location of the bounding box that would encompass the shape.
- **Width**: The x value of the rectangle or bounding box
- **Height**: The y value of the rectangle or bounding box
- **Area**: The complete area covered by the shape (not the bounding box)

- **Rotation:** If a region has been rotated, the degrees of rotation from the original position will be noted
- **Shape:** Denotes the shape tool used to create the region

In the right-hand corner of the table, the operator can choose to display the size measurement values for the regions in either **pixels** or **um** (microns). When **um** is chosen, the computations are based on the currently selected objective lens.

Basic Region Math

If the operator clicks the right mouse button over any of the defined regions in the region table, a pop-up menu will appear that will allow the operator to very quickly set up some basic region math to modify the analyzed shape. This could be simply specifying that region 2 should be subtracted from region 1, or possibly subtract region 2 from all other regions.

When a mathematical relationship is defined via the pop-up menu, the region name will indicate the relationship. If the relationship is defined as subtracting region 2 from region 1, the label in region 1 would change from '1' to '1-2'.

If **Region Alone** is selected in the pop-up menu, then any defined mathematical relationship for that region will be removed.

All Alone will remove all previously defined mathematical relationships.

Other Ways of Generating Regions

The **Import Regions** and **Export Regions** buttons below the table of regions allow the operator to save a set of region definitions to a file for recalling later. This could be useful if the operator wishes to use consistently sized regions over multiple data sets. The user could save a set of regions using the **Export Regions** button and then when a new data set is loaded, use the **Import Regions** button to load the regions. The region locations could then be manually set where desired.

The **Import From PA** button brings up a dialog to allow the user to import masks drawn as [Photoactivation](#) masks for use as BOT regions.

The **Generate** button allows the user to call previously saved Actions to auto-draw regions. For more information about actions, see the [Actions](#) subsection in the Tools section in this document.

The **Remove Region** button deletes the selected region from the table, whereas **Clear Regions** will clear all regions from the table.

Region Groups

To create a Region Group, first create the regions in the Image window via the **Create Regions As** panel. Next, place a check in the box next to the region number in the **Display?** column for each region to be included in the region group. Uncheck any regions to be excluded from the group. Now press the **Create Group** button and a group will be created that consists of the specified regions.

To change a group definition, first check/uncheck the desired regions in the region table. Then select the desired group by left clicking on the group to highlight it. Press the **Update Group** button and the group definition will be changed accordingly.

Remove Groups and **Clear Groups** will delete selected or all groups, respectively.

By clicking on the checkbox next to the group number, the user can alter what region graphs are displayed.

Region Functions

Located below the Region Groups is a set of controls for Regions Functions. This section allows the user to create graphs that are mathematical manipulations of the data from one or more defined region. This is in addition to the predefined mathematical options presented by right clicking the mouse over a region definition as outlined above. Region Functions will display as new graph data.

To create a Region Function, click on the **Create Function** button; the button will turn green to indicate that this mode is activated. To exit this mode without actually entering a formula or equation, simply click on the **Create Function** button again.

Activating the **Create Function** button will activate a control below the buttons that allows the user to select from a list of pre-defined mathematical functions (e.g. f/f_0). Also activated is a control below the list of functions that allows the user to modify the pre-defined equation, or to enter a custom equation.

Located just below the control/field where the equation is shown is a string shown in blue that reads 'Need help with function syntax?'. Clicking on this string causes a help dialog to appear with additional information about the proper syntax for the mathematical functions.

Some sample equations are as follows:

Instead of looking at the average intensity of a region over time (standard graph of a region), the user may want to see the relative change in the region intensity information over time. That is, the user wants to look at the difference between the intensity values from one frame to the next. If the region of interest was region 2, the equation would be $\{2\} - \{2:-1\}$.

Many standard mathematical functions are available by using the following syntax: `Math.Log()`, `Math.Min()`, etc. For example, to compute the logarithm for the data in region 3, the syntax would be `Math.Log({3})`. To generate the absolute value of subtracting the value of region 1 from region 2, the syntax would be `Math.Abs({2} - {1})`.

Saving BOT Data

Each BOT acquisition is saved in its own folder. The location of the folder is determined by the Save Path, which can be viewed or changed by clicking the ... button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the ... button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, Misc, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data.

When viewing or performing a BOT measurement of a previously acquired image sequence in Playback Mode, additional buttons appear for saving data. **Save Changes** will save the current regions, definitions and data in place of the original data. **Save Changes As** will create a new set of files with the current regions, definitions, data, and a screenshot of the current image window in the same directory as the original file. This includes creating a new .xml file that is associated with the defined regions so different sets of regions, definitions, and data can be recalled for the same data set.

Acquisition / Analysis

Press **Start** to begin collecting BOT data on a new acquisition. BOT measurements can also be performed on previously saved data by performing the BOT while in Playback Mode. To update the graph when in playback mode, create/edit the regions and press **Start**. The program will cycle through the current image sequence and generate the graph of data.

By checking **Start with Input Trigger**, the user can set up a BOT measurement to begin when a TTL trigger is received from external equipment.

BOT Experiments can be synchronized to run alongside a [Voltage Output](#), [Voltage Recording](#), and [Mark Points](#) experiment by selecting the appropriate experiment from the drop down menus to the left of the **Start** button. For more information on these experiments, see their respective sections in this document.

BOT within a T-Series

A Brightness Over Time experiment can be embedded in a T-Series by checking the **BOT** box in the desired cycle. See the [T-Series](#) Tab section of this manual for more information about T-Series experiments.

Files Created by the BOT Experiment

Running a BOT experiment creates a directory in the location specified by the file path in the BOT window. In this directory are the files that contain the definition of the experiment along with the results from the experiment. First and foremost, the directory contains a configuration file, which has a name ending in Config.cfg, that lists every microscope setting and its value at the beginning of the BOT experiment. Additionally, within the References subdirectory, an image is saved that contains anything that was in the image window at the beginning of the BOT experiment, including the image itself, along with any overlays, such as the BOT regions. Next, there is a Brightness Over Time XML file which defines the BOT experiment. Here you will find the definitions of any regions, groups, and functions, along with imaging parameters and timestamps. In addition, an image of each frame in each channel that was part of the BOT experiment is saved in the directory. Finally, a comma-separated-value file is saved containing the timestamp of each frame along with the region measurement at that frame. Optionally, if [Voltage Recording](#), [Mark Points](#), or [Voltage Output](#) was synchronized with the BOT experiment, their corresponding files will be present in this directory. See the appropriate sections in this manual for information on these files.

BOT Drive Signals

BOT drive signals are custom analog outputs synchronized with the brightness of a particular BOT region in near real time; there is still some processing involved in calculating the brightness of a BOT region and updating the analog output signal. These outputs require a PCI-6713 option card for additional analog outputs synchronized with image data collection which can drive a total of 8 analog outputs.

BOT drive signals are set up on the **Custom Outputs** tab of the Configuration Utility. To enable a **BOT Drive Signal** simply click its checkbox to enable it and fill in the following fields:

- **Default** determines the value of the output when a BOT acquisition isn't running, this is an integral DAQ value where a value of 204.8 is equivalent to one volt
- **BOT Region** is the number of the BOT region driving the analog output, these numbers start with one and match the numbering displayed in Prairie View
- **Scaling Factor** is a floating point value multiplied by the BOT region's average brightness value to generate a 12-bit DAQ value, similar to the **Default** a value of 204.8 is equivalent to one volt

Quick Reference ([Basic Overview](#))

Note: **Bold text** is entered as is; *grey italicized text* needs to be replaced with actual values before entered.

[Intensity Values](#)

{Region Number} - Current intensity of specified region number.

{Region Number:Frame Offset} - Intensity of specified region number at a relative frame offset.

{Region Number,Frame Number} - Intensity of specified region number at an absolute frame offset.

[Timestamps](#)

{t} - Current timestamp in seconds.

{t:Frame Offset} - Timestamp at a relative frame offset in seconds.

{t,Frame Number} - Timestamp at an absolute frame offset in seconds.

[Frame Number](#)

{f} - Current frame number.

[Operators & Functions](#)

You can use any common operators such as +, -, / or * as well as any valid C# syntax such as **Math.Max(Expression #1,Expression #2)**, **Math.Abs(Expression)** and **Math.Log(Expression)**. Note that expressions are **case sensitive**.

[Intensity Values \(\[Top\]\(#\)\)](#)

There are three different ways to include intensity values in your functions: by region number, by region number and a relative frame offset and by region number with an absolute frame number.

To include the current intensity value in your function simply enclose the region number you are interested in within curly braces, {Region Number}. For example, to subtract the intensity values for region one from those of region two the function would be the following, '{2} - {1}'. These intensities will vary as the function is recalculated for each frame.

To include intensity values from past and/or future frames enclose the region number followed by the frame offset separated by a colon in curly braces, *{Region Number:Frame Offset}*. For example, to calculate the change in intensity for region one over each frame the function would be the following, ' $\{1\} - \{1:-1\}$ '. This takes the intensity for region one for the current frame and subtracts from it the intensity value for the previous frame.

If an offset should reference a nonexistent frame either before the first frame or after the last frame then the values for the first and last frames are used instead respectively.

So in the example the function will always evaluate to zero for the first frame since the value for the first frame is substituted for the value of the previous frames values since there is no frame before the first frame. This substitution also occurs when updating a function during a live acquisition for any positive frame offsets, since the data has yet to be collected for any future frames the value of the current frame is used instead.

Finally to include intensity values for a specific frame enclose the region number followed by the frame number separated by a comma in curly braces, *{Region Number,Frame Number}*. For example, to calculate F over F_0 for region one where F is the current intensity value and F_0 is the average of the first 5 frames the function would be the following, ' $\{1\}/(\{\{1,1\} + \{1,2\} + \{1,3\} + \{1,4\} + \{1,5\}\} / 5)$ '. This takes the current intensity of region one and divides it by the average intensity of the first 5 frames.

Timestamps ([Top](#))

There are three different ways to include timestamp values in your functions: for the current frame, for a relative frame offset and for an absolute frame number. All timestamps are all in seconds.

To include the timestamp value for the current frame in your function simply enclose a lowercase T within curly braces, *{t}*. For example, to display the timestamp value for each frame the function would be the following, '*{t}*'. The timestamp will vary as the function is recalculated for each frame.

To include timestamp values from past and/or future frames enclose a lowercase T

followed by the frame offset separated by a colon in curly braces `{t:Frame Offset}`. For example, to display the frame period the function would be the following, '`{t} - {t:-1}`'. This takes the timestamp value for the current frame and subtracts from it the timestamp value for the previous frame which will generate a horizontal line.

If an offset should reference a nonexistent frame either before the first frame or after the last frame then the values for the first and last frames are used instead respectively.

So in the example the function will always evaluate to zero for the first frame since the value for the first frame is substituted for the value of the previous frames values since there is no frame before the first frame. This substitution also occurs when updating a function during a live acquisition for any positive frame offsets, since the data has yet to be collected for any future frames the value of the current frame is used instead.

Finally to include timestamp values for a specific frame enclose a lowercase T followed by the frame number separated by a comma in curly braces, `{t,Frame Number}`. For example, to display the current frame's timestamp relative to the 2nd frame's timestamp the function would be the following, '`{t} - {t,2}`'. This takes the timestamp of the current frame and subtracts is from the timestamp of the 2nd frame.

Frame Number ([Top](#))

To include the current frame number in your function simply enclose a lowercase f within curly braces, `{f}`. For example to display the current frame number the function would be the following, '`{f}`'. This will result in a diagonal line increasing by one each frame. Not all too exciting on its own, but used in combination with other variables can prove invaluable.

Operators & Functions ([Top](#))

Any of the four standard arithmetic operators can be used on the variables mentioned in this document: addition (+), subtraction (-), division (/) and multiplication (*).

The modulus operator (%) can also be used.

In addition to variables and operators any numeric constants can be used as desired.

If basic operators, variables and constants are insufficient the entire C# math library is available as well as any other standard C# libraries. Keep in mind that C# is **case sensitive** which is also true for any expressions entered for functions. A few commonly used math functions are:

Math.Abs(Expression) - Calculates the absolute value of given expression.

Math.Min(Expression #1,Expression #2) - Calculates the minimum value of two expressions.

Math.Max(Expression #1,Expression #2) - Calculates the maximum value of two expressions.

Math.Log(Expression) - Calculates the natural log of given expression.

Math.Log(Expression,10) - Calculates the log (base 10) of given expression.

Math.Sqrt(Expression) - Calculates the square root of given expression.

Math.Pow(Expression #1,Expression #2) - Calculates the value of the first expression raised to the power determined by the value of the second expression.

Math.Cos(Expression) - Calculates the cosine of given expression in radians.

Math.Sin(Expression) - Calculates the sine of given expression in radians.

Math.Tan(Expression) - Calculates the tangent of given expression in radians.

Basic Overview ([Top](#))

Functions can be used to display raw brightness over time data in different ways during acquisitions or after data has already been collected in playback mode.

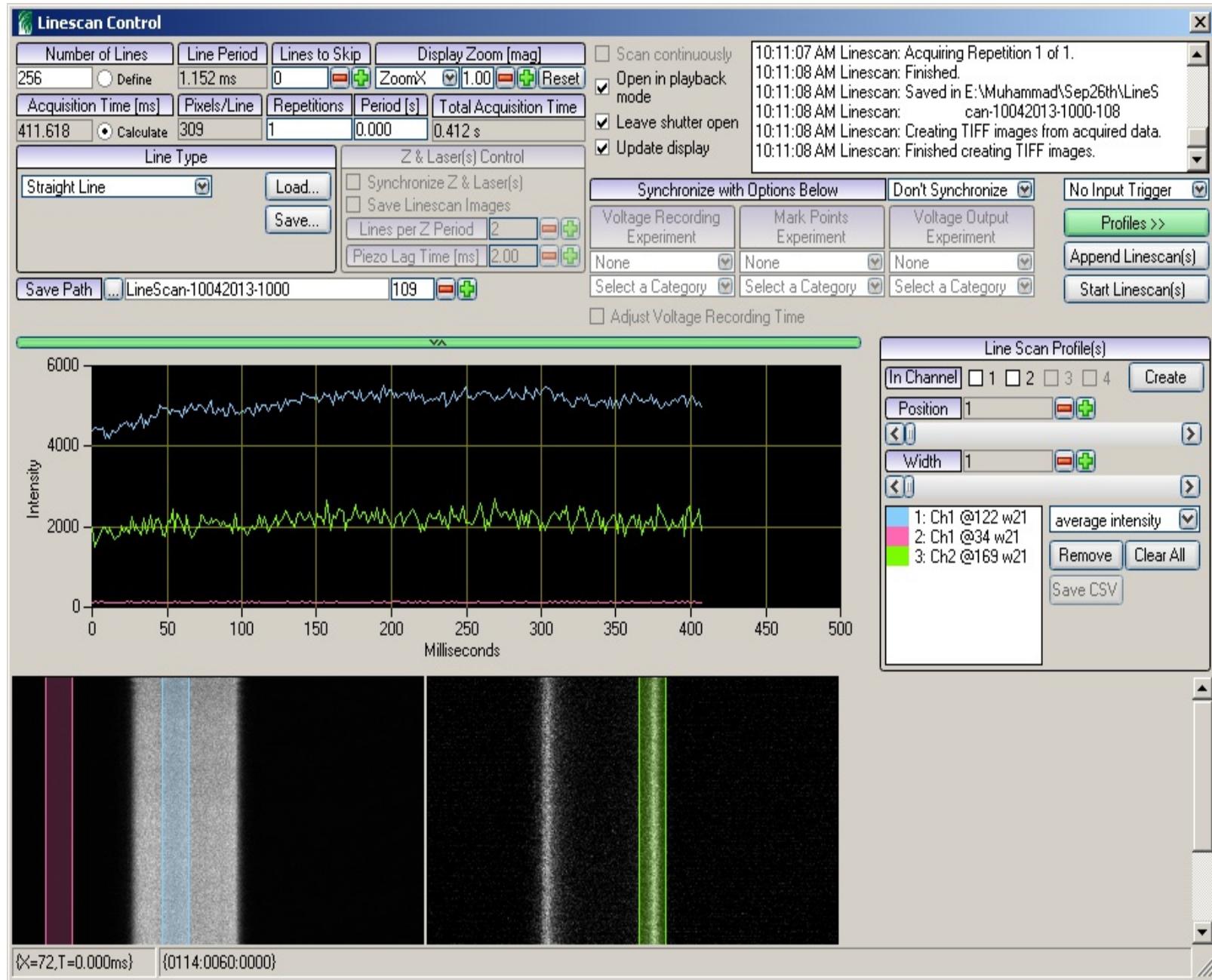
Functions consist of a few variables used to access intensity data consisting of region and frame numbers enclosed in curly braces { } in combination with some basic arithmetic operators and optionally some C# syntax for advanced users.

Functions are added by clicking the Create Function button which will display a dialog where an expression defining the function can be edited (syntax is explained in the rest of this document). When the expression is complete either press Enter or click the Accept button at which point the expression will be evaluated for correctness and saved. To cancel simply click the Cancel button or close the form. If the expression is found to be incorrect a message box will pop up at which point the expression can be fixed and accepted again. Behind the scenes the expression is actually being compiled into a small program of its own for speed.

There is an additional list of templates available when creating or editing functions used to quickly add frequently used expressions. Templates can be created and edited while editing a function and are saved independently of the function being edited. To create a template containing whatever you've placed in the function text box so far press the Create Template button and enter a name for the template. To edit a template select it from the list by clicking and its contents will show up in the function text box, make any changes and press the Save Template button. It is also possible to delete a template by selecting it and clicking the Delete Template button.

Line Scan

The Linescan Control dialog opens when the user clicks the **Line Scan** button on an Image window. This window is used to define a one-dimensional acquisition along a user-defined line.



The Linescan Control window is divided into three main sections. The top section contains controls for the user to define the acquisition. The middle section of the window allows the user to define profiles to display intensity data in a graphical format; reveal this section by clicking the **Profiles** button in the upper control section. The bottom section of the window displays the acquired data as an image. These sections are described in detail below.

Defining a Line Scan

Parameters of the Definition

The user can choose to define the **Number of Lines** or the **Acquisition Time** for the scan; the other value is calculated automatically by the software. Use the **Define** and **Calculate** radio buttons to select which parameter text box to activate.

Line Period is based on the line defined by the user and the current scan settings defined in the main control window. Hovering over this field will display a tooltip showing which portion of the line period is spent scanning vs. retracing.

The **Pixels/Line** field displays the number of pixels scanned in the current definition.

During a line scan, the system will repeatedly scan the defined line. If the user wants to collect intensity data less frequently, a number of **Lines to Skip** can be defined. This number must be an integer. Scanning continues during these skipped lines, but laser power is set to minimum levels. For example, if the user sets the **Number of Lines** to 200 and the **Lines to Skip** to 1, the system will scan the line 400 times but only apply laser power and collect data every other line. The **Line Period** field will update to show the time from the start of one acquired line to the start of the next acquired line (taking into account the time for skipped lines).

The user can define a number of **Repetitions** for the experiment, and define a **Period** for those repetitions. For example, 3 repetitions with a period of 10 seconds would perform the defined number of lines 3 times, with 10 seconds between the start of the first repetition and the start of the second repetition. **Leave shutter open** determines whether the hard shutter will be closed between repetitions (if not checked), or left open until the entire acquisition is complete (if checked). The **Total Acquisition Time** field will update to display the amount of time acquiring data and waiting between acquisitions for the defined period.

For some line types (described below), the user can check the box to enable the **Scan continuously** option. The line will be scanned in both directions; essentially, the defined pattern will be mirrored back on itself. For example, if the user draws a Freehand line 500 pixels long and checks **Scan continuously**, the scanned line will be 1000 pixels long, where the scan will go from start to end to start as one line. If the user selects a Sprial line, the scan will go from the outside to the center and then back from the center to the outside. The Line Period and Acquisition Time fields will be updated to reflect the new scan parameters. Note that Line Period does not double when checking **Scan continuously**, as there is no retrace time in a continuous scan. This option is automatically checked for Circle and Lissajous modes, and is not available for Line mode.

Line Type

The **Line Type** drop-down menu allows the user to choose the type of line to be scanned.

- **Straight Line** defines a horizontal line across the image. The two vertical bars define the portion of the line that is acquired. Reposition the line vertically on the image by dragging either of the outer boxes on the Image window. Change the lateral range of the

acquisition by dragging the two inner vertical bars. This is the only type available when using the Resonant Scanner or AOD to scan the sample. Use the Scan Rotation controls in Galvo mode to change the orientation of the sample relative to the horizontal line on the Image window.

- **Circle** defines a continuous circular scan pattern. In the Image window, left click and drag in the square markers to change the size and position of the circle, right click and drag to translate circle. The first pixel of the circle is indicated by an arrow head marker, which also indicates the direction of the scan.
- **Freehand** allows the user to define a custom line. Click and drag the left mouse button in the Image window to draw the line; hold shift to make straight segments. A green box marks the start of the line, and a red box marks the end. Click the left mouse button in either of these boxes and drag to extend the line. Click the right mouse button in either of these boxes to move the line.
 - The speed and accuracy of a Freehand line is limited by the response of the galvanometers scanning the line. Tight corners will be followed more closely at slower dwell times and higher pixel densities. To prevent over-driving the galvanometers, a Minimum Freehand Dwell Time parameter is defined in the Scan Settings. This parameter is defined at a 512x512 image size and scaled accordingly for other image sizes. The Line Period and other fields account for this minimum during setup. Additionally, if the user defines a Freehand line with a dwell time below the minimum time, a message will appear in the information box on the right side of the window to indicate that the dwell time was temporarily increased. The parameter is set during installation; contact Bruker Fluorescence Microscopy support personnel for assistance or to request changes to the setting on the system.
- **Action(s)** allows the user to import Freehand line definitions from external software programs, such as MatLab. See more information about [Actions](#) elsewhere in this manual, and contact Bruker Fluorescence Microscopy support personnel for more assistance.
- **Spiral** defines a spiral pattern over the image. Two additional controls allow the user to define the spiral. The **Shape** slider adjusts the shape of the pattern, with 1.0 being a circular spiral and smaller values making the scan more of a square shape. The **Spirals** slider adjusts the number of spirals, affecting the length of the line and the density of the scan. The Minimum Freehand Dwell Time parameter (described above) also applies to Spiral line scans.
- **Lissajous** is another continuous pattern. The **Freq** slider adjusts the density of the pattern.
- **Segmented** is a pattern of individual, unconnected straight line segments. The **Auto Order Segments** check box switches between scanning the segments in a user defined order, or an order optimized for scan speed. The **Show Separators** check box turns on and

off the overlays in the linescan window image. Individual segments can be deleted, or their scan order can be changed using the context menu available when right-clicking one of the ends of a segment. Line scan profiles are automatically created for each channel and every segment when enabling line scan profiles.

Click the **Save...** button to save the defined line, which can be recalled again later by clicking the **Load...** button.

Collecting the Line Scan

Click **Start Linescan(s)** to start the acquisition. The message box in the upper right corner of the window will update to show the status of the acquisition.

Each Linescan acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the ... button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the ... button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, Misc, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and - buttons. Note that decreasing the counter value can result in overwriting data.

A reference image is saved along with the line scan acquisition. The reference image is a TIFF image of the contents of the Image windows, with any overlays (including the defined line) currently displayed on the image. The channels active in each Image window will be listed in the name of the corresponding reference images.

Each defined Repetition will be saved as a separate file in the folder for the current line scan data set.

The **Append Linescan(s)** button starts the defined acquisition but does not start a new dataset. Instead, the acquisition is treated as one or more additional repetitions in the previous acquisition, reflected in the acquisition count messages in the message box in the upper right corner of the window.

When the **Update display** box is checked, newly acquired intensity data will be displayed in the bottom section of the Linescan Control window.

When the **Open in playback mode** box is checked, newly acquired intensity data will be

displayed in the Image window(s) at the end of the acquisition. Some features of Linescan Profiles (described below) are available only in playback mode.

Triggered Line Scans

A Line Scan can be started on its own as described above, or set to respond to input triggers from other equipment. Triggering options are selected via a drop-down menu in the Linescan Control window.

If **No Input Trigger** is selected, then the acquisition will start immediately when the Start Linescan(s) or Append Linescan(s) button is pressed.

If **Trigger First Rep.** is selected, then after Start or Append Linescan(s) is pressed, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first repetition. All subsequent repetitions are acquired automatically.

If **Trigger Each Rep.** is selected, then after Start or Append Linescan(s) is pressed, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first repetition. This process is then repeated for each subsequent repetition in the defined acquisition.

Triggers sent to the system should be 5V TTL-style signals on a BNC cable. If the system is configured with a GPIO box, input triggers are received by the Trig 1 In through Trig 8 In connections in the middle row on the front on that box.

Synchronizing Functions With Linescan

It is possible to synchronize Voltage Recording, Mark Points, and Voltage Output experiments with a line scan acquisition. The [Voltage Recording](#), [Mark Points](#), and [Voltage Output](#) experiments are configured in their own control windows; more information is provided elsewhere in this manual. The synchronization with the line scan is defined in the Synchronize with Options Below section of the Linescan Control window.

The drop-down menu next to Synchronize with Options Below allows the user to choose when the synchronized experiments run:

- **Don't Synchronize** means that no Voltage Recording, Mark Points, or Voltage Output experiments will be called from the Linescan Control window
- **Once at Start** means that the synchronized experiment(s) will be started along with the first repetition of the line scan
- **Each Repetition** means that the synchronized experiment(s) will be started at the start of every repetition of the defined line scan acquisition

After a synchronization option is chosen, the experiment selection options become active. Use the drop-down menus to select the Current or previously saved experiment for Voltage Recording, Mark Points, and/or Voltage Output.

Checking the **Adjust Voltage Recording Time** changes the acquisition time of the selected Voltage Recording experiment to match the time required for the line scan and synchronized Voltage Output and/or Mark Points experiments.

The **Total Acquisition Time** field will be updated to reflect the duration of the line scan including the synchronized experiment(s).

It is recommended that a user make a global decision on where to configure Voltage Recording and Voltage Output experiments for a given line scan acquisition. It is possible to configure a Mark Points experiment that calls a Voltage Recording or Voltage Output experiment from inside the Mark Points configuration. If using this option, it is not advised to also call Voltage Recording or Voltage Output from the Linescan Control window.

Synchronized Voltage Recording and Voltage Output experiments will start as defined in the Linescan Control window and not wait for any trigger defined in the Voltage Recording or Voltage Output windows. Mark Points experiments will override a trigger on the very first point of the experiment (first point of first repetition of first line), if defined, but will respect all other triggers defined in the Mark Points Series.

More information about Mark Points, Voltage Recording, and Voltage Output can be found in other sections of this manual.

Z & Laser(s) Control

The controls in this section allow the user to drive a Z-piezo device during a line scan acquisition. The goal is to collect intensity data from a volume in the sample at higher speeds than are possible when performing a Z-Series with traditional raster imaging. Alternatively, high-speed volume imaging can be carried out using the Z-Series feature with a Z-piezo and Resonant Scanner or AOD.

This feature requires specific hardware and software configurations which must be implemented by Bruker Fluorescence Microscopy personnel. Additionally, analysis and reconstruction of position and intensity data must be carried out using third-party software tools. Contact Bruker Fluorescence Microscopy support personnel for more information about this feature.

In this mode, the galvanometers are driven along a user-defined path defined as a Freehand, Spiral, or Lissajous line, while the Z-piezo device is driving in a sinusoidal pattern by analog signal control. Laser power can also be adjusted as a function of depth.

The intended data acquisition method for this type of scan is to record position and intensity signals on analog inputs in the Voltage Recording window, for reconstruction and analysis later

in other software packages. Positional information is obtained by recording feedback signals from the X and Y galvanometers and the Z-piezo device. Intensity data is acquired by routing the Preamplifier output signals to Voltage Recording inputs (rather than to the typical Preamplifier inputs used for other imaging).

A Custom Output must be defined for the Z-piezo drive signal, and an additional Custom Output must be defined for each laser to be synchronized with the acquisition.

Define a Voltage Recording experiment to collect analog signals routed from the X and Y galvanometer feedback, Z-piezo feedback, and Preamplifier signal(s) for the channel(s) from which intensity data will be acquired. Choose a sampling frequency (Samples/Second) appropriate for the data resolution needed in the analysis.

Define the Z range and laser power gradient by defining a Z-Series in the Z-Series tab.

In the Z & Laser(s) control section of the Linescan Control window, configure the synchronization of Z and XY control with the following parameters:

Check the **Synchronize Z & Laser(s)** box. Lines to Skip parameter of the line scan definition will be automatically set to zero.

Save Linescan Images can be checked if the user wishes to save the image segments generated in Prairie View software during the 3D acquisition. Intended operation will leave this option un-checked, as Prairie View does not reconstruct the 3-dimensional data acquired, and both the intensity and positional information must be recorded through Voltage Recording and reconstructed using other software packages. Thus, saving the images takes up disk space with files which will not be used.

Lines per Z Period is the number of XY line scan traces that should be executed for each Z period. One Z period is movement of the Z-piezo device from its start to stop position and back to its start position.

Piezo Lag Time allows the user to define the time difference between the application of the drive voltage to the piezo and the piezo achieving the intended location. The parameter is associated with and saved as part of the calibration for the currently selected Objective Lens.

Synchronize the Voltage Recording experiment to run with the line scan acquisition, and check the box to **Adjust Voltage Recording Time**.

Start the acquisition by clicking **Start Linescan(s)** or **Append Linescan(s)**.

Profiles

Line Scan Profiles allow the user to define regions of the line from which to plot intensity data in a graph during acquisition. Intensity data from these plots is saved as a CSV file in the directory containing the line scan data. Click the **Profiles** button in the Linescan Control window to reveal the profile definition section of the window. For **Segmented** line scans

profiles corresponding to all channels and every line segment are automatically created and acquired when the profiles interface is visible. For other line types the user has to define the profiles of interest manually.

To define a profile, check the box for one or more channels and click the **Create** button. One definition will be created for each channel whose check box was activated. Then drag the **Position** and **Width** sliders to adjust the definition of the profile. The profile definition(s) will be displayed in the list box below the definition controls. The name of the profile reflects the channel, position, and width information for the profile, and a colored rectangle indicates the color of the trace and the overlay used to display the profile. The profile definitions are displayed along the line on the Image window and overlaid on the data displayed at the bottom of the Linescan Control window.

Within the list of profiles, the currently selected definition will be displayed with a green background behind the text. Any changes to the Position and Width sliders will be applied to the currently selected definition.

A drop-down menu allows the user to define whether the intensity information recorded is the average or sum of the pixels encompassed by the profile definition. Buttons in the interface allow the user to **Remove** the selected profile or **Clear All** profile definitions.

During acquisition, intensity information for each profile is plotted in the graph within the Linescan Control window. Clicking the green bar above the graph reveals controls for zooming and panning within the graph display.

Profiles can be moved by clicking and dragging the position of the colored markers in the Image window or in the data display at the bottom of the Linescan Control window. The rectangle marking the profile definition will move to the new location defined by the user. Dashed lines in the same color will mark the original position of the profile, corresponding to the trace still visible in the Profiles section of the window.

If the user is viewing the line scan data in Playback mode, moving a profile definition will cause the intensity graph to be recalculated. The user can save the new intensity plot data by clicking the **Save CSV** button. If the user is not viewing the data in Playback mode, the intensity graph will not be recalculated, but the new profile position will still be used in subsequent acquisitions.

Profile data can also be displayed in the Voltage Recording window. Defined profiles will appear as buttons next to each Voltage Recording plot. This allows users to overlay intensity data with electrical recordings that were synchronized with the collection of the line scan. Note that the Previous, Average, and History display options will be applied to line scan profiles acquired with a Voltage Recording, but not to stand-alone line scan acquisitions.

Data Display

Intensity data for each channel is displayed as an image in the bottom section of the Linescan

Control window. The horizontal axis is the position along the acquired line. The vertical axis is the line number, which can be interpreted as time. Each channel active during the acquisition is displayed. A scroll bar on the right side of the window allows the user to view more of the data without expanding the window.

The user can get information about a particular pixel in the displayed data by hovering the mouse over that pixel. Information boxes at the bottom of the Line Scan window display the position of that pixel along the line (X) and the start time (T) for that trace of the line, as well as the intensity of that pixel in each channel.

Click the left mouse button on the displayed data to display a vertical yellow cursor. A yellow box will appear on the Image window showing the position of this pixel on the reference image. These yellow markers can be used to correlate locations along the defined line scan with intensity information from the acquisition. The user can reposition the yellow markers by clicking elsewhere in the data display or dragging the yellow box in the Image window.

The position and width of any defined profiles are displayed on the image(s) as colored rectangles corresponding to the color used in that profile's definition and trace. To move a profile to a new location for the next acquisition, click and drag the rectangle in the data display at the bottom of the Linescan Control window. The rectangle marking the profile definition will move to the new location defined by the user. Dashed lines in the same color will mark the original position of the profile, corresponding to the trace still visible in the Profiles section of the window.

Long acquisitions are broken into multiple files during acquisition and reconstructed at the end of the scan. In these cases, only the last portion of the acquisition will be displayed in the Linescan Control window. To see the entire data set in the Image window, open the file in Playback Mode. This will happen automatically after any acquisition where the **Open in playback mode** option is checked; otherwise, load the data set from the File menu.

Point Scan

The point scan feature of Prairie View allows the user to plot the intensity of multiple pixels in the image as a function of time. By selecting points in the image and setting some of the parameters accessible via the point scan window, the response of these points to stimuli can be plotted in real time.

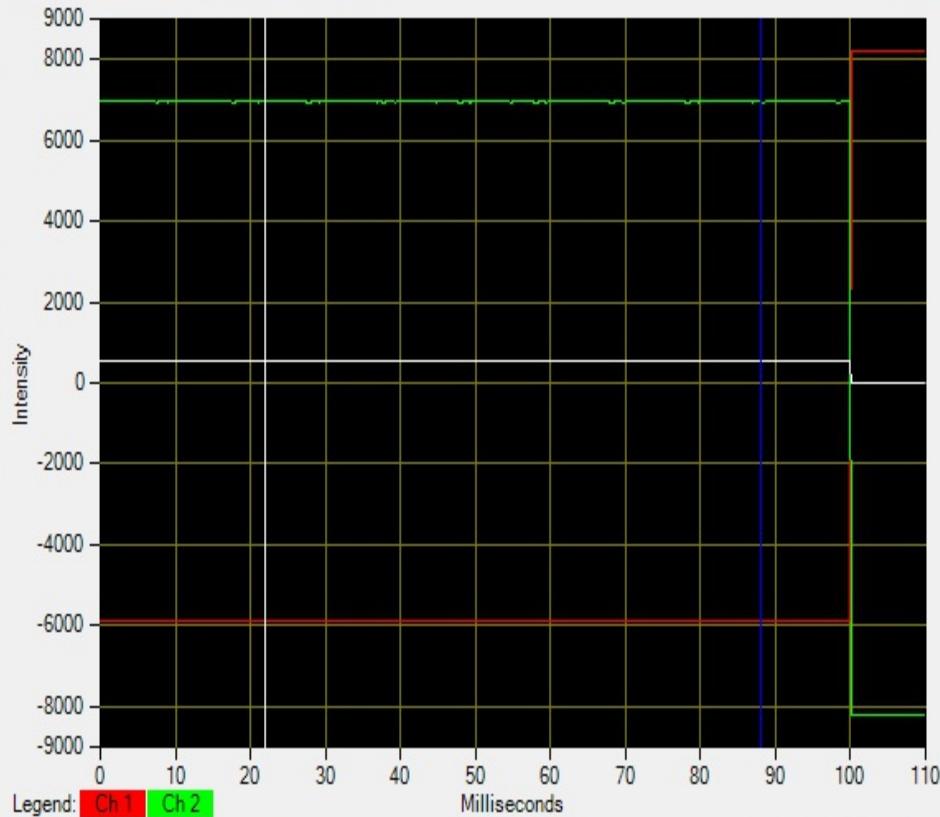


Image Window Options

Show Points as Crosshairs

Point Color Red

Show Point Indices

Left Click Action
Hold Shift for straight lines, release & hold for segments.

Select/Move Points
 Add Grid of Points
 Add Line of Points

Right Click Action
Redraw line along new path.

Add Individual Points

Points/Groups		
Name	Indices	Type
Grid 1	1-9	Grid
Line 1	10-12	Line
Point 1	13	Point
Point 2	14	Point
Point 3	15	Point
Point 4	16	Point
Point 5	17	Point
Group 1	13, 15, 17	Group

Partially selected rows are highlighted in yellow, see image window for specific point selections.

Selected Points/Groups

Point Count/Density	2 <input type="color"/> 2 <input type="color"/>
N/A	N/A
Point Order	Forward <input type="color"/>
Remove	Split
Create Group	
Export...	
Add to Point Series	
Clear All	Import...

Graph Options

Horizontal Scale	Vertical Scale	Show Average For
<input checked="" type="radio"/> Best Fit	<input checked="" type="radio"/> Best Fit	1 <input type="color"/> Samples
<input type="radio"/> Fixed	<input type="radio"/> Fixed	<input checked="" type="checkbox"/> Update Display
Maximum [ms]	Maximum	Enter Playback Mode After Scan
110.000 <input type="color"/>	9000.000 <input type="color"/>	<input type="checkbox"/>
Minimum [ms]	Minimum	Save Graph...
0.000 <input type="color"/>	-9000.000 <input type="color"/>	

Cursor Information

Frequency (1/Δ Time)	Left Time	Right Time	Δ Time
15.133 Hz	21.952 ms	88.032 ms	66.080 ms
Data Source	Left Intensity	Right Intensity	Δ Intensity
Ch 1	-5875.0	-5875.5	-0.5
Ch 2	6956.2	6949.2	-7.0
Function #1	-5875.0	-5875.5	-0.5

Scan at Crosshair

These values will also be used as defaults in the point series grid.

Use Imaging Galvos	Synchronize with Options Below
Initial Delay (ms)	Inter Point Delay (ms)
10.00 <input type="color"/>	10.00 <input type="color"/>
Interval (ms)	Duration (ms)
110.00 <input type="color"/>	100.00 <input type="color"/>
Dwell Time (μs)	4.0 <input type="color"/> 2.5 MHz
Use Uncaging Galvos <input checked="" type="checkbox"/>	
Initial Delay (ms)	Inter Point Delay (ms)
50.00 <input type="color"/>	0.12 <input type="color"/>
Interval (ms)	Duration (ms)
750.12 <input type="color"/>	750.00 <input type="color"/>
Uncaging Laser	405nm Uncaging <input type="color"/>
Uncaging Laser Power	0 <input type="color"/>
Live Scan Period	
Live Scan	1.0 <input type="color"/> Second(s)

Voltage Recording Experiment
None
Select a Category

Voltage Output Experiment
None
Select a Category

Wait for Trigger
Never

Repetitions
1
 Scan until stopped
 Hide Crosshair
Start Point Scan

Imaging Point/Group	Imaging Indices	Initial Delay (ms)	Inter Point Delay (ms)	Interval (ms)	Duration (ms)	Dwell Time (μs)	Parameter Set	# Reps	Wait for Trigger	Uncaging Point/Group	Uncaging Indices	Initial Delay (ms)	D
Grid 1	1-9	200.00	150.00	200.00	50.00	4.0	CurrentSettings <input type="color"/>	1	Never <input type="color"/>	Point 1 <input type="color"/>	13	100.00 <input type="color"/>	<input type="color"/>
Line 1	10-12	0.22	50.00	150.00	100.00	0.4	CurrentSettings <input type="color"/>	2	Never <input type="color"/>	Point 3 <input type="color"/>	15	20.00 <input type="color"/>	<input type="color"/>
Group 1	13, 15, 1	100.00	0.22	20.22	20.00	0.8	CurrentSettings <input type="color"/>	1	Never <input type="color"/>	Point 2 <input type="color"/>	14	30.00 <input type="color"/>	<input type="color"/>
Point 4	16	0.22	75.00	150.00	75.00	8.0	CurrentSettings <input type="color"/>	3	Never <input type="color"/>	Grid 1 <input type="color"/>	1-9	0.12 <input type="color"/>	<input type="color"/>

Add Row Remove Row Copy Row Clear All

Repetitions 1 Pause Between Reps (ms) 0

Save Path ... PointScan-12112014-1301 55

Leave Shutter Open Start Point Series

The simplest type of point scan is controlled using the point scan window's "Scan at Crosshair" panel on the right hand side of the window. This point scan can run both an imaging point intensity recording and a point uncaging experiment simultaneously in parallel. This way, by carefully choosing the timing options for the uncaging and imaging panels, an experiment can be defined in which recording is performed in conjunction with uncaging. To run this experiment, follow the steps below:

1. Ensure that the **Hide Crosshair Overlay** checkbox is not checked.
2. Position the Imaging crosshair over the pixel for which intensity is to be recorded.
3. If uncaging is desired, position the Uncaging crosshair (denoted with a U) over the point in the image which will be uncaged.
4. In the "Use Imaging Galvos" panel, set the desired **Initial Delay** in ms. This is the time to wait from when the experiment is started to when intensity recording should begin.
5. In the same panel, set the **Duration** to the length of time for which the intensity is to be recorded, in ms.
6. In the same panel, set the **Inter Point Delay** to the amount of time that should be elapsed between the end of one repetition and the beginning of the next, or set the **Interval** to the amount of time that should elapse between the start of one repetition and the start of the next. These fields will be ignored if there is only one repetition.
7. In the same panel, set the **Dwell Time** to the time desired between recording samples, in us.
8. If uncaging is desired, ensure that the checkbox on the right side of the label of the "Use Uncaging Galvos" is checked. If uncaging is not desired, skip to step 15.
9. In the "Use Uncaging Galvos" panel, set the desired **Initial Delay** in ms. This is the time to wait from when the experiment is started to when uncaging should begin.
10. In the same panel, set the **Duration** to the length of time for which the uncaging laser is powered, in ms.
11. In the same panel, set the **Inter Point Delay** to the amount of time that should be elapsed between the end of one repetition and the beginning of the next, or set the **Interval** to the amount of time that should elapse between the start of one repetition and the start of the next. These fields will be ignored if there is only one repetition.
12. In the same panel, set the **Uncaging Laser** to the laser that will be used for uncaging.
13. In the same panel, set the **Uncaging Laser Power** to the desired value representing the power of the laser used for uncaging.
14. If it is desired to run a voltage output and/or recording simultaneously with this experiment, choose either First Repetition or Every Repetition in the **Synchronize with Options Below** drop down menu, depending on whether the voltage output or recording is desired to run once at the start of the experiment, or with every repetition of the point

scan. Additionally, choose the voltage output and/or recording experiment which is to be run simultaneously using the **Voltage Recording Experiment** and **Voltage Output Experiment**. More information about these features can be found in the [Voltage Output](#) and [Voltage Recording](#) sections of this manual.

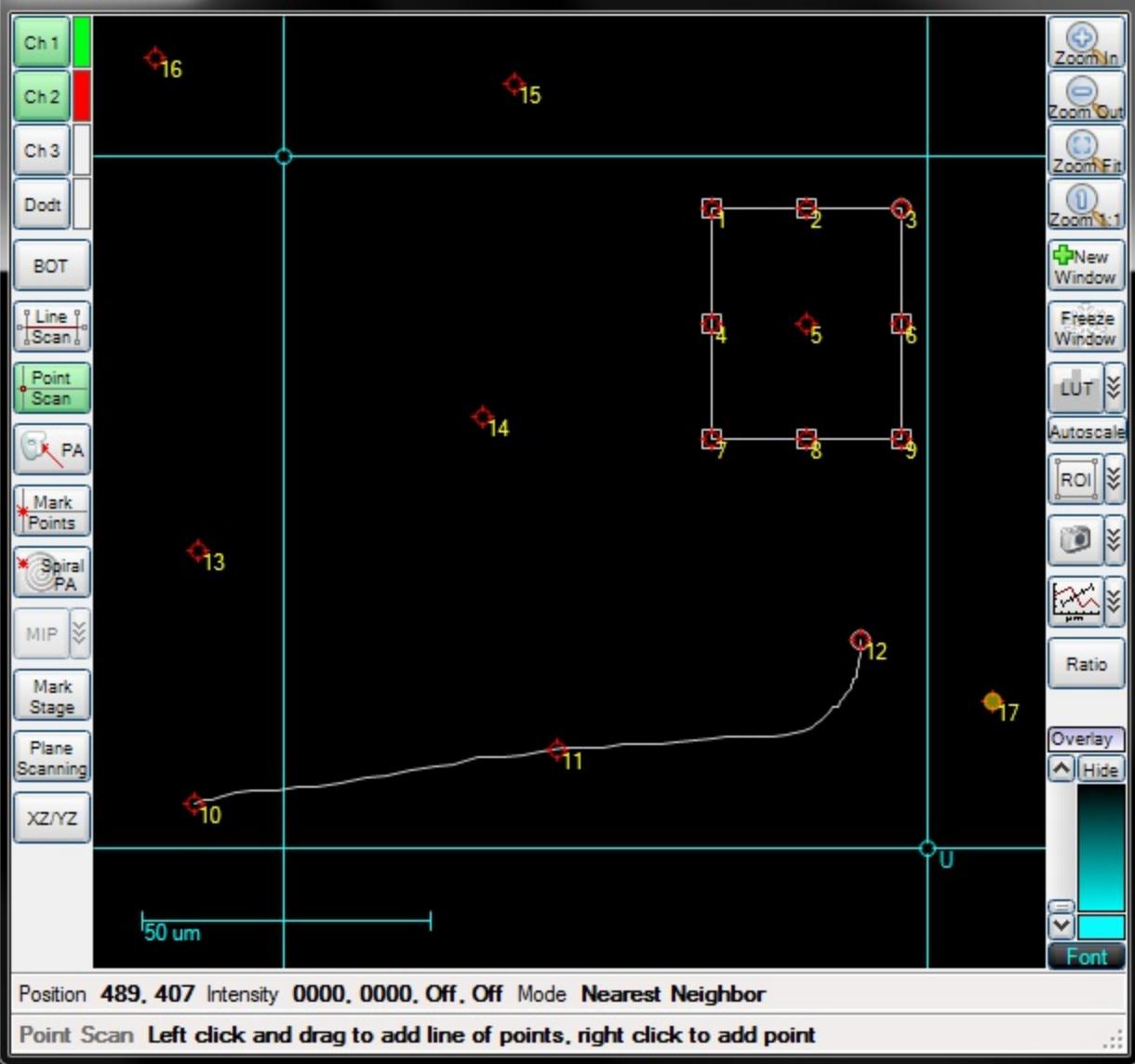
15. If the beginning of the point scan experiment should be started by another stimulus, choose the appropriate selection in the **Wait for Trigger** drop down menu.
16. Choose the number of times the experiment should run by changing the value in the **Repetitions** control.
17. Choose the **Save Path** using the control at the bottom left of the point scan window.
18. Press the **Start Point Scan** button to begin the experiment.

If, instead of running the point scan experiment a set number of times, the experiment should run indefinitely until a stop button is pressed, check the **Scan Until Stopped** checkbox prior to pressing the **Start Point Scan** button. In this case, the **Start Point Scan** button will change to become **Abort Point Scan**. Press this button to finish the experiment. This type of experiment will run the procedure defined by the values entered in the textboxes once, then continually record the intensity at the imaging crosshairs until the **Abort Point Scan** button is pressed. Once the **Abort Point Scan** button is pressed, acquisition is stopped, and the data is saved to the appropriate file.

If it is desired to monitor the intensity of the imaging crosshair pixel in real time, the **Live Scan** feature can be used. In this case, the **Live Scan Period** should be chosen to match the amount of time for which previously acquired data should be displayed, then press the **Live Scan** button. For example, if the **Live Scan Period** is chosen to be 2 seconds, the graph will display the most recent 2 seconds of intensity values recorded.

Choosing Points to be Scanned

Image Window - 1



Defining Point Locations

The first step in running a more complex point scan experiment is to choose the points which will be scanned. This can be achieved using one of three methods, two of which are chosen on the left side of the point scan window under the 'Left Click Action' heading. To perform any of these methods, both the Image window and the Point Scan window should be open. The first of these methods is to simply right click on an image window at the desired location of a new point and it will be added. Additionally, if a point has been placed, and it is then determined that the point needs to be moved, a left-click and drag on the point will allow the point to be repositioned in the image. The second method accessed under the 'Left Click Action' heading mentioned earlier, **Add Grid of Points**, allows for a grid to be defined initially by left-clicking at the top left corner of the grid, and dragging to the bottom right corner. When the mouse button is released, a 3x3 grid of points will be created over the desired area. Near the middle of the point scan window is a section entitled **Point Density**, which allows for a more densely defined grid. By changing the density in the X and/or Y directions, the resolution of the grid can be changed to include more or fewer points than the original 3x3 layout. The third and final method, **Add Line of Points**, allows for the points to be defined along a line created by left-clicking and dragging arbitrarily over the image. By default, the line created is not restricted to

be a straight line, but will follow any path the mouse takes. Conversely, by holding the <Shift> key while drawing the line, the line is coerced to be a straight line with the endpoints defined by the beginning and end of the drag operation. Once this line is created, it will by default have three points. The number of points can be increased or decreased by changing the X Point Density near the middle of the Point Scan window.

Point Representation

In addition to these methods for defining the location of the points, their representation can be chosen using the “Image Window Options” box on the left hand side of the point scan window. The first drop down menu allows for selection of the representation of these points between a crosshair, a dot, and no representation (i.e. the points are not shown on the image). Immediately beneath this drop down menu is another which allows for the color of the point representations to be chosen. Finally, underneath the two drop down menus is a checkbox which enables or disables display of the point indices alongside the point representations in the image.

Point Organization / Grouping

Immediately to the right of the “Image Window Options” panel is a table which lists the Name, Index, and Type of each point defined in the window. This table can be used to create groups of points or remove individual points from the image. To create a group of points, control-click on individual points or shift-click on the endpoints of a contiguous list of the points which are desired to be grouped together. Then, click the **Create Group** button to the right of the table. Points which should be grouped together can also be chosen using their representation in the image window. Using the mouse, it is possible to left-click and drag to create a box which surrounds multiple points. This will select all of the points encompassed by the box. Alternatively, control-clicking individual points will select multiple points. With the desired points selected, the **Create Group** button can be pressed to group the points together.

Intragroup Scanning Order

Any group of points, including a defined grid or line, will by default number the points in ascending order in which the points were drawn. If this behavior is not desired, the order of points can be changed using the **Point Order** control to the right of the point table. The options presented here are forward, which is the default behavior, reverse, which puts the points in the opposite order of the default behavior, non-neighbor, which maximizes the distance between successive points, and custom, which allows the order of the points to be defined using the point names in the table. If the custom option is chosen, the textbox underneath it becomes active, and the order of points can be entered. In this scenario, use the number given in the point name to define a point. Continuous lists of points can be entered using the hyphen, such as 2-6 to represent points 2, 3, 4, 5, and 6. Otherwise, separate entries using a comma followed by a space.

Once the points and their order are defined, one or multiple points can be selected in the table, and the **Add to Point Series** button pressed to add a line to the point scan series table near the bottom of the point scan window. Additionally, the **Clear All** button will remove all points currently defined in the image. Finally, the **Import** button allows for points to be

imported from the current line scan, or from an external application using the Tools>Actions dialog.

Running a Series of Point Scans

When multiple points are defined, a series of point scans can be run using the point scan series table near the bottom of the point scan window. Each step of the series is defined using one row of the table. These rows can be initialized by pressing the **Add Row** button below the point scan series table, or by choosing a point or a group of points in the point table and pressing the **Add to Point Series** button. Each row can define one imaging experiment and one uncaging experiment, each of which can operate on a single point or a group of points. The imaging and uncaging experiments run simultaneously in parallel such that uncaging and imaging can be synchronized by carefully choosing the time parameters in the row of the table. In addition, each row can be run multiple times using the **#Reps** column in the table. In this scenario, the experiment will wait for both the imaging and uncaging portions of the experiment to finish before repeating the row. When a row is added, follow these steps to set the associated parameters for the point scan:

1. In the **Imaging Point/Group** column, choose the desired point or group for which intensity will be recorded.
2. Set the desired **Initial Delay** in ms. This is the time to wait from the end of the previous row's recording (or the beginning of the experiment) to the time the intensity recording for this row should begin.
3. Set the **Duration** to the length of time for which the intensity is to be recorded at each point, in ms.
4. Set the **Inter Point Delay** to the amount of time that should elapse between the end of recording the previous point and the beginning of recording the next point. Multiple points can be present in a row by including a group of points and/or specifying a number of repetitions greater than one. Alternatively, the **Interval** can be set to the amount of time that should elapse between the beginning of one point and the beginning of the next point..
5. Set the **Dwell Time** to the time desired between recording samples, in us.
6. Choose the **Parameter Set** which corresponds to the imaging settings desired for recording intensity at the point/group.
7. Set the **# Reps** to the number of times that the row should run before moving onto the next row.
8. If desired, set the **Wait for Trigger** option accordingly to wait for a stimulus prior to starting the row.
9. If uncaging is desired, choose the desired point or group at which uncaging should occur using the **Uncaging Point/Group** column. If uncaging is not desired, choose "None" and

skip to step 15.

10. Set the desired **Initial Delay** in ms. This is the time to wait from when the experiment is started to when uncaging should begin.
11. Set the **Duration** to the length of time for which the uncaging laser is powered, in ms.
12. Set the **Inter Point Delay** to the amount of time that should elapse between the end of uncaging the previous point and the beginning of uncaging the next point. Alternatively, the **Interval** can be set to the amount of time that should elapse between the beginning of one point and the beginning of the next point.
13. Set the **Uncaging Laser** to the laser that will be used for uncaging.
14. Set the **Uncaging Laser Power** to the desired value representing the power of the laser used for uncaging.
15. If it is desired to run a voltage output and/or recording simultaneously with this experiment, choose either First Repetition or Every Repetition in the **Synchronize with** column, depending on whether the voltage output or recording is desired to run once at the start of the experiment, or with every repetition of the point scan. Additionally, choose the voltage output and/or recording experiment which is to be run simultaneously using the **Voltage Recording Experiment** and **Voltage Output Experiment**. More information about these features can be found in the [Voltage Output](#) and [Voltage Recording](#) sections of this manual.
16. Repeat the above steps for every row in the table.
17. Choose the number of times the experiment should run by changing the value in the **Repetitions** control found beneath the series table, slightly to the right of the center of the window.
18. If desired, enter a value into the **Pause Between Reps** field to set a time to wait from when one repetition of the table is finished to when the next repetition should begin.
19. Choose the **Save Path** using the control at the bottom left of the point scan window.
20. Press the **Start Point Series** button to begin the experiment.

If, when populating the series table, it is found that one of the rows is unnecessary, click on any value in that row to make it the active row. This is indicated by a black triangle appearing to the left of the row. Then, click the **Remove Row** button to eliminate this row from the series. Additionally, if it is desired to run a similar point scan as a previously defined row, select the row, again causing the black triangle to appear next to the row, and click on the **Copy Row** button to create an exact copy of the row in the series table. Finally, if all rows in the table are unwanted, press the **Clear All** button to remove all rows from the table.

Visualization of Point Scan Results

Graph Overview

When a point scan is run, the intensity recorded at each point is plotted in the graph in the upper left hand corner of the point scan window for visualization of the result. This graph plots the recorded intensity on the y axis versus time elapsed on the x axis. The number of primary plots in the graph corresponds to the number of channels active in the Image window, and each is color coded according to the legend displayed at the bottom left hand corner of the graph panel in the Point Scan window.

Zooming and Panning

At the top of the graph panel are the graph controls used in all Prairie View graphing applications. The farthest left button, the reset button, is used to restore the axes to their original position. Next is the panning hand, which is used to drag the graph in any direction, which translates the graph in the desired direction. The next two controls are similar, except that they are limited to horizontal and vertical translation, respectively. The fifth control is the zoom function. To use this tool, draw a box on the graph by clicking and dragging the mouse around the area to be zoomed to. The last two controls are also zooming tools, but are limited to horizontal and vertical zoom, respectively. On the far right of this row is the graph tooltip, which explains all of the controls mentioned herein.

Axis Scaling

In the upper right hand corner of the point scan window are several controls to change the display of the graph to the left. The first panel controls the Horizontal Scale of the graph, and has two options. Choosing **Best Fit** sets the x axis limits to show all of the recently acquired data. Conversely, choosing **Fixed** and entering values into the **Maximum** and **Minimum** boxes allows for custom defined limits on the x axis. The panel immediately to the right, the Vertical Scale panel, functions in exactly the same way as the Horizontal Scale, except it affects the y axis instead of the x axis. On the far right, the displayed data can be filtered using an averaging filter with the number of samples equal to any value entered into the **Samples** textbox. Note that for the real time display, the acquired data is down-sampled to 1 kSamples/second, and thus choosing a number of samples to average of less than 2500 will not change the displayed time between samples, although the filtering is still being applied. When a previously run experiment is opened in playback mode, this number will auto-adjust to the smallest value which will still allow the entirety of the experiment to be displayed in the graph. Below this box are two checkboxes. The first, **Update Display**, toggles whether to update the graph with the data acquired from the next experiment. If this checkbox is left empty, the experiment will run, saving the intensity recordings into their appropriate files, but the graph will not show any of the data. On the other hand, if it is desired to visualize the data acquired in real time, ensure that this checkbox is enabled. The second, **Enter Playback Mode After Scan**, causes Prairie View to automatically load the acquired point scan data, whether from a “Scan at Crosshairs” or point series experiment, into playback mode. The last control in this panel, the **Save Graph** button, exports an image of the current graph as a tiff file for later review.

Quantitative Analysis

The final panel in the point scan window is the “Cursor Information” panel, which displays information about the acquired data. After a point scan experiment, two cursors are shown on

the graph which can be moved to correspond with any time value on the graph. When the cursors are positioned, their corresponding time points can be seen in the **Left Time** and **Right Time** textboxes. Furthermore, the difference between these two time points can be seen in the **Δ Time** textbox and the corresponding frequency can be seen in the **Frequency** textbox. In addition to the time information related to the cursors, the information related to the data can be found in the table immediately below. This table consists of four columns. The first column lists the **Data Source** of the row, which is either one of the acquisition channels or a user defined function. The second column, **Left Intensity**, shows the intensity of the data corresponding to that row's **Data Source** at the time point corresponding to the left cursor. Similarly, the **Right Intensity** shows the intensity of the data at the time point corresponding to the right cursor. Finally, the last column, **Δ Intensity**, shows the difference between the **Left Intensity** and the **Right Intensity**.

Data Evaluation

When the green button next to the “Cursor Information” panel is pressed, the “User Defined Functions” panel is displayed. Here, the user may enter custom mathematical functions to perform on the data. By clicking **Create Function**, a new window opens which allows for entry of a new function. Additionally, the dialog contains a link to help the user enter a function in the proper format. When the function has been defined, pressing <Enter> or clicking **Accept** will store the function in the table and add it to the plotted data for the next graph. If a slight change should be made to the function, the function can be highlighted, and the **Edit Selected** button pressed to re-open the function entry dialog with the previously defined function, so that a change can be made. On the other hand, if it is decided that a function is no longer wanted, the **Remove Selected** button will delete the active function from the list. Finally, if none of the defined functions are wanted, the **Clear All** button will delete all functions from the list. Once a function has been defined, it will be added as a **Data Source** in the “Cursor Information” panel, and its value will be plotted on the next point scan.

Files Saved by the Point Scan Experiment

A Point Scan experiment saves files that define the experiment as well as contain its results in the directory chosen as the filepath in the Point Scan window. Firstly, a configuration file is saved which contains the state of all microscope settings at the beginning of the Point Scan experiment. This file has a name ending in Configuration.cfg. Furthermore, the Point Scan experiment has an associated XML file which contains its definition. In this file, you can find the imaging and uncaging settings corresponding to the Point Scan experiment, as well as the timestamps of each point. Additionally, two sets of images are saved with the experiment. In the main directory, the raw image from the beginning of the experiment is saved for each channel. In the “References” subdirectory, a snapshot of each image window is stored with the current overlays. This image shows the locations of the points used in the experiment. Finally, each point imaged is associated with a comma-separated-value file which contains a relative timestamp as well as the intensity in each channel of that point at the corresponding time. If Voltage Recording, Mark Points, or Voltage Output was synchronized with the Point Scan

experiment, their associated files will be present here as well. See the appropriate subsections for more information on these files.

Quick Reference ([Basic Overview](#))

Note: **Bold text** is entered as is; *grey italicized text* needs to be replaced with actual values before entered.

[Intensity Values](#)

{*Channel Number*} - Current intensity of specified channel.

{*Channel Number:Sample Offset*} - Intensity of specified channel at a relative sample offset.

{*Channel Number,Sample Number*} - Intensity of specified channel at an absolute sample offset.

[Timestamps](#)

{t} - Current timestamp in seconds.

{t:*Sample Offset*} - Timestamp at a relative sample offset in seconds.

{t,*Sample Number*} - Timestamp at an absolute sample offset in seconds.

[Sample Number](#)

{s} - Current sample number.

[Operators & Functions](#)

You can use any common operators such as +, -, / or * as well as any valid C# syntax such as **Math.Max(Expression #1,Expression #2)**, **Math.Abs(Expression)** and **Math.Log(Expression)**. Note that expressions are **case sensitive**.

[Intensity Values \(\[Top\]\(#\)\)](#)

There are three different ways to include intensity values in your functions: by channel, by channel and a relative sample offset and by channel with an absolute sample number.

To include the current intensity value in your function simply enclose the number of the channel (1 thru 4) you are interested in within curly braces, {*Channel Number*}.

For example, to subtract the intensity values for channel one from those of channel two the function would be the following, '{2} - {1}'. These intensities will vary as the function is recalculated for each sample.

To include intensity values from past and/or future samples enclose the number of the channel (1 thru 4) followed by the sample offset separated by a colon in curly braces, *{Channel Number:Sample Offset}*. For example, to calculate the change in intensity for channel one over each sample the function would be the following, '**{1} - {1:-1}**'. This takes the intensity for channel one for the current sample and subtracts from it the intensity value for the previous sample.

If an offset should reference a nonexistent sample either before the first sample or after the last sample then the values for the first and last sample are used instead respectively. So in the example the function will always evaluate to zero for the first sample since the value for the first sample is substituted for the value of the previous samples values since there is no sample before the first sample. This substitution also occurs when updating a function during a live acquisition for any positive sample offsets, since the data has yet to be collected for any future sample the value of the current sample is used instead.

Finally to include intensity values for a specific sample enclose the number of the channel (1 thru 4) followed by the sample number separated by a comma in curly braces, *{Channel Number,Sample Number}*. For example, to calculate F over F_0 for channel one where F is the current intensity value and F_0 is the average of the first 5 sample the function would be the following, '**{1}/(({1,1} + {1,2} + {1,3} + {1,4} + {1,5}) / 5)**'. This takes the current intensity of channel one and divides it by the average intensity of the first 5 samples.

Timestamps ([Top](#))

There are three different ways to include timestamp values in your functions: for the current sample, for a relative sample offset and for an absolute sample number. All timestamps are all in seconds.

To include the timestamp value for the current sample in your function simply enclose a lowercase T within curly braces, *{t}*. For example, to display the timestamp value for each sample the function would be the following, '**{t}**'. The timestamp will vary as the function is recalculated for each sample.

To include timestamp values from past and/or future sample enclose a lowercase T

followed by the sample offset separated by a colon in curly braces `{t:Sample Offset}`. For example, to display the sample period the function would be the following, '`{t} - {t:-1}`'. This takes the timestamp value for the current sample and subtracts from it the timestamp value for the previous sample which will generate a horizontal line.

If an offset should reference a nonexistent sample either before the first sample or after the last sample then the values for the first and last samples are used instead respectively. So in the example the function will always evaluate to zero for the first sample since the value for the first sample is substituted for the value of the previous samples values since there is no sample before the first sample. This substitution also occurs when updating a function during a live acquisition for any positive sample offsets, since the data has yet to be collected for any future sample the value of the current sample is used instead.

Finally to include timestamp values for a specific sample enclose a lowercase T followed by the sample number separated by a comma in curly braces, `{t,Sample Number}`. For example, to display the current sample's timestamp relative to the 2nd sample's timestamp the function would be the following, '`{t} - {t,2}`'. This takes the timestamp of the current sample and subtracts is from the timestamp of the 2nd sample.

Sample Number ([Top](#))

To include the current sample number in your function simply enclose a lowercase S within curly braces, `{s}`. For example to display the current sample number the function would be the following, '`{s}`'. This will result in a diagonal line increasing by one each sample. Not all too exciting on its own, but used in combination with other variables can prove invaluable.

Operators & Functions ([Top](#))

Any of the four standard arithmetic operators can be used on the variables mentioned in this document: addition (+), subtraction (-), division (/) and multiplication (*). The modulus operator (%) can also be used.

In addition to variables and operators any numeric constants can be used as desired.

If basic operators, variables and constants are insufficient the entire C# math library is available as well as any other standard C# libraries. Keep in mind that C# is **case sensitive** which is also true for any expressions entered for functions. A few commonly used math functions are:

Math.Abs(Expression) - Calculates the absolute value of given expression.

Math.Min(Expression #1,Expression #2) - Calculates the minimum value of two expressions.

Math.Max(Expression #1,Expression #2) - Calculates the maximum value of two expressions.

Math.Log(Expression) - Calculates the natural log of given expression.

Math.Log(Expression,10) - Calculates the log (base 10) of given expression.

Math.Sqrt(Expression) - Calculates the square root of given expression.

Math.Pow(Expression #1,Expression #2) - Calculates the value of the first expression raised to the power determined by the value of the second expression.

Math.Cos(Expression) - Calculates the cosine of given expression in radians.

Math.Sin(Expression) - Calculates the sine of given expression in radians.

Math.Tan(Expression) - Calculates the tangent of given expression in radians.

Basic Overview ([Top](#))

Functions can be used to display raw intensity data in different ways during acquisitions or after data has already been collected in playback mode.

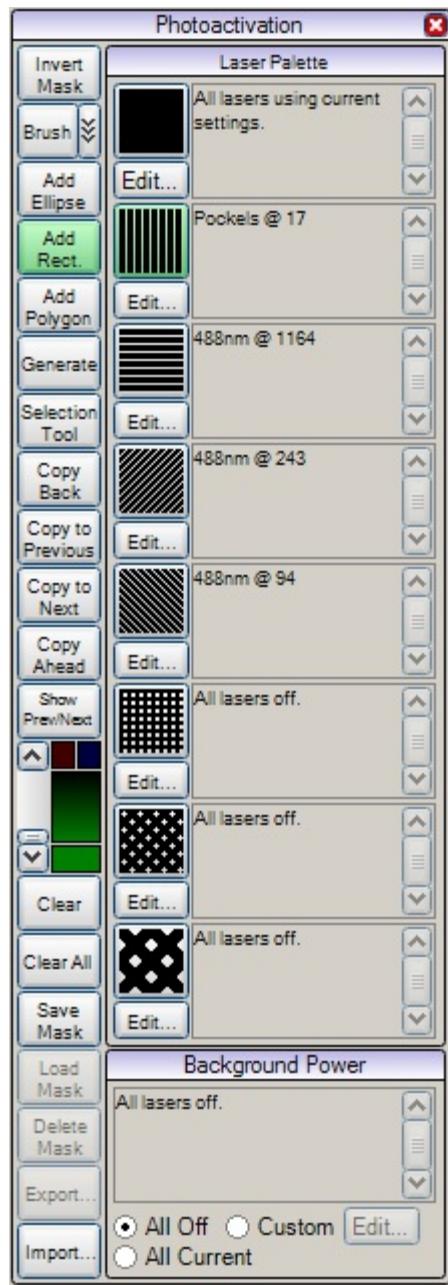
Functions consist of a few variables used to access intensity data consisting of channel and sample numbers enclosed in curly braces { } in combination with some basic arithmetic operators and optionally some C# syntax for advanced users.

Functions are added by clicking the Create Function button which will display a dialog where an expression defining the function can be edited (syntax is explained in the rest of this document). When the expression is complete either press Enter or click the Accept button at which point the expression will be evaluated for correctness and saved. To cancel simply click the Cancel button or close the form. If the expression is found to be incorrect a message box will pop up at which point the expression can be fixed and accepted again. Behind the scenes the expression is actually being compiled into a small program of its own for speed.

Photoactivation (PA)

The Photoactivation tool allows the user to define masks in the scan area to determine where laser power will be applied. These masks can be applied to a single focal plane, specific slices of a Z-Series, or all slices of a Z-Series. Laser power will be applied only to those areas included in the mask, and power will be modulated as specified by the mask's definition.

The photoactivation button  opens the photoactivation mask editor dialog. On point scanning systems, PA masks are carried out using the imaging galvanometers. In order to use this feature on an SFC or Camera system, the system must be configured with a Photoactivation/FRAP module or Ultima scanner.



When using a Photoactivation Mask, the galvos scan a square or rectangular region, but laser power is applied only in the areas defined by the mask. In some cases, the scanned region is the entire field of view. In other cases, the scanned region is the smallest rectangular region needed to encompass the entire mask.

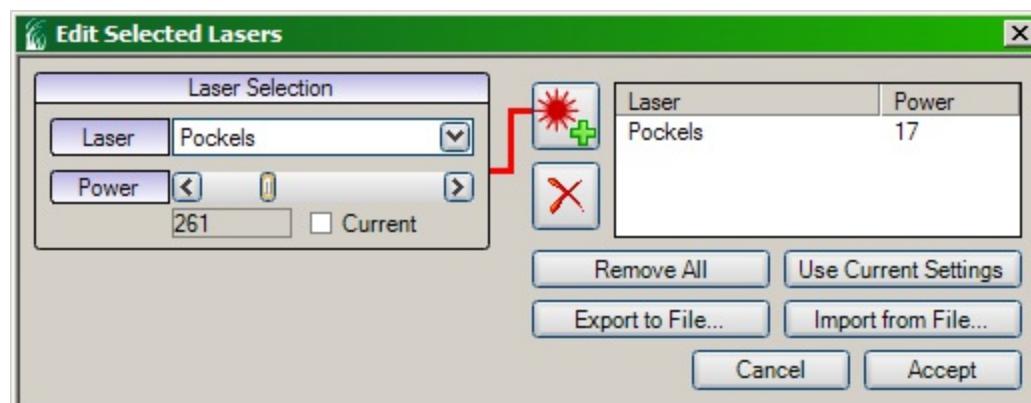
In the Preferences menu, the option to Save Images Generated With Photoactivation determines whether TIFF files are made when a PA mask is being scanned. In most cases, these files do not contain useful images of the sample. However, enabling the option provides the user with a quick reference image to see the position of the mask on the image.

Photoactivation Editor Controls

Palettes

The right side of the editor dialog consists of 8 Laser Palettes. Each palette definition is a power setting for one or more lasers on the system, and draws with a particular pattern on the image. The pattern allows the user to distinguish between regions drawn with different palettes, but does not reflect a pattern or texture to the scan itself.

To set the laser power(s) that define a particular palette, click the associated **Edit** button to open the Edit Selected Lasers dialog.



The power setting for each laser in the palette definition appears in the box on the right side of the window. To change the power setting for one of these lasers, choose that laser from the drop-down on the right side of the dialog. Move the Power slider to the desired setting, or check the **Current** box to use the power setting made on the Laser, PMT, DAQ tab of the Main

Control window. Click the button to add that new setting to the definition in the box on the right side of the dialog. To add another laser to the definition, choose the new laser from

the drop-down, set the power, and click the button. To use all configured lasers at their current power settings, click the **Use Current Settings** button below the list of defined laser powers.

To remove a laser from the definition, select it from the box on the right and click the button. To clear the entire definition, click the **Remove All** button.

Palette definitions can be exported to a permanent file with the **Export to File** button, and later recalled with the **Import from File** button.

Click **Accept** to save the changes and close the dialog, or click **Cancel** to exit without saving changes.

To select a palette, click the pattern icon next to the palette definition; the selected palette's

pattern icon will appear with a green background.

Note: Palettes are only present for systems with certain laser configurations. If no such lasers are configured, this section of the dialog will not appear. Drawing tools are still available, and masks will use the laser power(s) defined on the Laser, PMT, DAQ tab of the Main Control window.

Drawing Options

Choose a drawing tool to begin drawing a mask on the image.

Invert Mask will swap the masked and unmasked areas of the mask. This option is not available if the current mask uses more than one palette.

The **Add Ellipse**, **Add Rect.**, and **Add Polygon** buttons allow the user to add these ellipses, rectangles, or polygons by clicking and dragging within the Image window. With the ellipse tool, click the desired center position for an ellipse and drag outward to expand. With the rectangle tool, click one corner of the desired rectangle and drag outward to expand. With the polygon tool, click to add corners to the shape, and right click to close the region after making the last corner.

The **Brush** tool allows the user to paint areas of the image. A small arrow next to the tool allows the user to choose from three brush sizes (Small = 2% of the display area, Medium = 4%, Large = 8%) or an Erase option to remove part of a previously drawn mask.

Generate allows the user to call previously saved Actions to auto-draw regions. [Actions](#) are described elsewhere in this manual.

The **Selection Tool** can be used to select a rectangular region of an existing mask. Left click inside the selected region to drag it to a new location. Right click inside the selected region to copy the selection and drag the copy to a new location. Left click and drag one of the handles on the selection box to resize the selected area. Click the **Invert** button to swap the masked and unmasked areas of the selection. Click the **Clear** button to delete the mask inside the selected area.

When drawing a 3D mask in Playback of a Z-Series, clicking **Copy Back** (or **Copy Ahead**) adds the mask elements on the current slice to all slices between the current slice and the start (or end) of the Z-Series. Clicking **Copy to Previous** or **Copy to Next** adds the mask elements on the current slice to one adjacent slice in the Z-Series. The **Show Prev/Next** button displays masks on adjacent slices in a different overlay color, allowing the user to scroll through a Z-Series and see masks for the previous, current, and next slice on the same image.

The scroll bar allows the user to define the transparency of the mask overlay on the Image window. This can be helpful when visualizing the structures covered by the mask. This scroll bar applies only to PA masks on the Image window. The Overlay scroll bar on the Image window itself will dim PA masks as well as all other overlays on the window. Note that the

Hide/Show option on the Image window will hide all PA masks as well; if the PA overlay is missing, check to be sure the Hide option is not enabled.

The color of the current, previous, and next mask overlays can be chosen by the user. Click the rectangle below the transparency slider to choose the color for the current slice; click the squares above the slider to choose the color for the previous and next slices.

The **Clear** button will clear the mask elements inside a selected region. If no selection has been made, a message box will appear to ask the user whether to clear all mask elements. The **Clear All** button will clear all mask elements in the Image window.

For systems configured to use Laser Palettes, the Background Power section allows the user to choose the laser power(s) to be used in any area of the image not covered by a mask. Radio buttons provide options for all lasers off, all lasers at current power, or a custom definition which can be set up with the **Edit** button like any other palette.

Click the **Save Mask** button and name the current mask. This will save the mask in a temporary file, allowing it to be re-loaded (with the **Load Mask** button) later in the same session. To keep a mask to use in later Prairie View sessions, use the **Export** button to save it to a permanent file, from which it can be **Imported** later.

Creating a Photoactivation Mask

A mask must be created on images of the same dimension on which it will be used. For example, a mask to be used in an ROI must be created in that ROI. A mask to be used on a 512x512 image must be made on a 512x512 image.

- To apply a mask to a single image, create the mask on an image of the same X,Y dimensions
- To apply a mask to every slice of a Z-Series, create the mask on a single image of the same X,Y dimensions that will be used in the Z-Series
- To make a 3D mask with different areas on different Z-levels, make the mask while in Playback of that Z-Series, using the same X, Y and Z dimensions as will be used when applying the mask (e.g. a mask to be used on a 10-slice Z-Series must be created on a 10-slice Z-Series).

For a single image:

1. Collect a single image using the same scan dimensions that will be used when applying the photoactivation mask (i.e. if planning to use an ROI during the experiment, use it here as well)
2. While the single image is in the Image window, click the  button on the left side of the Image window; this will bring up the photoactivation mask editor dialog.

3. Choose a laser palette from the list on the right side of the photoactivation mask editor dialog. The default palette is often set to “All lasers using current setting”, which will scan the drawn mask using the photoactivation laser at whatever power is set at the time the mask is used. Using the **Edit** button for each palette, the user can pre-set the laser power to be used for each mask; these settings will override the position of the laser power slider when the mask is used. Multiple palettes may be used in the same mask.
4. Click the **Brush**, **Add Ellipse**, **Add Rect.**, and **Add Polygon** buttons and draw masks in the image window. Additional buttons allow the user to select areas of the mask to clear, clear all parts of the mask, and invert the scanned and unscanned areas of the image. A slider bar allows the user to change the opacity of the drawn mask, enabling the image underneath to be seen more clearly; the opacity slider does not affect the photoactivation itself.
5. If multiple masks will be created during this Prairie View session, click the **Save Mask** button and name the current mask. This will save the mask in a temporary file, allowing it to be re-loaded (with the **Load Mask** button) later in the same session. To keep a mask to use in later Prairie View sessions, use the **Export** button to save it to a permanent file, from which it can be **Imported** later.
6. Close the photoactivation mask editor dialog

For 3D mask on a Z-Series:

Creating photoactivation masks for use in a Z-Series is similar to doing so for a single image, but with the added dimension of depth. Refer to the instructions above for creating masks for a single image; aspects unique to photoactivation in a Z-Series are described below.

1. Collect a Z-Series using the same scan dimensions that will be used when applying the photoactivation mask. Use the same number of slices that will be used when applying the mask. If an ROI will be used during the experiment, use it here as well. It is recommended that the user save the Z-Series definition.
2. Open the photoactivation mask editor dialog, choose palettes, and draw masks as described for single images. Each component of the mask will apply only to the slice on which it is drawn; the user can draw different masks on different slices. Use the copy buttons to propagate elements of the mask to other slices; **Copy to Previous** and **Copy to Next** add the mask element to an adjacent slice, while **Copy to Back** and **Copy Ahead** add the mask elements to all slices between the current slice and the start or end of the Z-Series.
3. The **Show Prev/Next** button allows the user to view the masks defined for the adjacent slices, in a different overlay color than that used for the mask defined on the current slice.
4. Save and/or export the mask as described for single image masks

5. Close the photoactivation mask editor dialog
6. Exit playback mode from the Z-Series on which the mask was being created

Using a Photoactivation Mask – Galvo Mode Acquisitions

Photoactivation Masks can be applied in a number of ways.

For a Single Image:

For a mask defined on a single image, the user can choose a mask from the **Photoactivation Mask** drop-down menu on the [Misc tab](#) of the Main Control Window. Only masks valid for the current scan settings (image size and dimensions) will appear in the list of saved masks. This mask will be applied to all subsequent Live Scans, Single Scans, Z-Series, and W-Series (but not T-Series). To stop using the mask, change the drop-down menu selection to None. The selection will automatically change to None if the user changes the image size to differ from that used to define the selected mask. The selection does not apply in SFC, Resonant, or AOD mode.

To apply a single-plane mask within a T-Series, add a Photoactivation or Image cycle to the T-Series and select the desired mask from the Photoactivation column drop-down menu. Only masks valid for the selected scan settings (image size and dimensions) will appear in the list of saved masks. Enter the number of reps and period desired, as well as any synchronization with other software modules.

For a Z-Series:

To apply a mask in a Z-Series, add a Z-Series cycle and select the desired mask from the Photoactivation column drop-down menu. Only masks valid for the current scan settings will appear in the list of saved masks. This includes masks defined on a Z-Series of the same X, Y and Z dimensions, as well as masks defined on a single image of the same X,Y dimensions (which would then be applied to all slices of the Z-Series). Enter the number of reps and period desired, as well as any synchronization with other software modules.

Using a Photoactivation Mask – SFC and Camera Mode Acquisitions

Photoactivation masks on SFC and Camera systems are used only within a T-Series. Implementation differs for single images and Z-Series. As mentioned previously, a mask must be created on images of the same dimension (number of slices, size of ROI) on which it will be used. Navigate to the T-Series tab to perform the photoactivation experiment. The T-Series containing the photoactivation can also contain other elements; for clarity, the examples below contain only the photoactivation cycles.

For a single image:

This use of a photoactivation mask is designed around the concept of a FRAP experiment. Two

lines of the T-series constitute a photoactivation cycle. The first line calls the photoactivation mask and is essentially a modifier of the second line, in which the images are collected.

1. Drag the **Photoactivation Time Per Pixel** slider to the desired dwell time for the photoactivation laser



2. Click **Clear All** to remove all cycles from previous T-Series
3. Click **Photoactivation** to add two lines to the T-Series
4. Configure the first line to call the photoactivation mask
 - a) **# Reps** is the number of times the mask will be rastered across the image
 - b) **Period** is the offset time between the start of the mask and the start of the imaging cycle called in the second line of the T-Series; positive numbers start the photoactivation before the imaging, while negative numbers delay the start of the photoactivation until after imaging has begun
 - c) Choose the desired photoactivation mask from the drop-down menu in the **Photoactivation** column
5. Configure the second line to collect images; at least one image must be collected for the photoactivation mask to be used
 - a) **# Reps** is the number of images that will be collected
 - b) **Period** is the time between the start of consecutive image repetitions; check the box under **Max Speed** to scan continuously
 - c) **Duration** is the total imaging time
6. Click the **Start T-Series** button to begin the photoactivation and acquisition

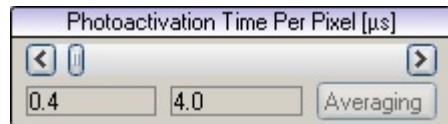
An example T-Series is shown below. Imaging will begin when the **Start T-Series** button is clicked, and will continue for 2 seconds. After 1 second of the imaging time has elapsed, the “Current” mask will be photoactivated one time.

	Cycle Type	# Reps	Period [s]	Max Speed	Duration [s]	Resource Selection	Photo-activation	BOT	External Trigger
	Async P	1	-1	---	0.12959	---	Current	---	---
▶	Images	40	0.05	<input checked="" type="checkbox"/>	2	---	Yes	<input type="checkbox"/>	No Trigger

For a Z-Series:

Photoactivation within a Z-Series takes place independent of imaging. Therefore, the T-Series need only have one line. No images are collected during the photoactivation. Images can be collected by adding additional lines to the T-Series or by collecting single images and/or Z-Series outside of the T-Series tab. Only the slices on which masks were defined will be scanned during the photoactivation; if masks are drawn on slices 4 and 6 of a 10-slice Z-Series, only those two slices will be scanned.

1. Drag the **Photoactivation Time Per Pixel** slider to the desired dwell time for the photoactivation laser



2. Click **Clear All** to remove all cycles from previous T-Series
3. Click **Z-Series** once to add one cycle to the T-Series
4. Configure the photoactivation Z-Series
 - a) **# Reps** is the number of times the photoactivation stack will be scanned
 - b) **Period** is the time between the start of consecutive repetitions
 - c) **Duration** is the total imaging time; move time between slices of the Z-Series is not included
 - d) Select your Z-Series definition from the drop-down menu in the **Z-Series** column
 - e) Select your photoactivation mask from the drop-down menu in the **Photoactivation** column
5. If desired, the user can collect images of the sample as part of the photoactivation T-Series by adding an additional **Z-Series** cycle and setting the number of repetitions, period, etc. This imaging cycle need not use the same Z-Series definition used to create and perform the photoactivation.
6. Click the **Start T-Series** button to begin the photoactivation

Additional Notes on Photoactivation

Photoactivation cycles can be embedded in T-Series with many other cycles. Thus, Parameter Sets, ROIs, Scripts, and other modifiers may change scan parameters before or after a photoactivation cycle. As always, scan dimensions (ROI, number of slices in a Z-Series) in place when a photoactivation mask is used must match those in place when the mask was originally created.

In some cases, the laser power applied during a photoactivation mask can result in the PMT signal becoming saturated. This is especially a problem with GaAsP detectors, which are more readily damaged than multi-alkali PMTs when exposed to bright light. Therefore, the user may opt to lower the PMT power while a mask is being applied. This can be done within a T-Series by including [Script](#) cycles before and after the mask is applied.

Mark Points

Mark Points is an application module designed to allow users to define point photoactivation protocols (uncaging, optogenetic stimulation). Mark Points can also be used to set up protocols for other types of photoactivation as well, such as photodamage/photoablation experiments.

Mark Points allows the user to define specific point locations that will be illuminated by a specific laser for a specific duration and intensity. The user also defines the time interval between individual points as well as groups of points.

The protocols defined by Mark Points can be synchronized with image acquisition (full frame, ROIs and line scans) as well as additional [Voltage Output](#) signals (that can be used to drive electrophysiology equipment or anything else which can be driven with an analog output), and [Voltage Recording](#) of analog signals from electrophysiology equipment or an device generating an appropriate signal.

[Mark Points Interface](#)

Mark Points Controller

X

Image Window Options

Show Points as Crosshairs

Point Color: Red

Show Point Indices

Left Click Action

Hold Shift to move all points, Ctrl to select individual points.

Select/Move Points

Add Grid of Points

Add Line of Points

Right Click Action

Redraw line along new path.

Add Individual Points

Points/Groups

Name	Indices	Type
Point 1	1	Point
Point 2	2	Point
Point 3	3	Point
Point 4	4	Point
Point 5	5	Point
Group 1	1-5	Group

Partially selected rows are highlighted in yellow, see image window for specific point selections.

Selected Points/Groups

▲
◀ ▶
▼
 Move Galvos
 Step (nm)

Mark Selected Point

These values will be defaulted in the grid below.

Laser	Initial Delay (ms)	Synchronize with Options Below
405nm Uncaging	0.30 <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Laser Power	Inter Point Delay (ms)	<input checked="" type="checkbox"/>
1000 <input type="button" value="-"/> <input type="button" value="+"/>	0.30 <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Duration (ms)	Interval (ms)	<input checked="" type="checkbox"/>
100.00 <input type="button" value="-"/> <input type="button" value="+"/>	100.30 <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Repetitions	1 <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Wait for Trigger	None <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Never <input type="button" value="-"/> <input type="button" value="+"/>	None <input type="button" value="-"/> <input type="button" value="+"/>	<input checked="" type="checkbox"/>
Check Box	Center Crosshair	<input type="button" value="Mark Point"/>

Uncaging Calibration

Default

Dr. Smith
2 Groups

Select Series to Load

Quick Load Mark Point Series

Point/Group	Indices	Initial Delay (ms)	Inter Point Delay (ms)	Interval (ms)	Duration (ms)	# Reps	Laser	Laser Power	Wait for Trigger	Trigger Selection	N =	Synchronization Frequency
Group 1 <input type="button" value="..."/>	1-5	100.00	200.00	1200.00	1000.00	1	405nm Uncag <input type="button" value="..."/>	1000	Never <input type="button" value="..."/>	---	---	Never <input type="button" value="..."/>

Add New Row Copy Selected Row Remove Selected Row Clear All Rows

Legend

- 2P Uncaging
- X Uncaging Galvo
- 405nm Uncaging
- Y Uncaging Galvo

Save Path MarkPoints-12172014-1141

Left click on the **Mark Points** on the Image window or select **Mark Points** from the **Electrophysiology** menu to launch the **Mark Points** interface.

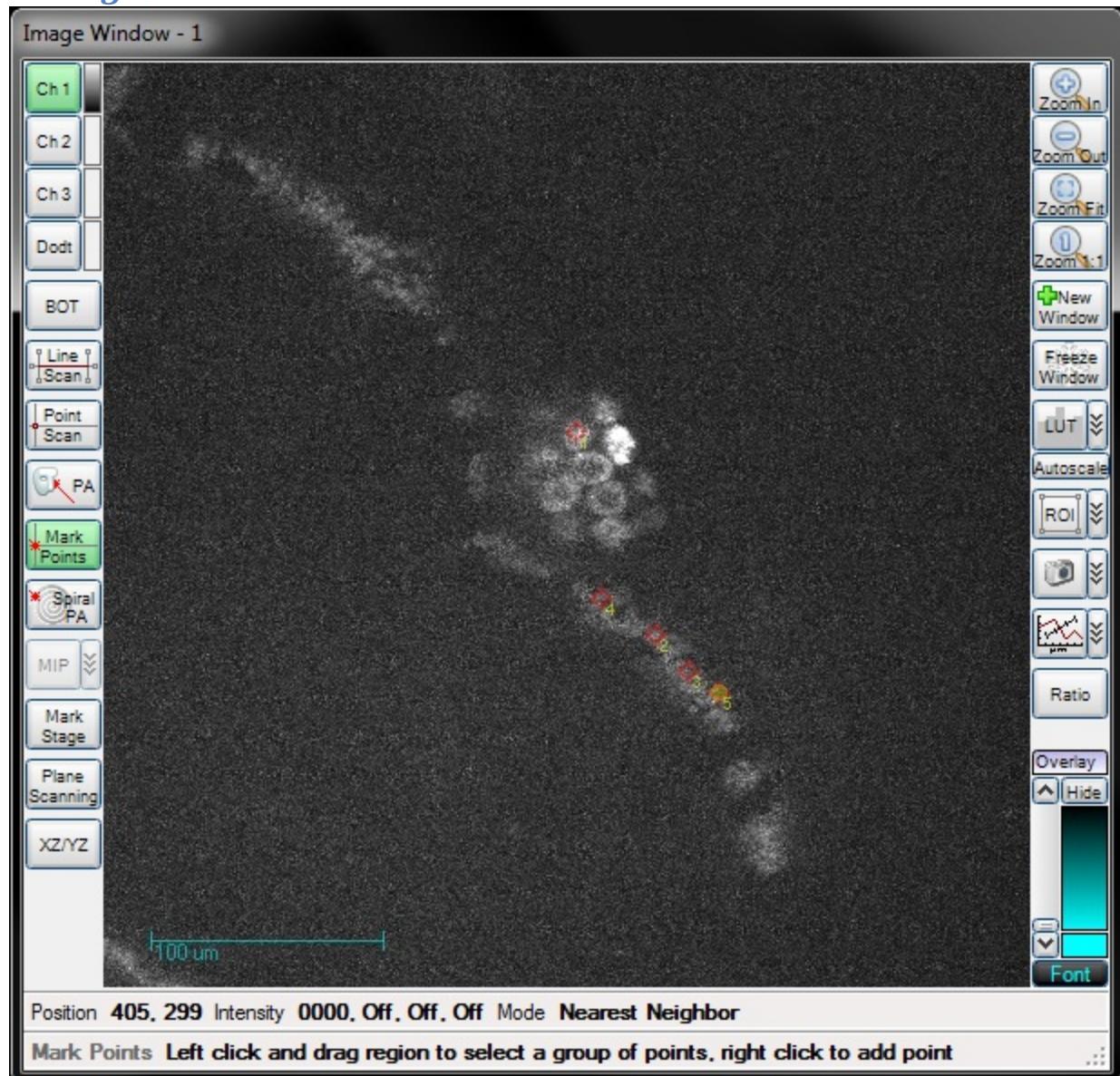
The **Mark Points** interface is divided into following sections:

- **Image Window Options** specify how image window overlays will appear (color and marker type) and how they can be interacted with (selecting points or adding lines or grids)
- **Points/Groups** shows all of the defined uncaging points and allows them to be worked

with in ways not possible on the image window, for example creating a group of points, changing the number of points along a line, defining a custom point order, or moving a point with sub-pixel accuracy

- **Mark Point at Crosshair** or **Mark Selected Point** (when crosshair is hidden) provides a place to set default values to be used when adding rows to a series in addition to providing a sandbox to test different combinations of laser, power and duration on a specific point before adding points to a series to be run together
- **Mark Point Series** defines the protocol to be run; groups, lines, and grids of points as well as individual points are added to the grid and the uncaging laser, intensity, pulse duration, delays, start trigger and any synchronization with additional analog output and/or inputs is specified for each row

Adding Points



After acquiring a reference image, the user clicks the **Mark Points** on the left side of the image window, or selects the **Mark Points** option from the **Electrophysiology** menu on the main form,

to open the Mark Points dialog.

Individual points (which can later be grouped) can be added by simply right clicking on the image. Adding lines or grids of points requires first selecting the appropriate **Left Click Action** in the **Image Window Options** section. Note that the image window can be zoomed to add/move points with greater accuracy. To move a point, left click in the point and drag to a new location. Points can be moved after a Mark Point experiment is run; this feature is useful in evaluating location based on response.

To add a line of points, select **Add Line of Points** in the **Image Window Options** under **Left Click Action**. To add a free hand line, click at the start of the line and drag the mouse along the desired path, and release the mouse button to stop drawing. The point density can then be set using the point density controls to the top right of the **Points/Groups** section. To draw a straight line, hold down the shift key while drawing the line. To move a line, click and drag any point on the line, with the exception of the last point which will instead rotate the line when clicked and dragged.

To add a grid of points, select **Add Grid of Points** in the **Image Window Options** under **Left Click Action**. Click and drag to draw a rectangle defining the outer limits of the grid. To move the grid click and drag any point that is part of the grid, also note that there are handles along the outside of the grid to resize and rotate the grid. To change the number of points in the grid the point density in both dimensions can be set using the point density controls to the top right of the **Points/Groups** section.

Groups of points can be created by selecting the points (line, grids and other groups will also work) which will become part of the group and then pressing the **Create Group** button on the right side of the **Points/Groups** section. There are multiple ways to select points, but one of the easiest is to select **Select/Move Points** in the **Image Window Options** under **Left Click Action** and simply drag a rectangle around the points which will become part of the group; it is also possible to hold down the Ctrl key while clicking a point to select or unselect a single point. Holding down the Ctrl key while dragging a region will toggle the selection of points within the region, and holding down the Shift key will add all of the points in the region to the selection. It is also possible to select, or deselect, points directly in the list of points in the **Points/Groups** section without using the image window.

Setting up an Experiment

Mark Point Experiments are defined in the **Mark Point Series** table. To add uncaging points to the experiment, first select the points (same process described above for [creating groups](#)) and click the **Add to Point Series** button in the **Points/Group** section. The selected points will now appear as separate rows in the point series table with the laser and timing settings defaulted from the **Mark Point at Crosshair/Mark Selected Point** section. To save time those default values can be set prior to adding points to the series.

Once the grid has been populated it is possible to make changes to the various columns:

- **Point/Group** indicates the uncaging point or points which will be illuminated
- **Indices** indicates the point indices which will be illuminated, these numbers match the numbers displayed on the image window overlay; this column is normally read-only, but is editable when the Custom Order option is selected in the **Point/Group** column (more information on that [below](#))
- **Initial Delay** is the amount of time to wait before illuminating the first point; note that the shortest possible delay is the galvo move time configured in the scan settings (contact a Bruker Fluorescence Microscopy representative before changing this value)
- **Inter Point Delay** is the amount of time to wait between points when more than one point is listed in the **Indices** column, or when there is more than one repetition in the **# Reps** column; this delay can also not be shorter than the galvo move time configured in the scan settings
- **Interval** is the total amount of time that elapses between illuminating points, or the sum of the **Inter Point Delay** and **Duration** columns; it provides another way to think about timing
- **Duration** is the amount of time to turn on the uncaging laser at each point; this column can be overridden by specifying the duration in the **Points/Groups** section (more information on this [below](#))
- **# Reps** is the number of times the points in the **Indices** column are illuminated
- **Laser** is the laser to be used to illuminate the points, different lasers can be selected for different lines, this column can be overridden by specifying the laser in the **Points/Groups** section (more information on this [below](#))
- **Laser Power** is the amount of laser power (same scale as slider on main form) to use when illuminating the points, this column can be overridden by specifying the laser power in the **Points/Groups** section (more information on this [below](#))
- **Wait for Trigger** specifies when a trigger will be required to continue illuminating points, these options are explained in more detail [below](#)
- **Trigger Selection** specifies which trigger will be used to start illuminating the points in the **Indices** column, note that the **Initial Delay** will occur after the trigger is received
- **N** = specifies the number of triggers to wait for when the Start After N Frame Triggers option is selected in the **Trigger Selection** column
- **Synchronization Frequency** specifies when to synchronize additional voltage outputs and/or inputs with the illumination of points
- **Voltage Recording Experiment** specifies which [Voltage Recording](#) experiment to run

while illuminating points

- **Voltage Output Experiment** specifies which [Voltage Output](#) experiment to run while illuminating points

To the right of the grid is a pair of buttons to move rows around within the grid and reorder portions of the experiment. Buttons below the grid allow rows to be added, removed, or copied. There are also buttons to save the current experiment or load a previously saved experiment. These saved experiments can also be selected in the **Quick Load Mark Point Series** section to quickly flip between experiments with a single click.

At the bottom of the Mark Points dialog there is a graph showing a preview of the signals which will be output when the experiment is run. There are buttons on the left side to focus on **Laser Power** or **Volts**. When viewing laser power all of the other signals will appear as dotted lines and only the laser drive signals will be emphasized. When viewing volts the galvo drive signals will be emphasized, along with any synchronized Voltage Outputs. Green regions will indicate an unknown delay for a trigger and red regions will indicate when a Voltage Recording is synchronized. This graph can be hidden or displayed by clicking the green bar underneath the **Mark Point Series** section.

[Running an Experiment](#)

Mark Point Series (or experiments) can be run in a number of ways, typically synchronized with imaging, in conjunction with electrophysiology recording, or both.

[From the Mark Points Dialog](#)

A Mark Point experiment can be run alone, or synchronized with a Voltage Recording, from the Mark Points dialog. This is typically done to verify that protocols are operating properly by using a uniform fluorescent sample to see which points are being hit. To run the current experiment, or series, simply click the **Run Mark Points** button at the bottom right corner of the Mark Points dialog.

To run a mark point experiment for a single point it is also possible to use the **Mark Point at Crosshair/Mark Selected Point** section and click the **Mark Point** button. If the **Hide Crosshair** option is unchecked this will use the location of the crosshair, but if the crosshair is hidden the location of the currently selected point will be used (if more than one point is selected the button will be greyed out).

[Synchronized with a T-Series](#)

A Mark Point experiment can be run during a T-Series between imaging sequences by pressing the **Mark Points** button to add a Mark Points cycle/row. This row will be executed sequentially and the next row will not start until the Mark Point experiment is finished.

If there are uncaging galvos present and configured it is possible to run a Mark Point experiment while imaging by filling in a few more columns for the imaging cycle/row.

Both of these options are described in more detail in the [T-Series](#) documentation.

Synchronized with a Line Scan If there are uncaging galvos present and configured it is possible to run a Mark Point experiment while acquiring line scan data by making a couple combo box selections. This is described in more detail in the [Line Scan](#) documentation.

Triggering Options

There are a number of choices when using the **Wait for Trigger** option, all of which will wait for a TTL signal before illuminating any (more) points:

- **Start with next scan (PFI0)** will start at the start of the next acquisition, it is possible (though discouraged for new users) to synchronize a mark points experiment across multiple acquisitions by having different sets of lines wait each for a separate start with next scan trigger; note that most raster based acquisitions start with a line retrace before pixel data is actually acquired
- **Start with external trigger (PFI1)** will start immediately after receiving an external trigger; this is the best way to synchronize with other equipment
- **Start after N frame triggers** will start after receiving N frame triggers, the number of frame triggers is specified nearby; note that it is possible to get into trouble when waiting for more frame triggers than are generated (i.e. waiting for 12 frame triggers when only 5 frames are collected). It is also possible to get into trouble using frame triggers more than once in the same experiment, or counting frame triggers in the middle of an experiment, it is possible in these cases that some frame triggers will be miscounted since Mark Point isn't counting triggers when it is running, and most acquisitions will generate extra frame triggers at the end. This option is not available without uncaging galvos present and configured, and requires [frame triggers](#) to be configured
- **Start with external trigger (Trig In)**, like PFI1, will start "immediately" after receiving an external trigger, but this is a software polled trigger and not a hardware trigger, so there is a delay on the order of 20us between receiving the trigger and acting upon it

Defining Pulse at the Point Level

By pressing the green button to the right of the **Points/Groups** section it is possible to define the laser pulse at the point/group level instead of at the experiment level. This makes it possible to use the same point, or group of points, multiple times in an experiment and always use the same laser, power, and duration. This is useful when the order of the points hit is more important than the how much laser power applied or how long that power is applied.

When this option is used additional columns are added to the **Points/Groups** section for laser, laser power and duration, and these columns become read-only elsewhere, or disappear entirely.

Summation Series

By clicking the dropdown button next to the **Add to Point Series** button in the **Points/Groups** section, or by right clicking either button, it is possible to access additional options for adding points to an experiment, most notably **Add to summation series (forward)** and **Add to summation series (reverse)**. Using an example with 5 points a forward summation series will create 5 rows in the Mark Point Series grid: one with point 1, one with points 1 and 2, one with points 1, 2 and 3, one with points 1-4 and one with points 1-5, each row hitting more points sequentially to compare the resulting response. The reverse summation series does the same thing, but instead of starting at the first point it starts at the last point and then proceeds with the last two points, last three points, etc., until it ends with points 5-1 on the last row.

Custom Point Order

In the **Point/Group** column in the Mark Point Series grid there are two Custom Order options which are used by the [summation series](#) feature, but can also be used on their own. The two options when selecting a custom order are to skip over missing indices, or to stop on the first missing index, either option enabled the Indices column so that a custom list of points can be specified. One option will just skip over any missing/invalid indices and proceed to the next index, and the other option will stop when encountering a missing/invalid index and proceed to the next row/repetition.

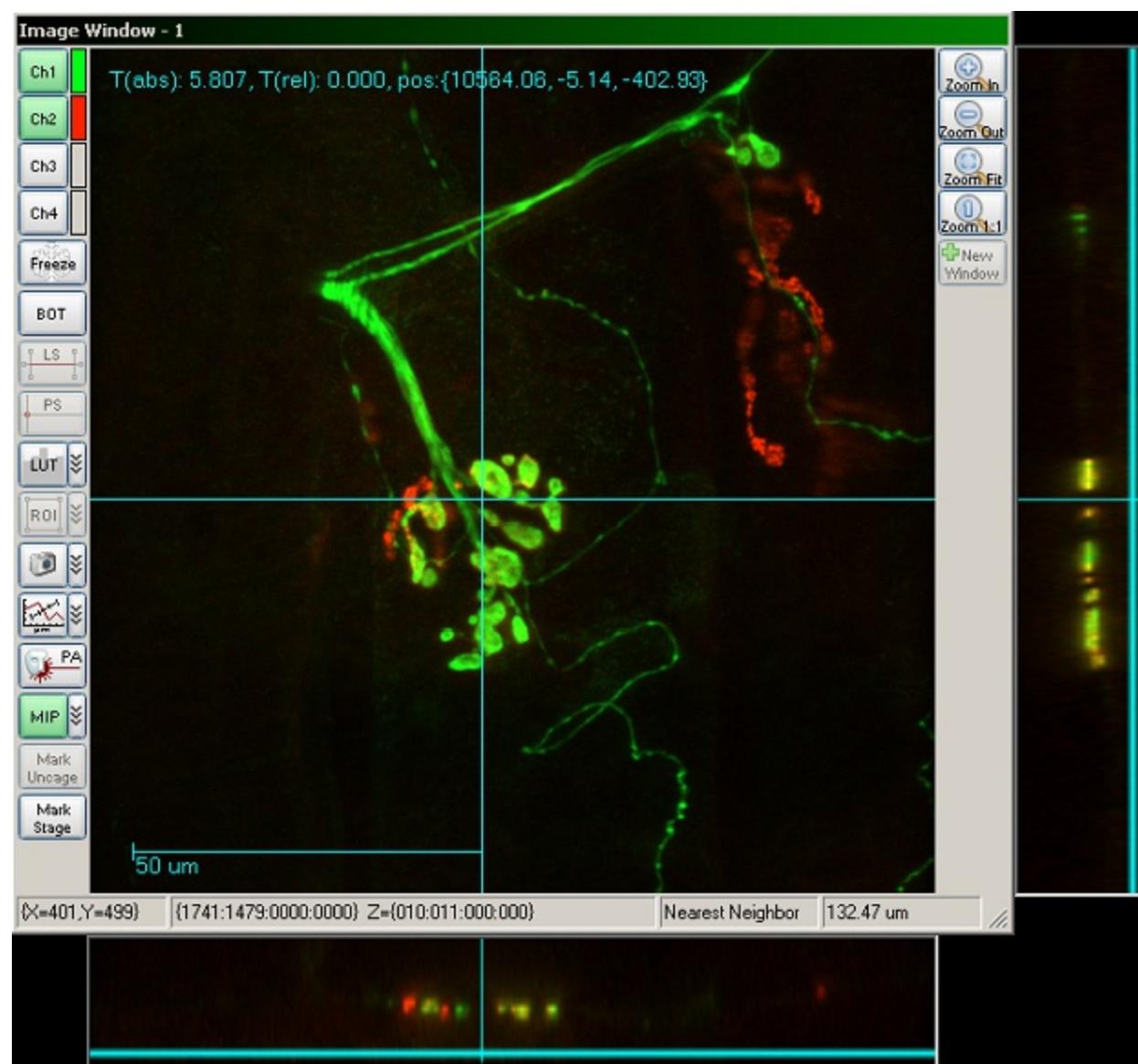
When [defining pulses at the point level](#) each index in the custom order will look to those values resulting in the potential for multiple lasers, powers and/or durations on the same line; the cells in these columns will display “Varies” in this case. This also makes it even easier to test different point orders without changing the pulse applied to each point, or to combine points which would otherwise have to be in separate rows or be contained in a group.

Maximum Intensity Projection (MIP)

This section of the Help file is under construction and has not been updated recently. The information below is from a previous version of the manual and may be somewhat out-dated. Please contact Bruker Fluorescence Microscopy support personnel with questions about these features.

While in Playback Mode (discussed in the [Playback Mode](#) section of this manual), the active image sequence can be shown as a Maximum Intensity Projection (MIP). The projection is a single image where each pixel value is the highest intensity pixel for that XY coordinate in all the planes of the Z-Series, or at all time points of a T-Series. To use MIP, a Z-series or T-Series must first be acquired. While in Playback mode, click the **MIP** button on the Image window. This places a cross hair cursor on the image and opens up a sidebar window to the right of and below the Image window, displaying the y-z and x-z projections, respectively. By moving the cursor to the x-y coordinate of interest, the user can observe changes in intensity in z. As the cursor is moved along the x-axis, intensity changes in the y-z plane are observed, while as the cursor is moved along the y-axis, intensity changes in the x-z plane are observed.

The user can see which Z-level each pixel in the XY projection came from by hovering the mouse cursor over that pixel and looking at the Z information at the bottom of the Image window. The intensity of that pixel in the MIP is shown for each of the four acquisition channels, and the slice number in which that pixel is the brightest is given in the “Z={...}” section.



MIP Menu

Options for display can be found by selecting the drop-down arrow to the right of MIP.

The user can choose to **Display XZ and YZ projections as 1:1**, which scales the projections based on the distance between consecutive slices of the Z-Series, or to **Display XZ and YZ as actual pixels**, which displays one pixel per slice of the Z-Series. The projections can be manually resized by clicking the edge of the window and dragging it to the desired size.

The **Thickness** option allows the user to choose a number of neighboring slices to be averaged in the XZ and YZ displays.

Color Depth applies a pseudocolor based on the slice in the projections.

The user can choose to display an **Average Projection** to show the average intensity for each pixel in the image sequence instead of a **Maximum Projection** showing the maximum intensity. **Cancel** allows the user to exit the MIP menu without making a selection.

Pre-Calculated MIP Tiff Generation And Playback

To enable creation of Maximum Intensity Projection Tiff files for smooth playback of

projections, enable the appropriate option in the [T-Series Preferences](#) dialog prior to conversion of raw data to Tiffs. Note average projection and color depth are not supported for fast playback of MIP images.

Mark Stage

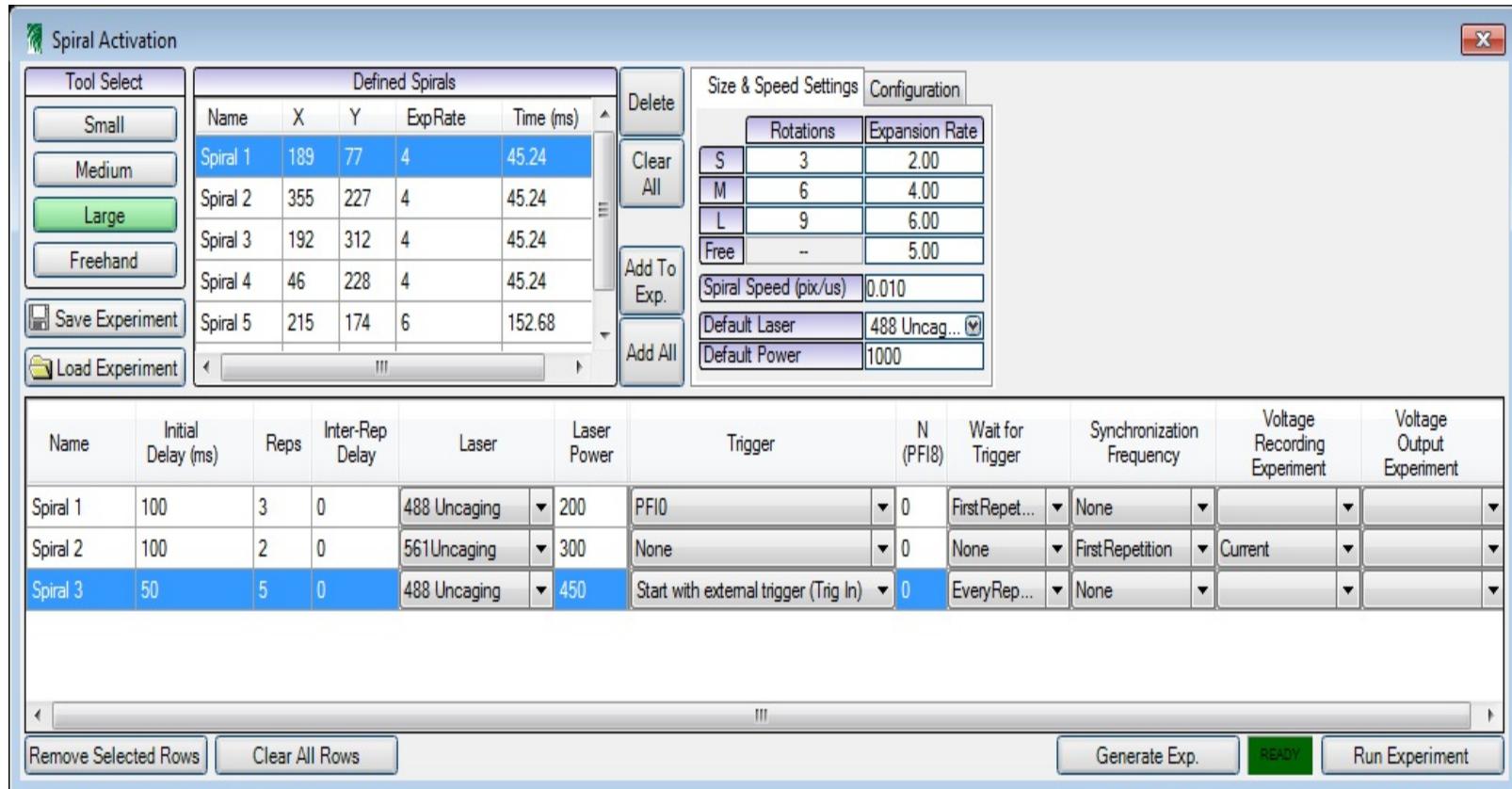
The **Mark Stage** button allows the user to dynamically move the stage and save stage locations while imaging. Click the **Mark Stage** button to activate it; the button will change from grey to green when it is active. While Mark Stage is active, the user can click in the Image window to move the sample and mark stage locations.

Clicking with the right mouse button will add the coordinate of the cursor to the list of saved locations in the [XY-Stage tab](#).

Clicking with the left mouse button will move the stage to put the clicked position in the center of the scan. This movement is dependent on selection of a properly calibrated objective, so that pixel size in the image can be accurately translated into the distance the stage moves in the x and y axes. If the stage moves in the wrong direction for one or both axes, change the checked/unchecked status of the **Invert X for ‘Mark Stage’** and/or **Invert Y for ‘Mark Stage’** options in the [XY-Stage tab](#) of the Prairie View main control window.

Spiral Activation

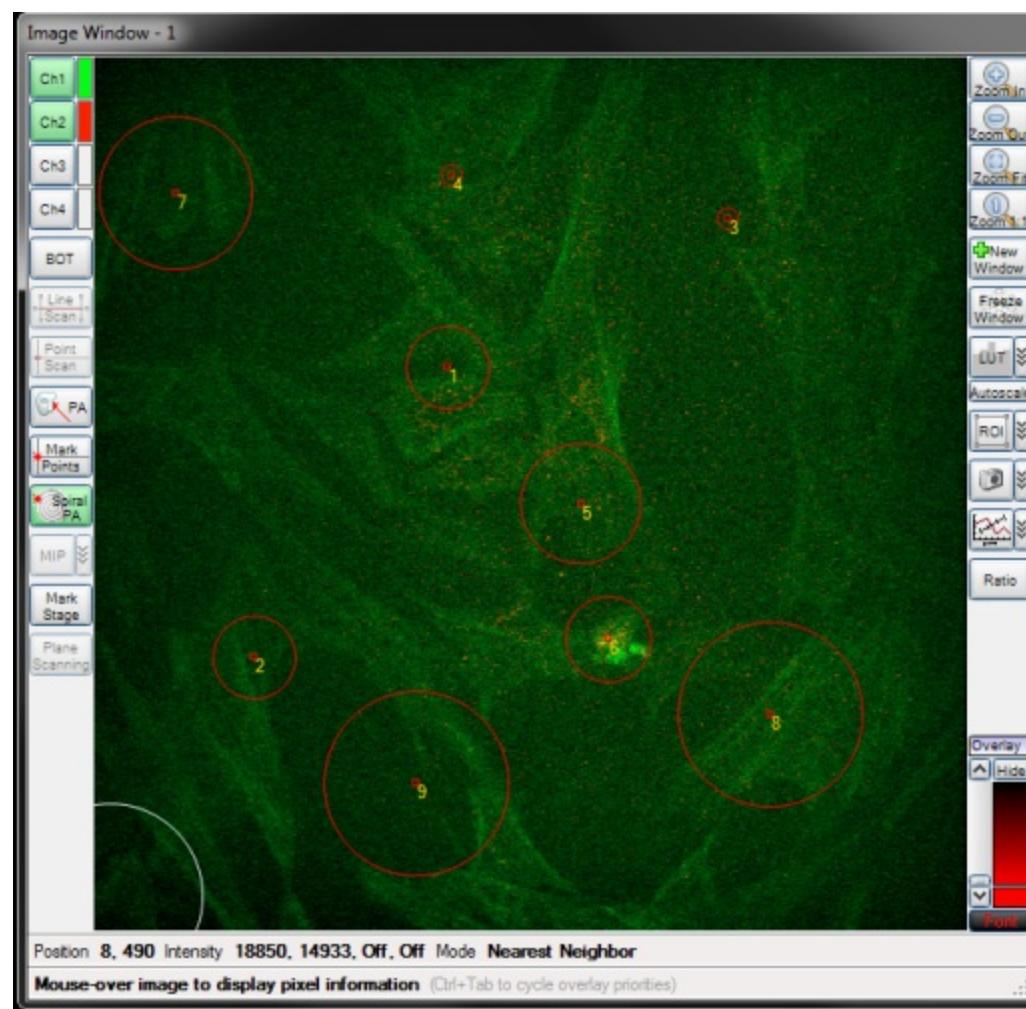
Spiral Activation is an application module designed to allow for high-speed photoactivation of circular areas. Spiral speed, size, location, and timing can be customized to achieve the desired illumination of a region. Spiral activation protocols can be run concurrently with imaging to visualize and record the effects. Spiral activation experiments may also be synchronized with Voltage Output and/or Voltage Recording experiments, often useful in electrophysiology setups to drive external hardware or record biologically-relevant signals.



1. To begin defining locations of and sizes of the spirals, first begin with the Tool Select.

Tool Select

Select the size of the desired spiral by selecting “Small”, “Medium”, “Large”, or the “Freehand” spiral sizes. The definition of each size can be found in the “Size and Speed Settings” tab in the upper right of the window. As the mouse cursor hovers over a pixel location on an image window, an overlay of the spiral area is displayed as long as a tool is selected. To define a spiral, left click on a pixel location in the image window to place a spiral centered at that location. With the freehand tool, click the desired spiral center and drag outward to the desired radius.



Defined Spirals Table

As spirals are placed on the image window, the defined spirals table is populated with the properties of each individual spiral.

Delete: Removes selected spiral(s) from the Defined Spirals table.

Clear All: Removes all spirals from the defined spirals table.

Add to Exp.: Adds selected spiral to the experiment table as a single line entry. If multiple spirals are selected with (Ctrl+Click) when this button is clicked, all selected spirals are added to the experiment table as a single line entry.

Add All: Adds all spirals to the experiment table with each spiral as its own line entry.

Size & Speed Settings

Rotations: The number of rotations in the spiral path for a given preset (small, medium, large). Changing this value affects the subsequent spirals placed, but does not affect previously defined spirals.

Expansion Rate: A value that describes the how fast the spiral path travels from the center to the edge of the spiral. The radius is a function of the expansion rate and the number of

rotations.

Spiral Speed: The speed at which the galvos trace the spiral path. A larger value corresponds to a faster travel through the spiral path, whereas a smaller value corresponds to a longer laser exposure time for the spiral.

Spiral Time in microseconds = $(\text{expansionRate} / (2\pi)) / 2 * (\text{Rotations} * 2*\pi)^2 / \text{Spiral Speed}$

Default Laser: The laser that is associated with the spiral(s) when added to the experiment table. The laser selection may be changed in the experiment table.

Default Power: The laser power that is associated with the spiral(s) when added to the experiment table. The laser power may be changed in the experiment table.

2. To create a Spiral Activation experiment, use Add to Exp or Add All to add defined spirals to the experiment table.

Experiment Table

Initial Delay: The amount of delay in milliseconds before the spiral(s) in this row begin. If this row is triggered, the initial delay is the amount of time after the trigger before the spiral begins.

Reps: The number of repetitions the spiral(s) in the row should be run.

Inter-Rep Delay: The amount of delay in milliseconds before the next repetition in the row should be run. If there is only a single repetition for the row, this value is ignored.

Laser: The laser used to photoactivate the spiral region(s) in this row. The dropdown menu lists all the available lasers configured on the uncaging light path for the system.

Laser Power: The amount of laser power to use for the photoactivation in this row.

Trigger: Source of the trigger for which the spiral(s) on the row wait.

PFI 0 is an internal trigger that occurs on the next scan.

PFI 1 and Trig In signals are external TTL trigger inputs found on the GPIO box

PFI 8 is a countable trigger that is often connected to the frame trigger signal.

If the Wait for Trigger option is chosen to be none for the row, the trigger source is ignored.

N(PFI 8): If the trigger source is chosen to be PFI 8, N refers to the trigger number on which the spiral(s) should begin.

Wait for Trigger: Options to choose how frequently to wait for the trigger.

First Repetition causes the all repetitions to run after receiving the appropriate trigger. Only the first repetition of the row waits for the trigger; all subsequent repetitions do not wait.

Every Repetition causes each repetition on the row to wait for a trigger. For example if there

are 4 repetitions defined for the row, triggered on every repetition, 4 triggers would be required to completely run the row.

Every Spiral causes each spiral on the row to require the appropriate trigger. This is most applicable if there is more than one spiral defined on the row. If there is only a single spiral defined on the row, this option behaves like the “Every Repetition” option.

Synchronization Frequency: Options to choose how frequently to synchronize the voltage output or voltage recording experiment.

First Repetition causes the first repetition to be synchronized with the voltage output and/or voltage recording experiment. If voltage output/recording experiment duration is longer than the duration of the photoactivation for the row, the system waits for the completion of the voltage output/recording before continuing to the next row.

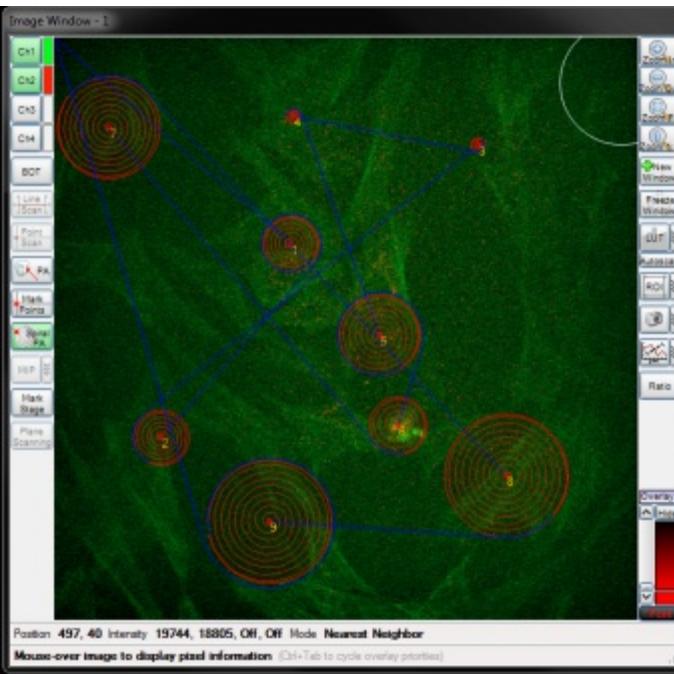
Every Repetition causes each repetition in the row to be synchronized with the voltage output/recording experiment. If the voltage output/recording experiment duration is longer than the duration of the repetition, the system waits for the completion of the voltage output/recording before continuing to the next repetition on the row.

Every Spiral causes each spiral on the row to be synchronized with the voltage output/recording experiment. This is most applicable if there is more than one spiral defined on the row. If there is only a single spiral defined on the row, this option behaves like the “Every Repetition” option. If the voltage output/recording experiment duration is longer than the duration of the spiral, the system waits for the completion of the voltage output/recording before continuing to the next spiral on the row.

Voltage Recording Experiment: Choose the current or saved voltage recording experiment to synchronize with the row. This option is ignored if the Synchronization Frequency option is chosen to be none.

Voltage Output Experiment: Choose the current or saved voltage recording experiment to synchronize with the row. This option is ignored if the Synchronization Frequency option is chosen to be none.

3. Generate and Run Experiment



Spiral Activation

Defined Spirals				Size & Speed Settings		Configuration	
Name	X	Y	ExpRate	Time (ms)			
Spiral 1	206	181	4	45.24	<input type="button" value="Delete"/>	<input type="button" value="Clear All"/>	<input type="button" value="Configuration"/>
Spiral 2	93.2	351	4	45.24	<input type="button" value="Add To Exp"/>	<input type="button" value="Add All"/>	<input type="button" value="Size & Speed"/>
Spiral 3	371	94.1	2	5.65	<input type="button" value="Save Experiment"/>	<input type="button" value="Spiral Speed (px/us)"/>	<input type="button" value="Default Laser"/>
Spiral 4	209	68.6	2	5.65	<input type="button" value="Load Experiment"/>	<input type="button" value="Default Power"/>	<input type="button" value="Default Power"/>
Spiral 5	285	261	5	76.97	<input type="button" value="4"/>	<input type="button" value="Run Experiment"/>	<input type="button" value="Run"/>

Tool Select: Small, Medium, Large, Freehand

Size & Speed Settings:

Rotations	Expansion Rate	
S	3	2.00
M	6	4.00
L	9	6.00
Free	-	5.00

Spiral Speed (px/us): 0.010

Default Laser: None

Default Power: 1000

Initial Delay (ms) Reps Inter-Rep Delay Laser Laser Power Trigger N (FFI#) Wait for Trigger

Spiral 1: 0	1	0	None	1000	None	0	None
Spiral 2: 0	1	0	None	1000	None	0	None
Spiral 3: 0	1	0	None	1000	None	0	None
Spiral 4: 0	1	0	None	1000	None	0	None
Spiral 5: 0	1	0	None	1000	None	0	None

Buttons: Remove Selected Rows, Clear All Rows, Generate Exp, Abort, Run Experiment

Generate Exp.

Generates and prepares the experiment described in the experiment table. If the spiral experiment table changes, generate will have to be clicked before running.

Run Experiment

Runs the generated spiral experiment. During the experiment the button becomes an abort button. If the abort button does not become the run button, it can be indicative of the system continuing to wait for a trigger.

Save and Load Experiment

Saving and loading an experiment allow the user to record and restore the defined spirals, spiral settings and the experiment table.

Configuration Tab: Advanced settings

Update Rate

The update rate of the galvo drive waveforms in Hz. Default is 100,000 Hz.

Fast Inter-Spiral Travel

If enabled, fast inter spiral travel causes the galvo to jump to the next spiral instead of traveling at the spiral speed to the next spiral. By default, this mode is disabled.

Downsample Spiral Graphics

If enabled, only a downsampled path of the spiral travel is plotted on the image window, which requires less processing. By default, this mode is enabled.

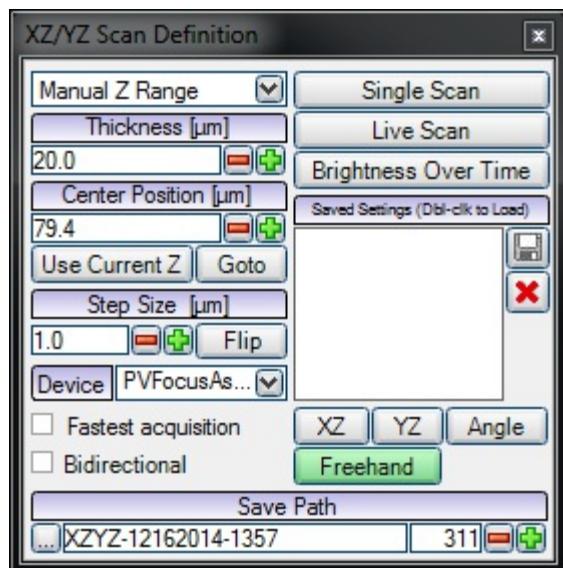
Plane Scanning

This section of the Help file is under construction. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

XZ/YZ Scanning

The XZ/YZ Scanning feature enables the user to acquire a vertical plane of data through the sample.

Note that use of this feature is supported by a sub-set of Z devices and requires additional connections to be made between the Z device(s) and the GPIO box. Supported Z devices include MAMC-driven Z motors and Bruker Fluorescence Microscopy Piezos. Contact Bruker Fluorescence Microscopy support personnel with questions about this feature.



There are three general steps to defining an XZ/YZ Scan:

1. Collect a frame scan in Galvo mode
2. Define the line in XY along which the scan will occur
3. Define the Z range and step size at which each XY scan will be performed

Defining an XZ/YZ Scan

Collect a frame scan using the controls on the Prairie View main control window.

Click the **XZ/YZ** button on the Image window to open the XZ/YZ Scan Definition dialog.

Choose the type of line to be drawn in the XY plane

- **XZ** defines a straight horizontal line, the position of which can be adjusted in the Image window by clicking and dragging the box in the middle of the line
- **YZ** defines a straight vertical line, the position of which can be adjusted in the Image

window by clicking and dragging the box in the middle of the line

- **Angle** defines a diagonal line, the position and angle of which can be adjusted in the Image window by clicking and dragging the boxes at the endpoints of the line
- **Freehand** defines a user-defined arbitrary line, which can be drawn on the Image window; click and drag the boxes at the endpoints of an existing line to extend the line

Device allows the user to select the Z device to use for the scan, if multiple Z devices are configured.

The Z range of the acquisition can be defined in one of three ways, using the drop-down menu at the top of the XZ/YZ Scan Definition window

- **Auto Z Range** sets the Z parameters to approximately fill the height of the Image window. After selecting Auto Z Range, the user can choose the Nyquist sampling option from the Tools menu to calculate the theoretical step size needed for Nyquist sampling.
- **Manual Z Range** enables the user to manually define the Thickness, Center Position, and Step Size of the scan.
- **Use ZSeries Range** imports the Thickness, Center Position, and Step Size from the current settings in the Z-Series tab in the main control window.

Based on the method selected for calculating the Z range, additional parameters may be defined by the user

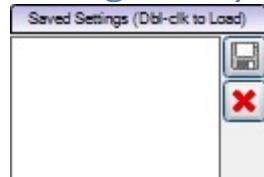
- **Thickness** is the height or depth of the scan in the Z direction (in microns)
- **Center Position** is the absolute position at which the scan is centered, for the selected Z device
 - **Use Current Z** changes the Center Position to be the current position for the active Z device
 - **Goto** moves the Z device to the position displayed in the Center Position field
- **Step Size** defines the Z distance between each line (in microns); the sign of this value determines the direction of the scan (top to bottom or bottom to top)
- **Flip** changes the sign of the Step Size to scan in the opposite Z direction, inverting the image

Fastest Acquisition induces a Bruker Fluorescence Microscopy Piezo Z device to operate in its calibrated mode. The acquisition itself is much faster than an acquisition that is not Fastest

Acquisition, though time is required for a new calibration to be performed whenever scan settings change.

Bidirectional collects consecutive scans in alternating Z directions, eliminating the need for the Z device to return to the start location between scans.

Saving an XZ/YZ Scan Definition



Multiple XZ/YZ definitions can be set up and saved for use during a Prairie View session; definitions are not preserved when Prairie View is closed. To save the current XZ/YZ definition, click the button. Give a custom name to the definition by clicking once on the name of the definition and typing a new name. To delete a definition from the list, select it and click the button. Saved definitions can be recalled to run as individual scans in this window by double-clicking the name of the definition. Saved definitions can also be referenced within a T-Series.

Running an XY/YZ Scan

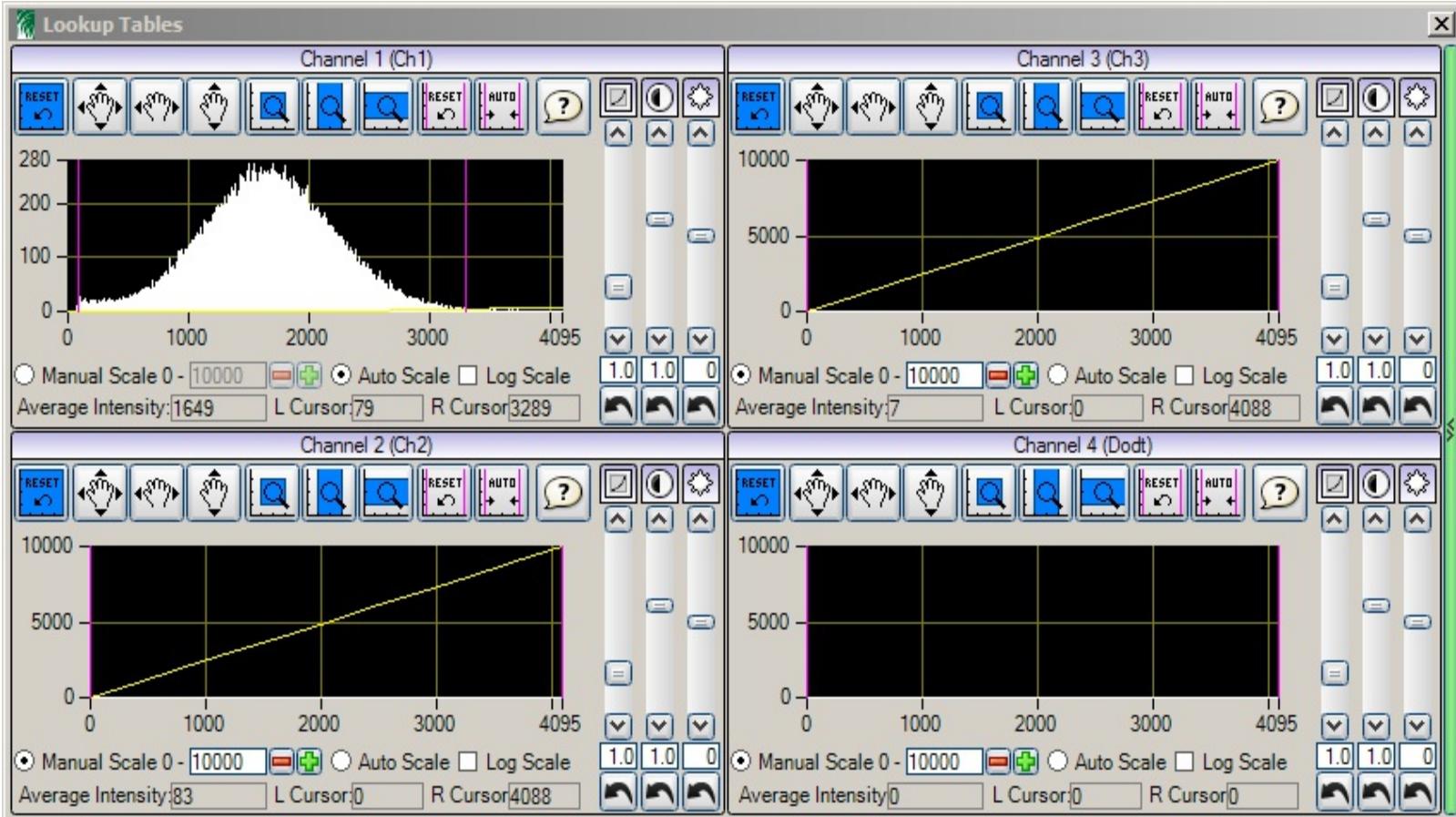
Three different acquisition types can be performed from inside the XZ/YZ Scan Definition window.

- **Single Scan** performs the defined XZ/YZ scan one time. The acquisition is saved in the folder defined by the Save Path on the main form. The directory name follows the following format:
XXYZ-[date]-[time Prairie View session started]-[iteration].
- **Live Scan** performs the current XZ/YZ scan continuously, until the user stops the acquisition with the Abort button. The last frame of the acquisition is saved in the folder defined by the Save Path on the main form. The directory name follows the following format:
XXYZ-[date]-[time Prairie View session started]-[iteration].
- **Brightness Over Time** can be performed if BOT regions have been defined on the previous XZ/YZ scan. Acquisition continues until the user clicks the Abort button. The acquisition is saved in the folder defined by the Save Path on the main form. The directory name follows the following format:
XXYZ-[date]-[time Prairie View session started]-[iteration].

Alternatively, the XZ/YZ scan can be embedded in a [T-Series](#). Use the XZ/YZ Image button to add the cycle, and select the current or saved scan definition from the Resource Selection column.

Look Up Tables

A Look Up Table (LUT) is the function that is used to color the display of the data to be displayed on the computer screen. Clicking the **LUT** button brings up the Lookup Tables window. If only 2 channels are visible in this window, click the vertical green bar on the right edge of the window to reveal the other two channels.



Images are digitized to the bit depth of the acquisition hardware. Images acquired on most systems are digitized as 12-bit data (0 for no signal up to 4095 for saturated signal). Systems with a Resonant Scanner acquire images as 13-bit data (0 to 8191). On a system with a Resonant Scanner, the user may choose to digitize data on a 12-bit scale to match that of systems without a Resonant Scanner; this option is found in the Preferences menu. For an SFC or Camera image, this is based on the bit depth of the camera; for example, data from a 16-bit camera will result in intensity values from 0 (no signal) to 65535 (saturated signal).

In a grayscale (black and white) LUT, values of 0 are usually represented as pure black and saturated values are usually represented as pure white. Since the display has only 256 grey levels, a function or LUT is used to define the display intensity scale. If these 256 display grey levels are used to display the full range of a 12-bit image with 4096 intensity levels, then each display grey level is equal to 16 image data intensity levels.

In the LUT graphs, the pixel intensities are displayed as a histogram, where the horizontal axis is the intensity level and the vertical axis is the number of pixels. Buttons above the graph allow the user to zoom in on sections of the histogram and pan around once zoomed in.

Controls below the graph allow the user to manually set the scale of the vertical axis or **Auto Scale** the axis. **Log Scale** allows meaningful information about the intensity scale to be viewed for images with a large dynamic range.

The pink vertical lines on the graph show the position of the minimum and maximum values of the Look Up Table; the L Cursor and R Cursor fields below the graph display the positions of these lines. The yellow line shows the shape of the function.

Since fluorescent images very often contain a large number of dark pixels, it is sometimes desirable to adjust the range of the LUT to exclude some of the dark pixels, thereby allowing the LUT to display smaller changes in the data intensity scale.

The user can drag the pink lines to adjust the high and low ranges of the LUT. The shape of the function (yellow line) can be adjusted with the sliders to the right of the graph, which control gamma, contrast, and brightness. The  button below each slider will reset that slider to the default value. These adjustments are specific to the channel for which they are made.

The **Reset**  button moves the LUT cutoffs back their minimum and maximum values.

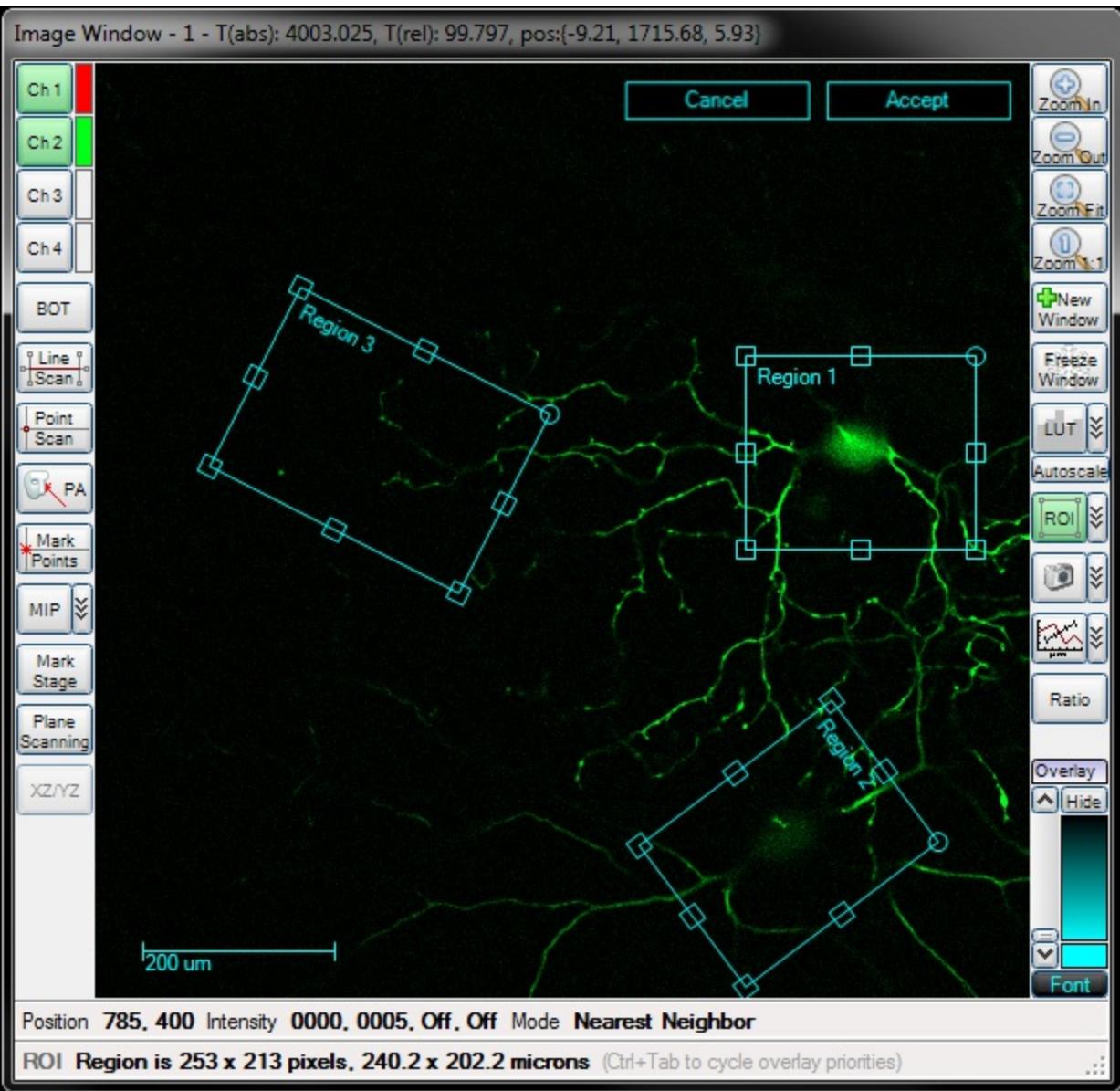
The **Auto**  button adjusts the LUT to maximize the contrast of the current image in the display. This is not a continuous adjustment, but rather an instantaneous one using the current frame's channel data. To continuously adjust the lookup tables click the **Autoscale** button below the LUT button on the image window.

Right-clicking on the **LUT** button itself brings up a menu from which the user can select to reset or automatically adjust the Look Up Table without opening the LUT window.

Adjustments to the LUT only affect the display of the image; the actual intensity values collected by the acquisition hardware do not change. Therefore, the LUT values in place during acquisition will not be saved as part of the data set or applied when opening the image again later.

LUT settings are automatically reset by default between sessions of Prairie View. However, the user can enable the Save/Recall LUT settings option in the Preferences menu. With this option enabled, the software will retain the LookUp Table settings in place when Prairie View is exited, to be recalled the next time Prairie View is started.

Regions of Interest (ROIs)



It is often useful to scan only a selected region of the field of view to optimize image speed. Using the **Region of Interest (ROI)** tool, it is possible to define a small portion of the sample to be imaged, which increases the scan rate. When this button is pressed, the cursor is used to define a rectangular area in which to limit scanning.

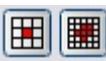
To exit out of the ROI and begin scanning the entire frame, click **ROI** again.

On point scanning systems, limiting the scan area increases the frame rate of the acquisition and limits laser exposure to the area of the sample within the ROI.

In SFC mode, limiting the scan area provides two advantages. First, it allows more sweeps of the selected area during the exposure time of the camera, which may allow the user to collect adequate signal intensity at lower exposure times. Second, by collecting data from fewer pixels of the camera, the camera can transfer and read out the frames more quickly. Due to camera transfer and readout geometry, this latter advantage often has more effect for ROIs near the top of the image than the bottom.

To define an ROI, select **ROI** and place the cursor at one corner of the area of interest. Click and drag the mouse, creating a rectangular ROI. The Image Window will automatically resize itself. Press **Live Scan** or **Single Scan** to refresh the display to reflect the ROI. The scan will be restricted to the area defined by the ROI.

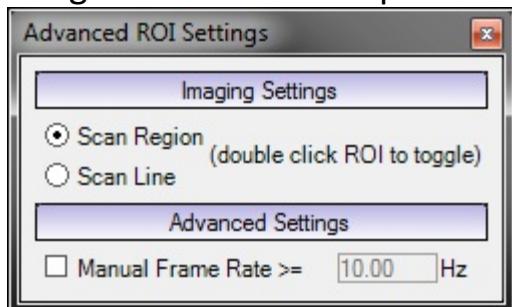
For point scanning systems, the pixel size used within the ROI will be the same as that of the image on which the ROI was drawn. To change the pixel size within the ROI, use the Increase/Decrease Pixel Size buttons in the Image Size section of the Main Control Window:



By right-clicking the **ROI** button or clicking on the drop-down button to the right of the **ROI** button, the user can bring up a context menu. The contents of this menu vary based on whether the **ROI** button is currently active.

- **Enter/Exit ROI** functions the same as clicking the **ROI** button – it allows a new ROI to be defined or closes the current ROI
- When ROI is not active, **Edit New ROIs** allows the user to draw a new ROI and edit it before accepting the new ROI
- When ROI is not active, **Edit Existing ROIs** allows the user to view and resize existing ROIs and draw new ROIs which can also be edited
- **Load** displays a list of saved ROIs which can be loaded by clicking on the desired region
- Selecting **Save** saves the current ROI as a new ROI or overwrites an existing saved ROI. Saved ROIs are not retained after Prairie View is closed. By default, ROI names include the pixel location of the upper left corner of the ROI and the dimension (in pixels) of the ROI. These values will change if the user changes the Image Size. To save a custom name for the ROI, enter the ROI and choose Save>New from the ROI menu; then type a name for the ROI and press Enter or click the green check mark.
- **Delete** displays a list of saved ROIs which can be deleted by clicking on the desired region
- **Delete All** deletes all saved ROIs
- **Import from File** imports a set of saved ROIs from a file
- **Export to File** exports the current saved ROIs to a file to be imported and reused at a later time. This allows the user to keep ROI definitions for a later Prairie View session
- **Save Reference Image** saves the Image window contents with an overlay of the saved ROIs marked on it
- Clicking to enable **Auto Save** places a check mark next to the menu option and causes all subsequently defined ROIs to be saved to the ROI list, to be loaded later in the same Prairie View session; click again to disable the option

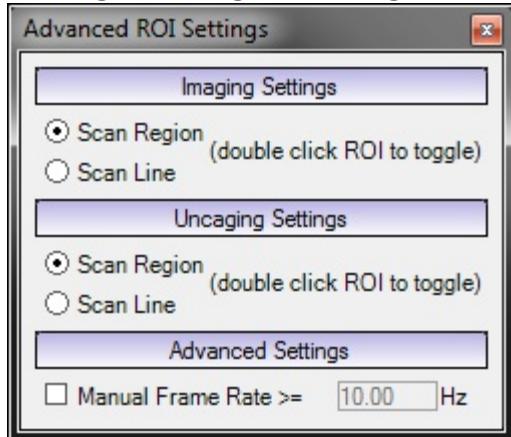
- Clicking to enable **Auto Edit** places a check mark next to the menu option and causes the ROI editor to open whenever a new ROI is drawn; click again to disable the option.
 - When Auto Edit is disabled, the system will immediately enter the newly drawn ROI.
 - When Auto Edit is enabled, the ROI editor allows the user to adjust ROIs before their definitions are finalized. Multiple ROIs can be drawn on the same image while the editor is open. Click the Accept button in the upper right corner of the image to accept the definition(s), or the Cancel button to exit the ROI editor without saving the defined ROI(s).
 - Click inside an ROI and drag to move it to a new location.
 - Click and drag square handles on the sides and corners of an ROI to change its size.
 - Rotate the ROI by dragging the circle indicator on the corner of the ROI. Delete the ROI by clicking the X near the corner of the definition.
 - To create multiple ROIs of the same size, click the right mouse button inside an ROI to clone the ROI, then click the left mouse button and drag the clone to a new location.
- Clicking to enable **Show Advanced Settings** places a check mark next to the menu option. When an ROI is drawn, the Advanced ROI Settings dialog will open next to the image window. This option does not apply to SFC mode.



- From this dialog, the user can choose from two options for scanning within the ROI
 - Scan Region will scan the ROI as defined
 - Scan Line will scan only one horizontal line across the center of the ROI. This single line will be scanned multiple times; the number of times is defined by the number of lines in the original ROI definition.
- The Manual Frame Rate option only makes sense when used in conjunction with the [Dual ROI](#) feature, otherwise it provides similar functionality to the period column in the [T-Series](#) grid. When using the Dual ROI option, where the imaging galvos are rastering a line while the uncaging galvos are simultaneously rastering a region, it is possible to continuously acquire line scan data while periodically

photoactivating a different region.

If the ROI is a [Dual ROI](#) then the dialog looks slightly different and includes separate settings for region being rastered by the uncaging galvos.



- **Use Z(focus) Values** saves the positions of all Z devices along with the ROI, so that different ROIs can be collected at different Z-levels. When loading the ROI, the Z device(s) will move to the position(s) stored with the ROI definition. In addition, ROIs can be drawn on various slices of a Z-Series while in Playback mode; the Z position of the slice will be saved as part of the ROI definition.

Note that this feature should NOT be used when a Z-Series will be performed within the ROI. The Z device positions defined in the Z-Series will over-ride the Z device position associated with the ROI. In these cases, rely on the top and bottom positions for the Z-Series to move the Z device(s) when entering or switching ROIs.

- **Cancel** closes the ROI context menu without loading or defining an ROI

On point scanning systems, an ROI must be at least 8x8 pixels, and the X dimension must be a number of pixels divisible by 4. These limits are automatically imposed by the software while drawing an ROI. Additional limits may exist for camera-based systems.

Saved ROIs can be called from within a T-Series by adding an ROI cycle and selecting the desired ROI from the **Resource Selection** drop-down menu. The ROI will be applied to all subsequent cycles of the T-Series, until another ROI (or “No ROI”) is selected in another ROI cycle.

Dual ROI Scanning

This feature is only available on systems with two sets of galvanometers (imaging and uncaging), additional analog outputs to drive the second set of galvos with the acquisition, and additional switching hardware to share control of the uncaging hardware; if those requirements are met, and the feature is configured, there will be a new option when right clicking the **ROI** button called **Enter Dual ROI**.

Defining a Dual ROI works a lot like defining a normal ROI, except after drawing the region two rectangles appear instead of one. One rectangle defines where the imaging galvos will raster and looks much like any other ROI region overly, and the other rectangle labeled **Uncaging** indicates where the uncaging galvos will raster. The two rectangles appear in the same location by default so one must be moved by clicking and dragging in order to see both regions clearly. The imaging region can be moved, resized and rotated like any other ROI, but the uncaging region can only be moved and scaled relative to the imaging region. Once the regions are in place accepting the ROI will cause future acquisitions to raster both sets of galvos while acquiring image data.

This feature has many potential uses, the most obvious of which is to scan two areas of the sample simultaneously, with significantly different emission wavelengths, and use a specific set of filters to acquire the data from the two regions as two different channels.

A more obscure use of the feature is to flip the imaging region to raster a line instead of a region and use the uncaging region to simultaneously photoactivate a different region. In this case the only interesting data is from the imaging region and the uncaging region is just used as a stimulus. The frequency of the photoactivation can be adjusted by adjusting the **Manual Frame Rate** on the **Advanced Settings** dialog described above.

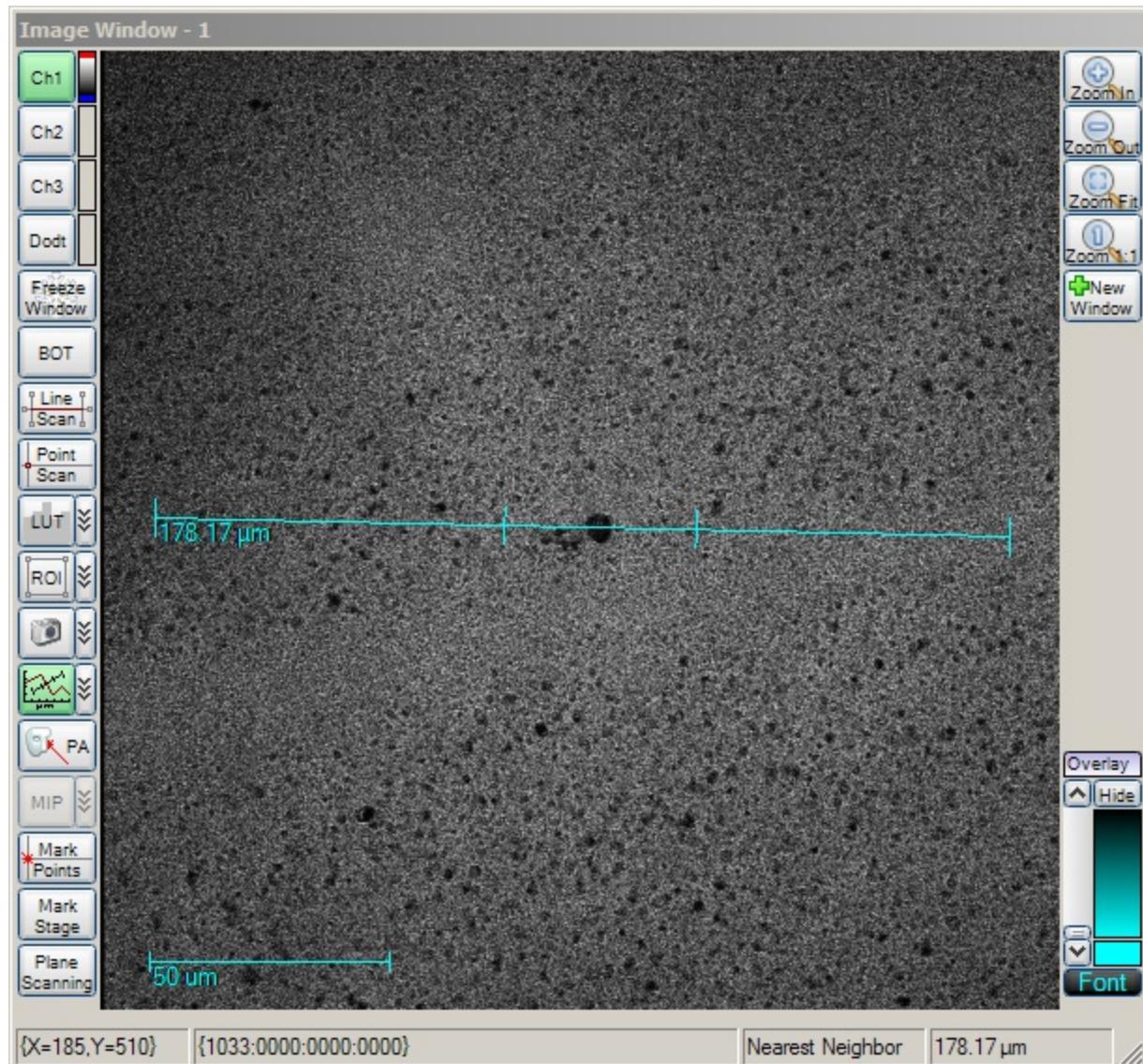


The snap button is used to save the current image displayed in an Image Window. The image will be saved to the filename and path specified in the Prairie View main form, and the incremental image counter on this tab will be increased by one to prevent over-writing data.

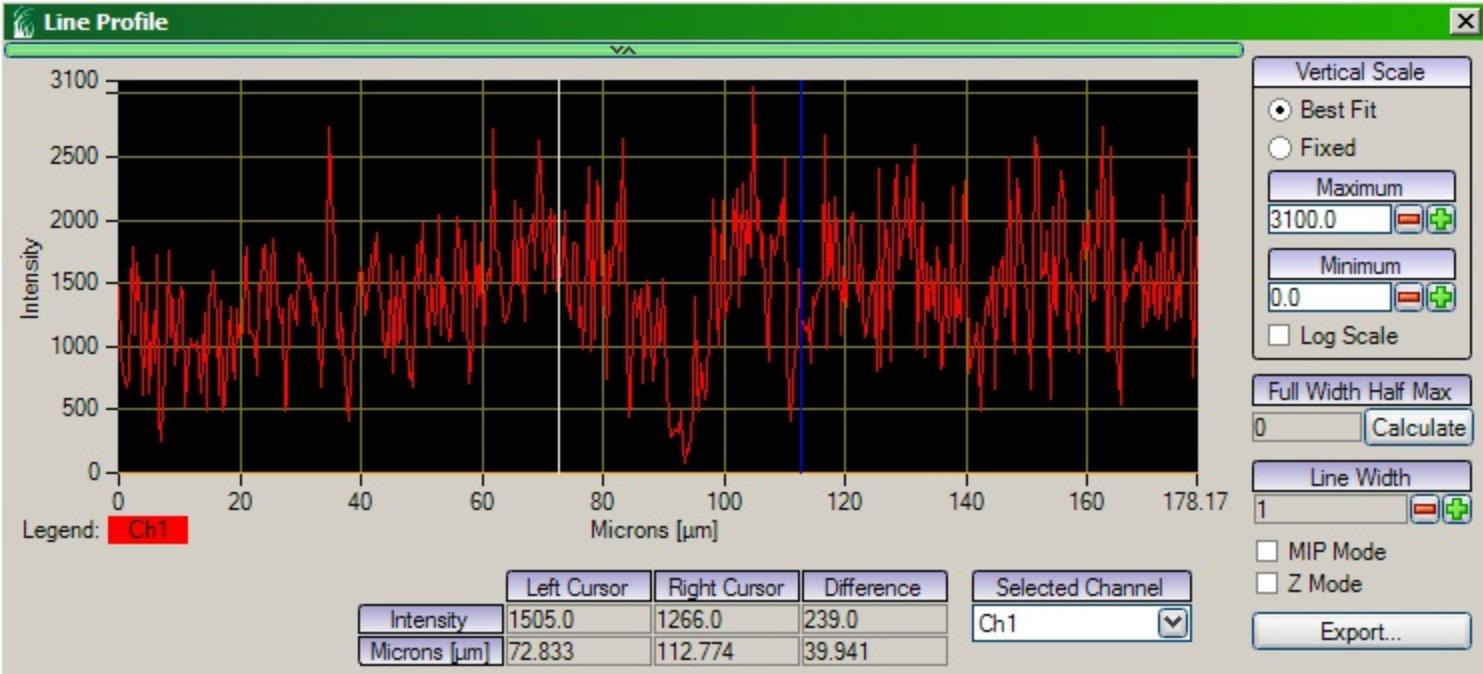
The displayed image will be saved as if it were a single image acquisition (a metadata file). If any overlays are present on the image, the exact displayed view (including display zoom) and overlays will be saved as an 8-bit TIFF.

Line Profile

The line profile tool  displays a plot of intensity along a user-defined line on each of the active channels. When the button is clicked, a line appears on the image window and the Line Profile dialog opens. By dragging the ends of the line in the image window, it can be positioned across the area of interest. Two additional markers along the line correspond to the white and blue vertical cursor marks on the line profile graph.



Spatial and intensity information for the two vertical cursors in the Line Profile dialog (corresponding to the inner markers on the line in the Image window) is displayed in a table below the intensity plot. For images acquired on multiple channels, the user can determine which channel to use for intensity information by choosing that channel from the **Selected Channel** drop-down menu.



By moving the white and blue lines in the Line Profile dialog or the corresponding marks in the Image window, the area of interest can be more sharply defined for FWHM calculations. To calculate the FWHM of the area between these cursors, choose the desired channel under **Selected Channel** and click **Calculate**.

When the Image window is displaying a Maximum Intensity Projection of a Z-Series (discussed in the [Maximum Intensity Projection](#) section of this manual), the **MIP Mode** checkbox in the Line Profile window allows the user to display a line profile from the projection image. The **Z Mode** checkbox on the Line Profile window can be used to acquire a profile or calculate FWHM along the z-axis at a point in X-Y (as defined in the MIP).

The vertical scale of the profile graph can be adjusted in the Line Profile dialog.

The user can adjust the **Line Width** to average the intensity profile of neighboring pixels.

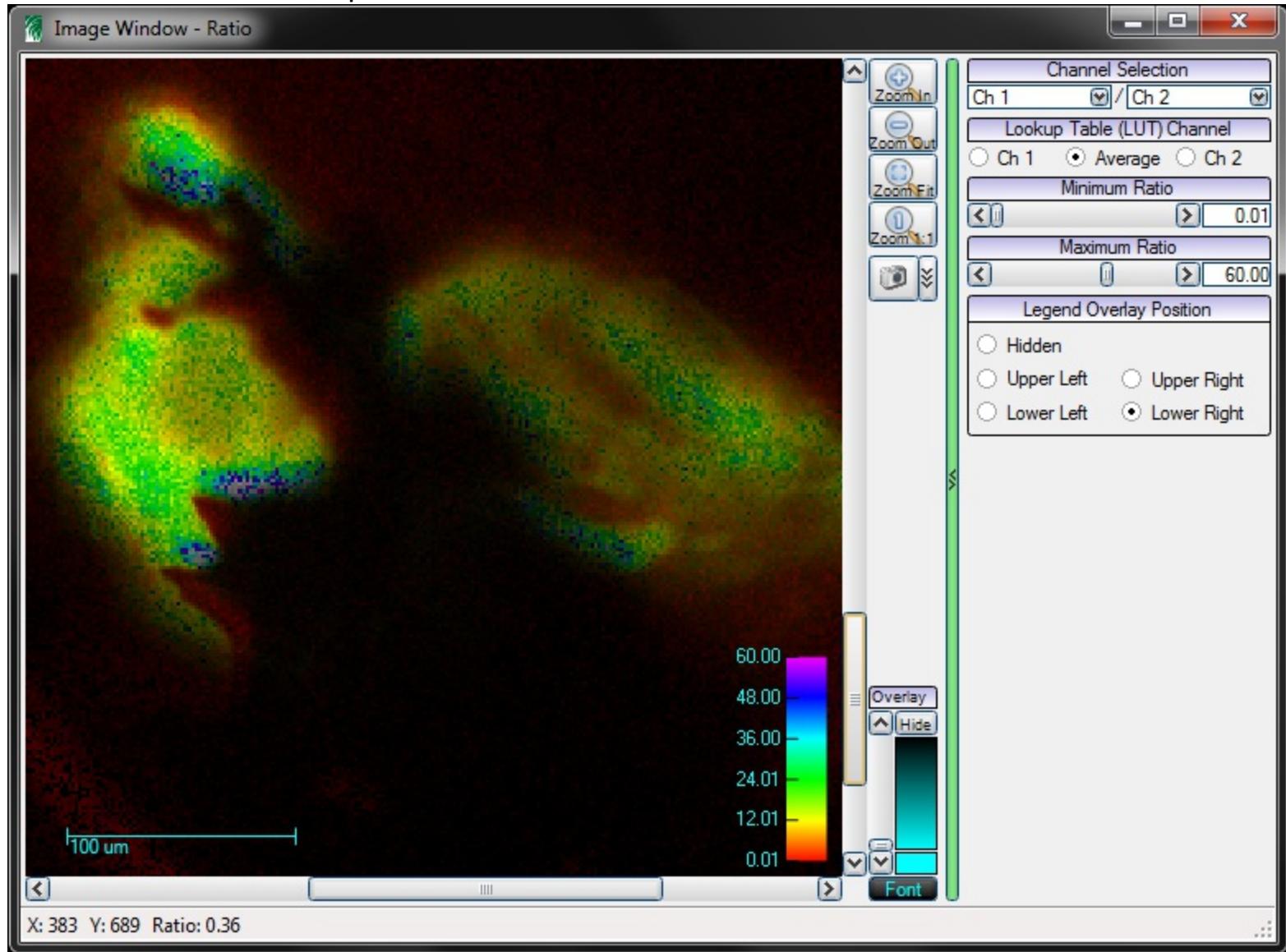
Intensity profile information can be saved as a comma separated data set by clicking **Export**.

By clicking the green bar above the line profile graph, the user can access buttons for zooming and panning within the graph display.

The drop-down button next to the line profile tool button allows the user to choose whether or not the Line Profile dialog will pop up when the line profile button is activated.

Ratio

The ratio image window behaves much like any other image window, but instead of being used to visualize the intensities of a combination of channels, it instead is used to visualize the ratio of intensities between a pair of channels.



The controls on the right side of the window are used to change what is displayed in the ratio image window. These controls include:

- **Channel Selection** combo boxes which specify which two channels will be divided to calculate a ratio at each pixel.
- **Lookup Table (LUT) Channel** selections to pick which channel, or an average of both, to use when determining the brightness of each pixel. A pixel with no visible intensity will appear black, a saturated pixel will display at full brightness, and intensities in between will use a linear brightness gradient.

- **Minimum Ratio** and **Maximum Ratio** sliders which map a range of ratios to a colored legend. The minimum ratio will display as red, the maximum ratio will display as magenta, and ratios in between will display with a color chosen from a smooth RGB gradient. Pixels with a ratio less than the minimum will appear black, and pixels with a ratio greater than the maximum will appear white; white displayed at different brightness levels will appear as greyscale.
- **Legend Overlay Position** selections specify which corner of the image to display the ratio/color legend in, or hide it from view.

To free up more space on the screen, these controls can be hidden by pressing the tall green button that runs top to bottom between the image and the controls.

Information about an individual pixel can be viewed by hovering the cursor over the pixel and looking below the image. Text there displays the X and Y pixel position of the cursor, and the ratio value for that pixel.

Playback Mode

After a multi-frame acquisition completes or a set of images is loaded from the file menu, Prairie View enters Playback mode indicated by the playback controls visible below the first image window.



An option in the [Preferences menu](#) allows the user to decide whether Playback will automatically open after an acquisition. In order for the images to display, the raw acquisition data must have been converted to image files. This can be done automatically after acquisition via an option in the [Preferences menu](#), or manually via the [Image-Block Ripping Utility](#) in the Tools menu. If the images files have not been created when Playback opens, a message box will give the user the option to convert the files at that time.

When in Playback mode, many controls associated with scanning become disabled until the playback mode is disabled by clicking **Exit**.

Playback Controls

Depending on the image sequence selected, one or both of two scroll bars may be available: one vertical for Z stack image data and one horizontal for time lapse image data.

Each image sequence represents a cycle of a T-Series, a simple Z-Series, or a single image. It is possible to navigate sequences by selecting one from the drop-down menu to the right of the horizontal scroll bar, or by using the arrow buttons next to the drop-down menu.

If the number of frames is the same for all sequences in the open data set, then both scrolls bars become active. The vertical scroll bar controls the selected frame while the horizontal scroll bar controls the selected sequence.

Arrows at the ends of the scroll bar allow the user to navigate in single-frame increments.

Next to each scroll bar is a set of five navigation buttons. The double-arrow buttons jump to the first or last frame/sequence. Single-arrow buttons play through the images in the indicated direction. The square button stops any playback currently in progress.

The **Loop** checkbox will continue playback in the reverse direction when the last/first image is reached. The **Wrap** checkbox will jump from the first to the last image or vice versa rather than reversing direction when the last/first frame is reached.

When playing through a set of images, the speed at which the images change is determined by the **Delay** text box (in milliseconds). Enter a new number in this box to change the speed of

playback.

When in Playback mode, information about the absolute and relative time of each frame's acquisition is displayed in the title bar of the Image window.

A text box in the lower right portion of the Playback window displays the file path for the acquisition. The user can open the directory containing the acquisition by clicking the  button on the right side of the window.

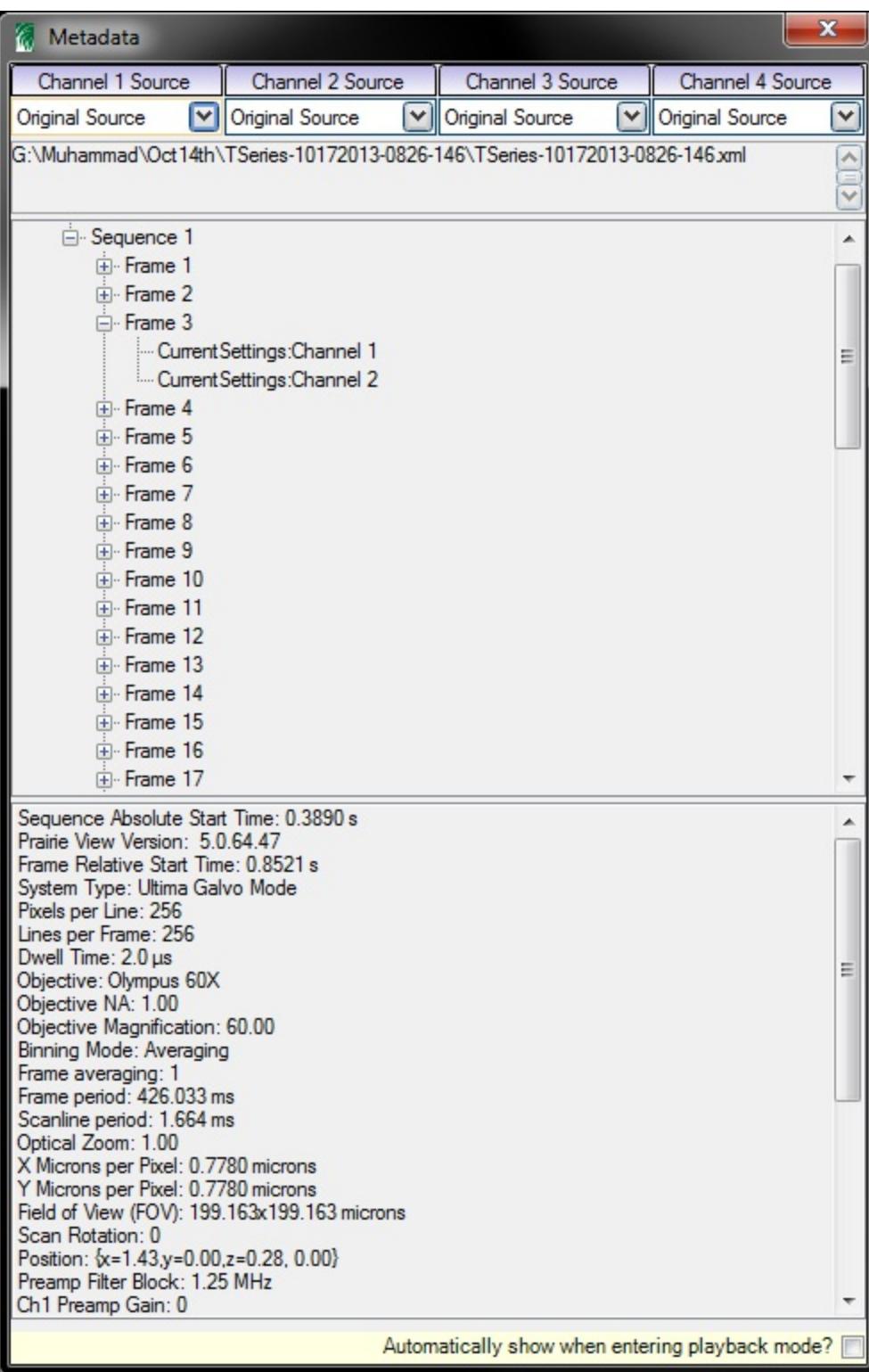
The user may choose a previously defined After Scan Complete/Playback action from the **Action** drop-down menu and execute it by clicking the **Perform** button. If the **Perform Action** box is checked, the action displayed in the drop-down menu will be executed every time the image in the Image window changes; that is, when changing slices in a Z-Series playback or changing time points or sequences in a T-Series playback. Actions are described [here](#).

The text box in the lower right corner of the playback controls will display an error message if the current image sequence is corrupt due to missing samples at the time of the acquisition. A red warning icon  will also appear in the lower right corner of the window. Due to hardware constraints while acquiring multiple channels with heavy processing, it is not always possible to keep up with the data throughput. Saving any heavy processing until after the data is acquired is a good practice in such cases.

While in Playback mode for a Z-Series or T-Series image sequence, the user can display projections of the images. This is discussed in the [Maximum Intensity Projection](#) section of this manual.

Metadata Window

The Metadata window displays a tree view of information about system settings such as laser power, motor positions, and scan timing for each frame. The Metadata window can be brought up at any time in playback mode by clicking **Info**. A check box in the lower right corner of the Metadata window allows the user to specify that the Metadata window open automatically each time Playback is open.



Channel Mapping in Playback Mode

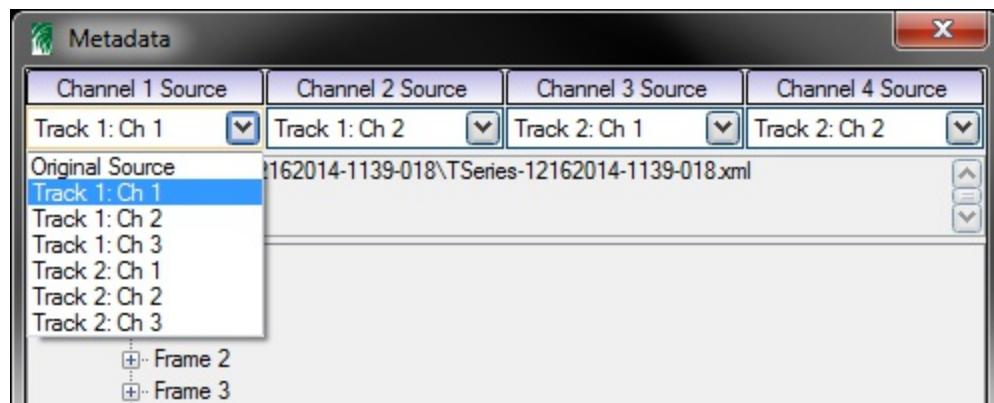
The Metadata window provides controls for mapping data associated with a certain acquisition channel, and an optional Parameter Set track, to a specific display channel. The playback mapping is important when looking at an image set that was acquired using multi-track Parameter Sets; the channels active when acquiring images within a specific track can be mapped to different display channels during playback.

The default choice for channel mapping is "Original Source", which is the most intuitive choice

and maps each channel of data to the channel in which it was acquired. However, in some cases it can be useful to change this mapping when viewing the data.

Consider an example where a T-Series has been acquired using a Parameter Set with two tracks (see further discussion in the [Parameter Sets Tab](#) section of this manual). Three physical channels of data were acquired for each of the two tracks, for a total of six logical channels of data to display. The user may wish to map these four of these logical channels (Track 1: Ch 1, Track 1: Ch 2, Track 2: Ch 1, and Track 2: Ch 2) to four different display channels to see them during playback.

While in Playback Mode for this T-Series, click the **Info** button on the right side of the Playback window. This will open the Metadata window. Across the top of this window are four drop-down menus which correspond to display channels 1 through 4. In this example, the data acquired on channel 1 with track 1, called “Track 1: Ch 1”, is mapped to appear in display channel 1 for playback.



Sometimes for a given sequence of frames there is no data for certain track/channel combinations so the image for that channel will be black.

SFC Settings Overview

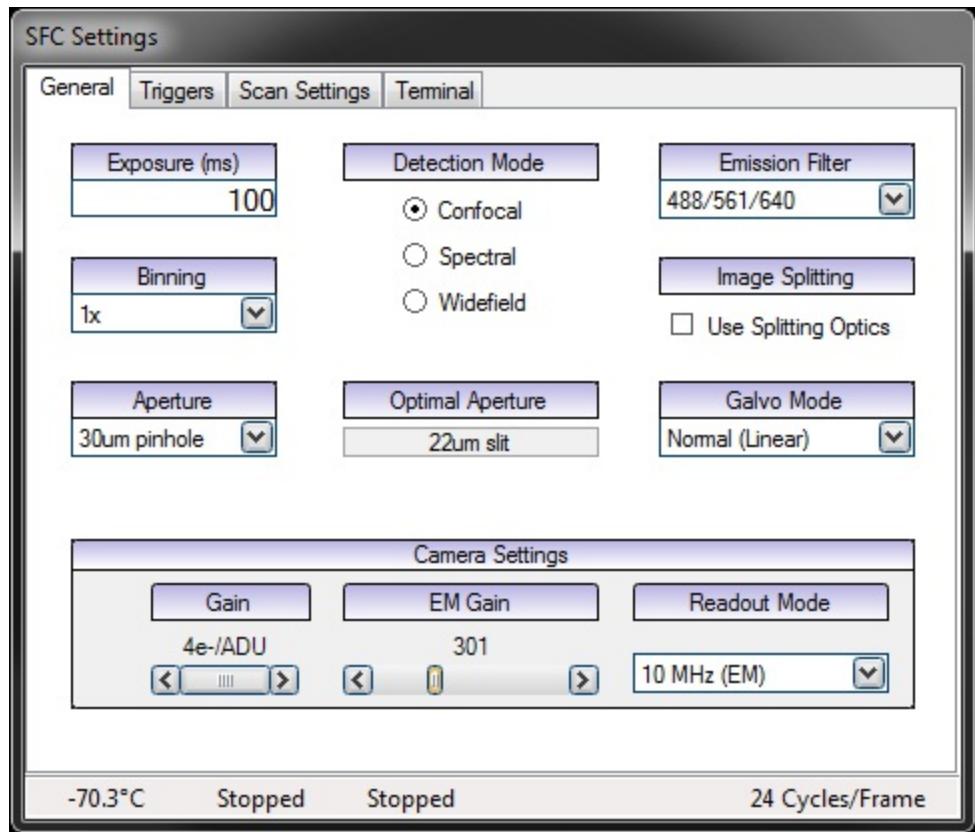
The SFC settings are controlled in the SFC Settings window. There are several different tabs for the components of the system and controlling the imaging parameters; these are described in the subsequent sections of this manual.

At the bottom of the window, an information bar provides status information about the system. From left to right, the information boxes report

- Temperature of the camera's internal sensor, if supported by the camera
- Stopped or Moving status of the Aperture plate
- Stopped or Moving status of the Emission Filter wheel
- Galvo periods (cycles) per frame of the image; one period means two sweeps across the sample

General Tab

The General tab includes the basic settings for creating an SFC scan, including apertures, filters, and camera settings.



The upper portion of this tab contains various settings for the SFC and camera:

- **Exposure time** is the amount of time the camera will collect photons from the sample for each active channel. Due to galvanometer and piezo movements and blanking of the laser, the sample is illuminated for 70-80% of the exposure time; the duty cycle depends on scan settings and galvo mode. The Frame Period reported by Prairie View equals the exposure time multiplied by the number of active channels. The minimum exposure time allowed is 1ms.
- The **Binning** dropdown allows the user to combine charge from adjacent camera pixels. Increasing the binning factor will increase speed and signal-to-noise, but will decrease spatial resolution.
- The **Detection Mode** selections allows the user to switch imaging modes: **Confocal** is normal swept field confocal imaging, **Spectral** will collect image data with 15 channels of spectral resolution (when available), and **Widefield** will bypass the swept field and image directly with the camera (when available).
- The **Emission Filter** dropdown allows the user to select the filter positioned in front of the camera. When the emission filter wheel is moving, the second motor indicator at the bottom of the window will change from "Stopped" to "Moving".

- **Use Splitter** is an option available on systems configured with splitting optics for simultaneous collection of multiple channels of data using a single CCD or CMOS image sensor. When the box is checked to enable this option, the software will separate a single camera image into multiple channels of data (either 2 or 4, depending on the splitting hardware). More information is available in the [Image Splitting Optics](#) section of this manual.
- The user can choose from 7 options in the **Aperture** dropdown. There are 3 pinhole diameters and 4 slit widths available. Each pinhole option is a linear array of 32 pinholes, which operate in parallel to perform a classic high resolution scan more quickly than a single point laser scanning microscope. Each slit option is a single slit that can be used to scan the sample at much higher speed than the pinhole arrays, with slightly reduced confocality. While the aperture plate is moving, the first motor indicator at the bottom of the window will change from “Stopped” to “Moving”.
- The **Optimal Aperture** field displays an aperture suggestion based on the current scan settings. This suggestion is calculated based on the excitation wavelengths in active channels and the magnification and numerical aperture of the selected objective lens. The suggested aperture is the one whose size (diameter or width) is nearest to the Airy disc diameter calculated with this information. No preference is given to the type of aperture (slit vs. pinhole).
- **Galvo Mode** describes the movement of the galvanometer in relation to image acquisition. **Normal (Linear)** mode is the standard operating mode, creating an image with uniform illumination using a linear galvanometer scan pattern. **Sinusoidal (Faster)** mode increases the number of galvanometer sweeps per image during short exposure times, but can result in an image where the edges are brighter than the center. **Harmonic (Fastest)** mode is useful with slit apertures to capture one image for every galvanometer sweep. In this mode, frame rate is often limited by the transfer speed of the camera. The minimum exposure time allowed by Prairie View is currently 1ms. The number of galvanometer cycles per image is displayed in the bottom right corner of the window.

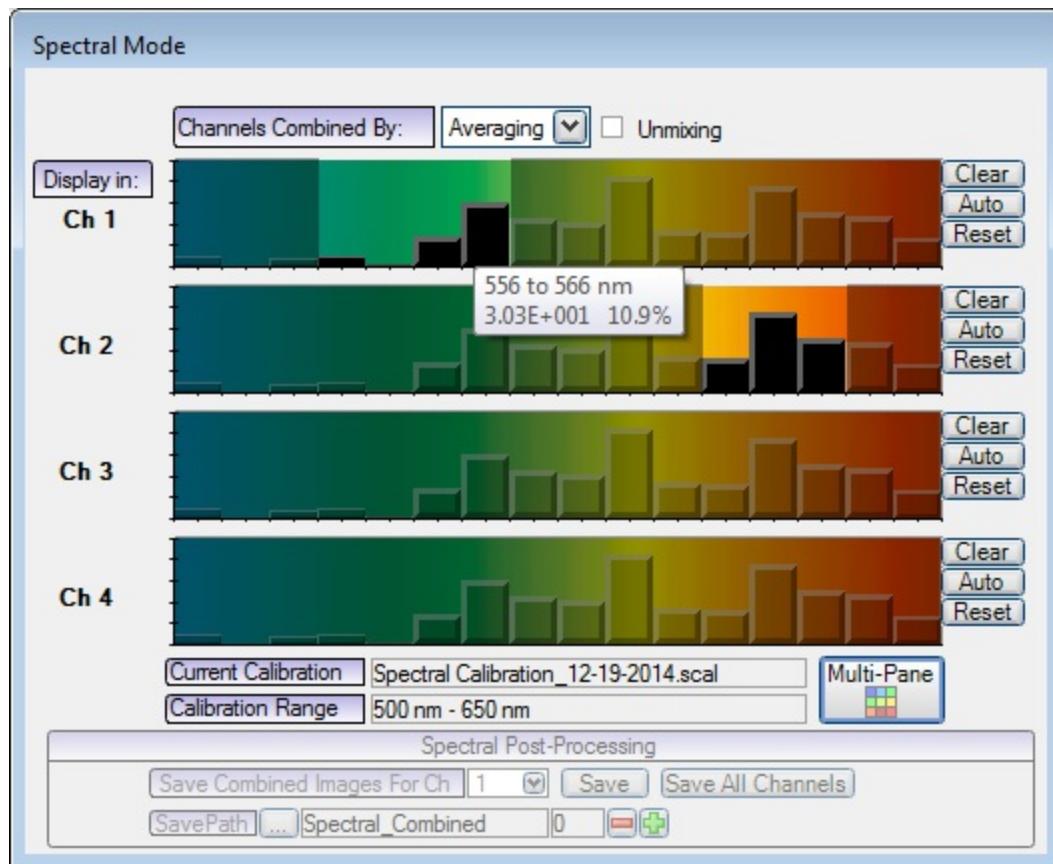
The lower portion of the tab contains various camera specific settings (when more than one camera is present multiple sets of controls will be shown, one set for each camera):

- Many cameras supported by Prairie View have two types of gain control, which amplify signal after it is acquired by the camera. Options in the first **Gain** slider or dropdown determine the way signal is digitized – they describe the number of photoelectrons that make up one unit of digital signal. The second gain control slider or dropdown allows the user to set the multiplication gain that is applied on the camera’s chip; this is called **EM Gain**.
- The **Port** or **Readout Mode** dropdown offers choices for the speed at which the camera transmits data to the computer, with or without multiplication gain, denoted by “(M)”.

Faster readout rates allow faster frame rates, but may affect the dynamic range and read noise of the signal. The chosen readout mode is also displayed in the bottom of the window.

Spectral Mode

The Spectral Mode main window provides controls for spectral imaging.

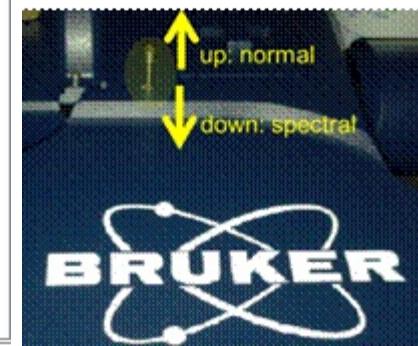


Spectral Imaging

The spectral imaging module is designed to allow for image collection with 16 channels of spectral resolution. In spectral mode, each frame consists of 16 discrete images with each image representing a wavelength range of approximately 10 nm. The total spectral range achievable is determined by the spectral bandpass filter(s) in the system, usually about 150 nm.

Setup

Exposure (ms)	Detection Mode	Emission Filter
100	<input type="radio"/> Confocal <input checked="" type="radio"/> Spectral <input type="radio"/> Widefield	488/561/640
Binning		
1x		
Aperture	Optimal Aperture	Galvo Mode
30μm pinhole	22μm slit	Normal (Linear)
Camera Settings		
Gain	EM Gain	Readout Mode
2.4e-/ADU	673	10 MHz (EM)
<input type="button"/> <input type="button"/> <input type="button"/>	<input type="button"/> <input type="button"/> <input type="button"/>	<input type="button"/> <input type="button"/>



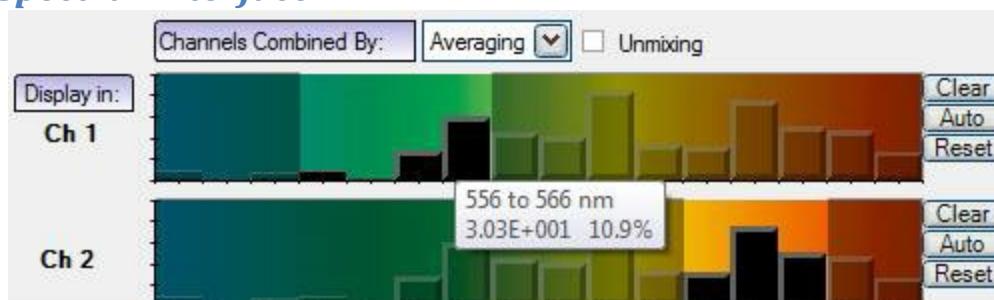
For spectral imaging, ensure that a pinhole aperture is selected along with the desired spectral bandpass filter position. In filter wheels that can accommodate multiple filters per position, the spectral bandpass position would be a combination of a laser blocking filter and a spectral limiting filter. In filter wheels that are only able to hold single filters per position, select the laser blocking filter as the emission filter and place the spectral limiting filter in an individual filter holder and in the camera lens chamber (under the lid with a brass knob). The Amici prism must also be inserted into the light path of the Opterra scan head.

Calibration

Current Calibration	Spectral Calibration_12-19-2014.scal
Calibration Range	500 nm - 650 nm

The current spectral calibration is displayed along with the wavelength range for which the calibration was determined. To calibrate the system, see [Spectral Calibration](#).

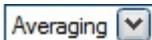
Spectral Interface



The spectral interface consists of 4 image window channels for visualization of the spectral data. The bar graph histogram updates in real-time and represents the amount of intensity data found in each spectral bin for the current frame. The spectral bins enabled in the first bar

graph will have its data displayed in image window channel 1, the second bar graph in image window channel 2 and so on. To enable display of a range of spectral wavelengths, the user can the desired wavelength bins and left mouse click the bar of the plot. Enabled spectral channels turn black while non-enabled channels remain transparent. To deselect a bin, the user clicks the enabled spectral bin.

Note: During an acquisition, all 16 channels of the spectral data are saved regardless of which channels are enabled for visualization.



If multiple spectral bins are selected for an image window channel, the spectral bins are combined by either averaging or summing the pixel values together. The mode can be selected by the drop down menu in the interface



Enable or disable [Spectral Unmixing](#) interface.



Each spectral histogram consists of three buttons that control behavior for that channel.

Clear will clear all the bin selections so that no spectral bins are selected.

Auto will autoscale the lookup table for that image window channel. This behaves the same as if the user clicks “Automatically Adjust Lookup Table Levels” on the Image window LUT context menu.

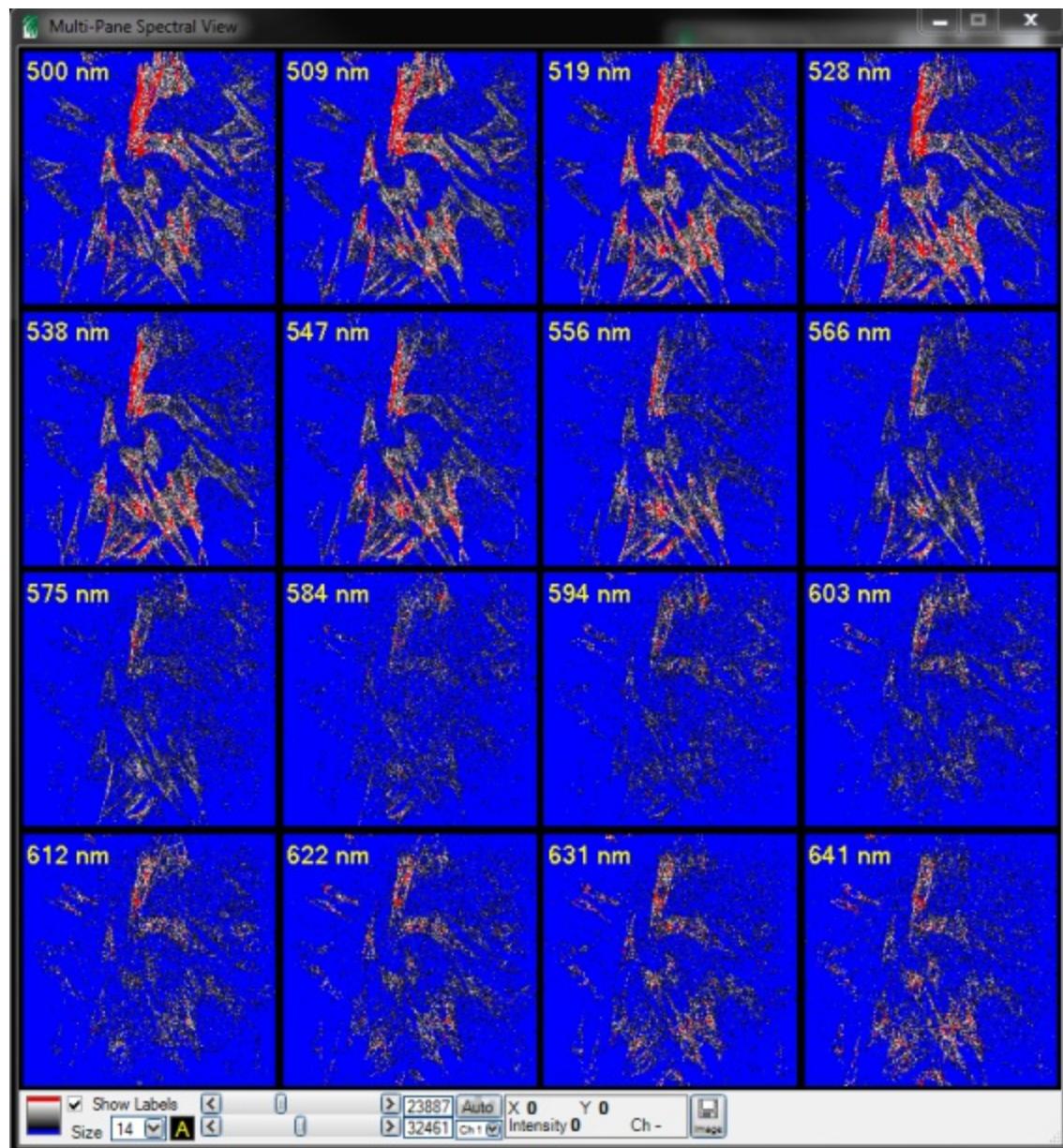
Reset will reset the lookup table for that image window channel. This behaves the same as if the user clicks “Reset Lookup Table Levels” on the Image window LUT context menu.

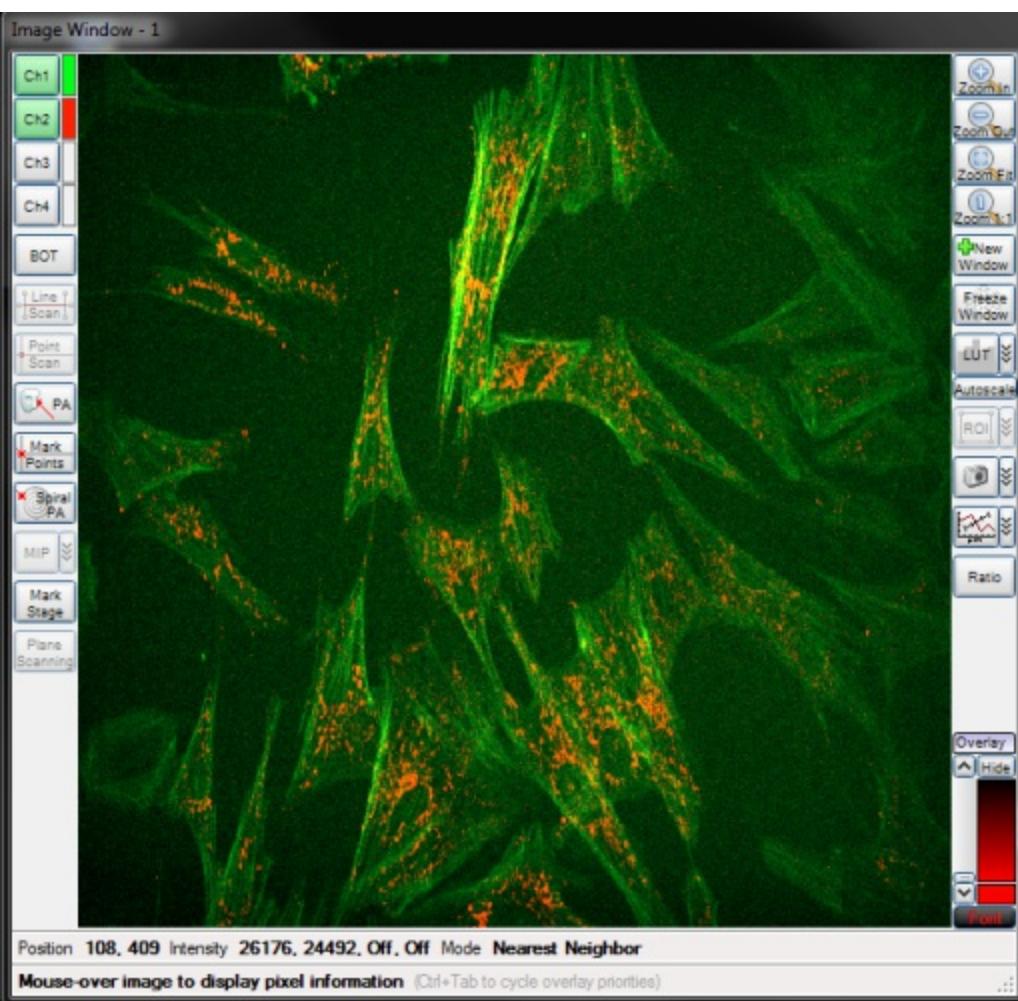


Enables the [Spectral Multi-pane View](#) where the user can visualize all 16 channels simultaneously.

Spectral Multi-Pane View

The Spectral Multi-pane View allows for simultaneous visualization of all 16 spectral channels in real-time. The look up table setting applies globally to all channels to facilitate balancing of laser power and gain to achieve the appropriate signal level in Spectral Mode.



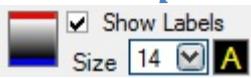


Global Look up Table Adjustment



The minimum (black level) and maximum (white level) of the global look up table may be adjusted with the sliders or values may be typed in. To auto-scale the look up table values by the intensity content of a certain channel, choose a channel from the dropdown menu and click Auto.

Color map and Wavelength Labels



The color map for the multi-pane view may be chosen by clicking the rectangle in the lower left corner of the window. To display the wavelength labels for each channel, check the “Show Labels” checkbox, choose a font size and the color for the text.

Pixel Information



The information for the pixel hovered over by the mouse cursor can be found in the above panel. X and Y coordinates, pixel intensity and channel number update as the mouse cursor changes position.

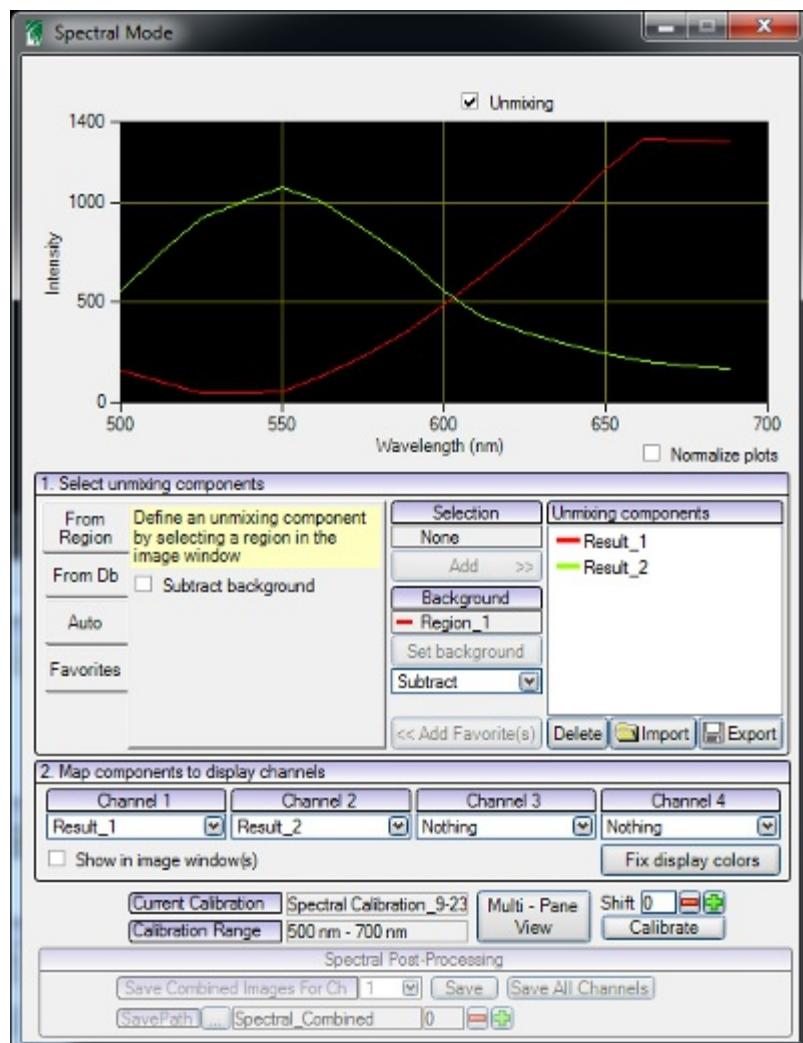
Save Multi-pane Snapshot



To save a snapshot of the multi-pane view as it is currently displayed, click the save image button and choose a save location.

Spectral Unmixing

A tool to help separate the spectral components present in a spectral image.



Workflow

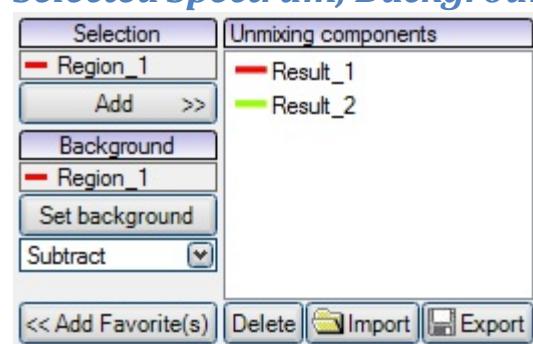
1. Define a [background component](#) with an [image region selection](#).
2. Define unmixing components with an [image region selection](#), from a [database](#), [automatically](#) or from a list of [favorites](#) by adding them to the [unmixing list](#) on the right.
3. [Map unmixed components to display channels](#).
4. In playback mode unmixed images can be saved using the Spectral Post-Processing save buttons at the bottom of the form.

Spectral Components Graph

Plots the currently selected component and the components in the unmixing list. The currently selected components is shown in a thicker line.

The **Normalize Plots** checkbox scales each curve such that its maximum value is 1. This is particularly useful when comparing components from images acquired at different intensities or when displaying components from the database since these are not scaled to the image intensity.

Selected Spectrum, Background and Unmixing Components List



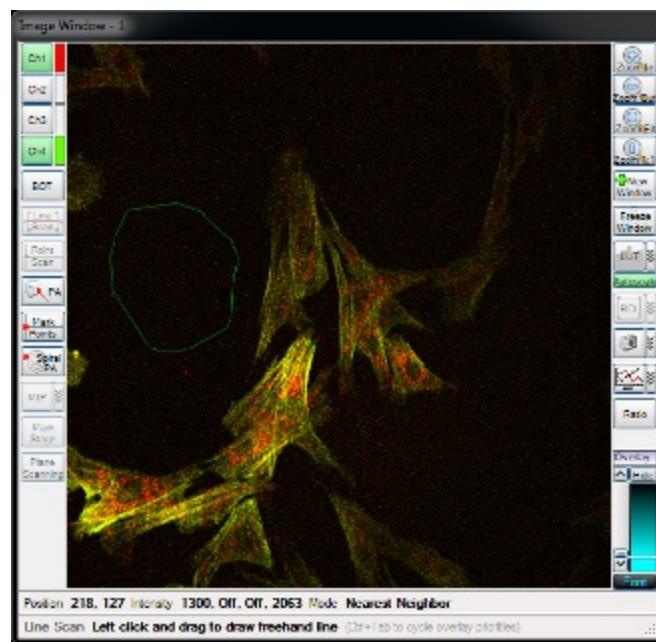
The **Unmixing components list** defines the components used to perform linear unmixing. Components can be renamed by double clicking. Multiple components can be selected using the shift or control keys. The **Export button** saves selected components to disk in a custom XML format. The **Import button** loads components from the custom XML export format, or a simple CSV format with two columns mapping wavelength to intensity.

The **Selection label** shows the component currently selected in one of the **From tabs** on the left. This component is plotted in a thick line style in the plot. The selection **Add button** adds the selected component to the unmixing components list.

The **Background label** shows the defined background component. The **Set background button** makes the currently selected component the new background component.

The **Background combo box** provides three background component strategies. The preferred method is **Subtract** which subtracts the background component from every pixel prior to unmixing. The **Unmix** method treats the background as a separate component to be unmixed by linear unmixing. The **Ignore** method performs no background processing, and is hence only suitable for images with negligible background signal.

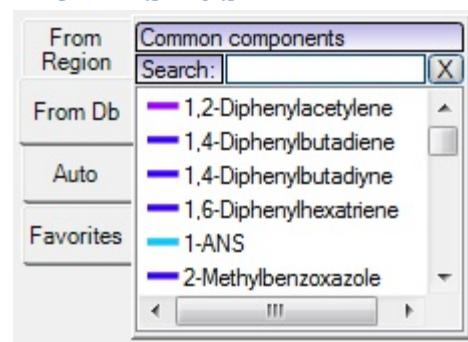
From Region Tab



Define a spectral component by selecting an image region containing a single component using a lasso tool. This is the preferred way to define the background component.

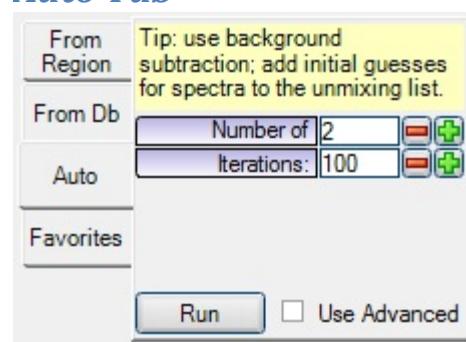
The **Subtract background checkbox** subtracts the background component from the component in the selected region. This should be selected when adding non-background components to the unmixing list.

From Db Tab



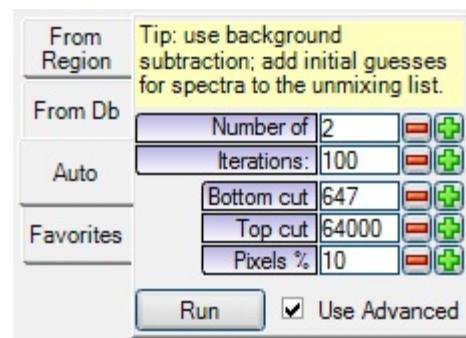
Provides a list of common fluorophores. The list can be filtered by entering text in the **search field** and pressing enter. The **X button** clears the filtering.

Auto Tab



Automatically detects components present in the current image. Define number of components to detect in the **Number of** text field and click the **Run button**. The numbers of iterations of the algorithm used to find the components can be set in the **iterations** text field. This defaults to 100 and can usually be left alone. Unmixing list components are used as initial conditions for the algorithm. Note that the contents of the unmixing list will be replaced with the results from the algorithm.

For best results define a background using the lasso tool and use the background subtract strategy prior to running the component detection algorithm



The automatic detection may fail to produce acceptable results for certain difficult images such as those with very few and small regions of real signal. Click the **Use Advanced checkbox** to fine tune the algorithm for better results in this case.

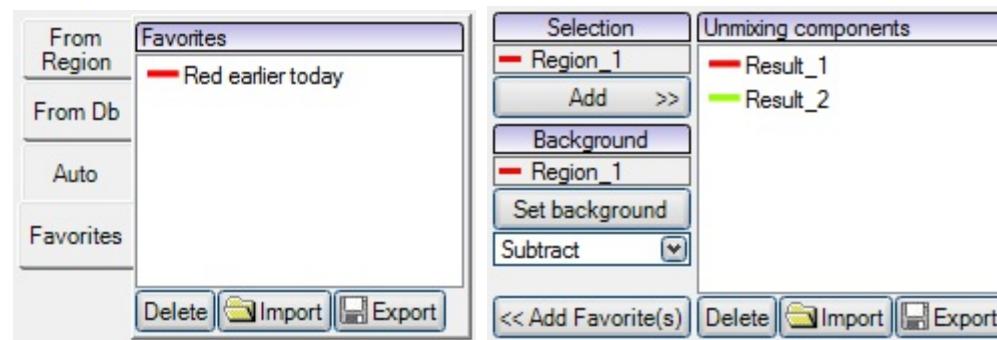
The **Bottom cut** text field sets the average per bin intensity below which pixels are ignored for the purpose of the detection algorithm. For images where only a few small areas are not background, the detection algorithm may be dominated by the noise present in background pixels, even after background component subtraction is performed. For example, in an image with only a small cell covering 1% of the image and an average background intensity of 1000, but intensities of 4000 or more in the cell, it would be advantageous to set the bottom cut to 2000 or so.

The **Top cut** text field sets the average per bin intensity above which pixels are ignored for the purpose of the component detection algorithm. This defaults to the camera maximum of 64 000. It is useful to exclude over-saturated regions of the image for the detection algorithm. This value should rarely be adjusted. If saturation is present in the image it should be dealt with at the time of acquisition by e.g. changing exposure or laser intensity. Saturated regions can never

successfully be unmixed, but the top cut can focus the detection algorithm on the non-saturated image regions for images which cannot be reacquired with more favorable settings.

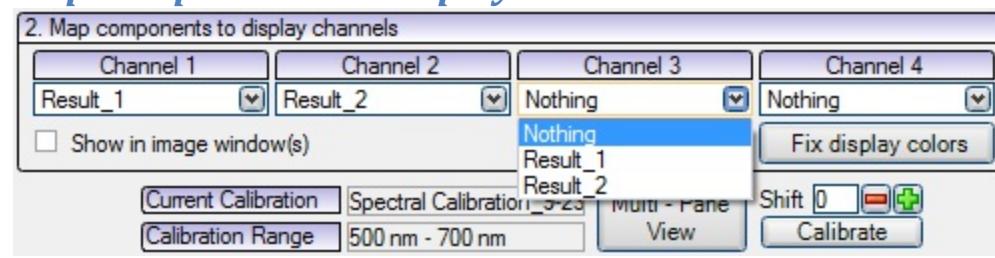
The **Pixels %** text field sets the fraction of pixels used by the algorithm to improve speed. This defaults to 10% which is adequate for normal images, but should be raised to 100% for images with little real data after an appropriate bottom cut was selected.

Favorites Tab



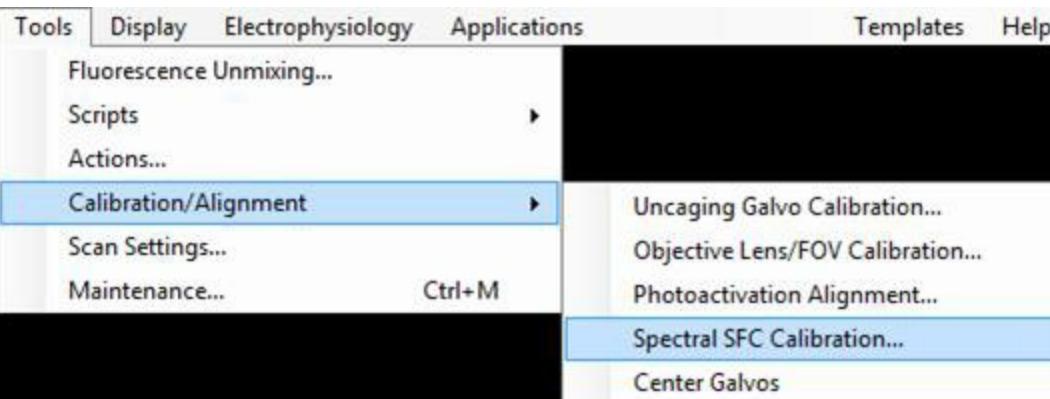
A place to save components determined in previous imaging sessions of for example control samples. To add components to the favorites list, select them in the unmixing list on the right, and press the **Add favorite(s) button**. To add a component from the favorites list to the unmixing list, select it in the favorites list and press the selection **Add button**. Components in the favorites list can be renamed, deleted, imported and exported in the same way as described for the unmixing list.

Map Components To Display Channels

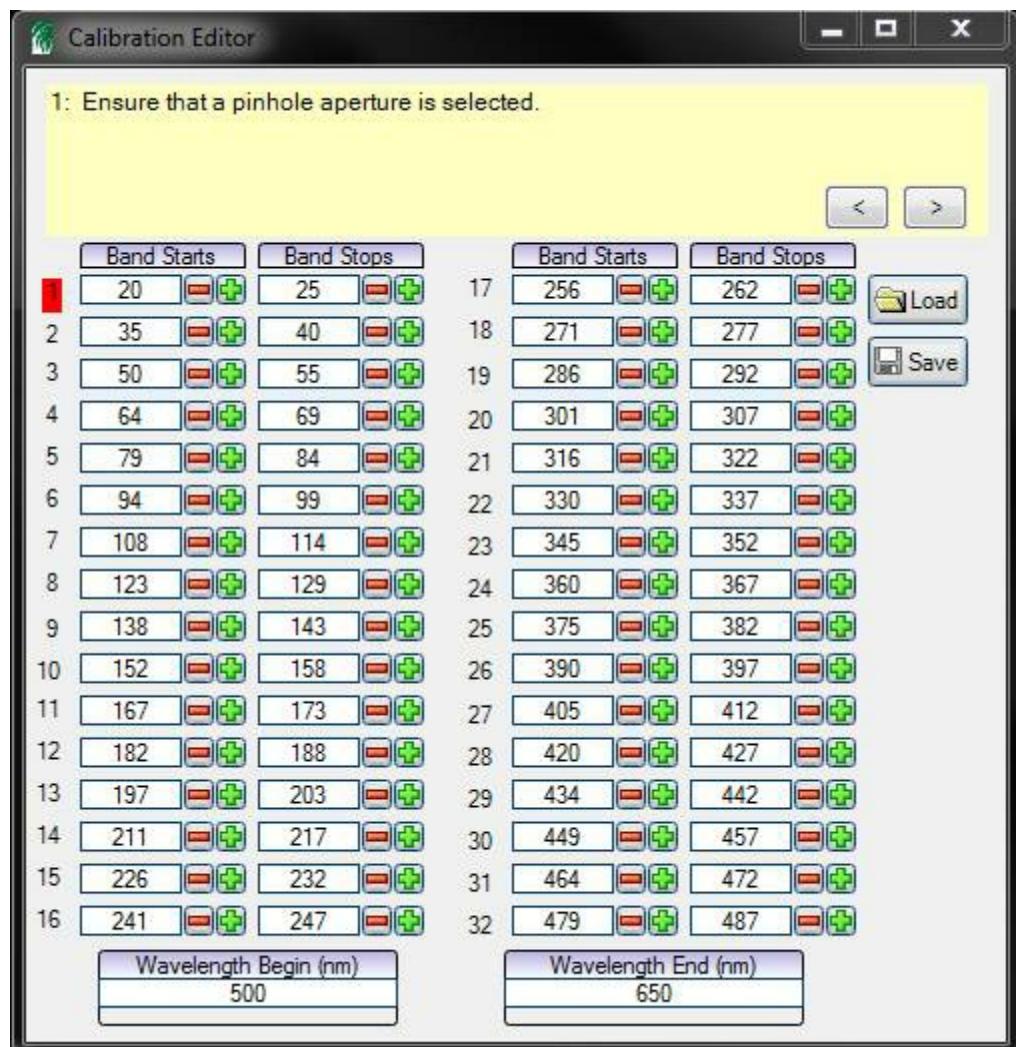


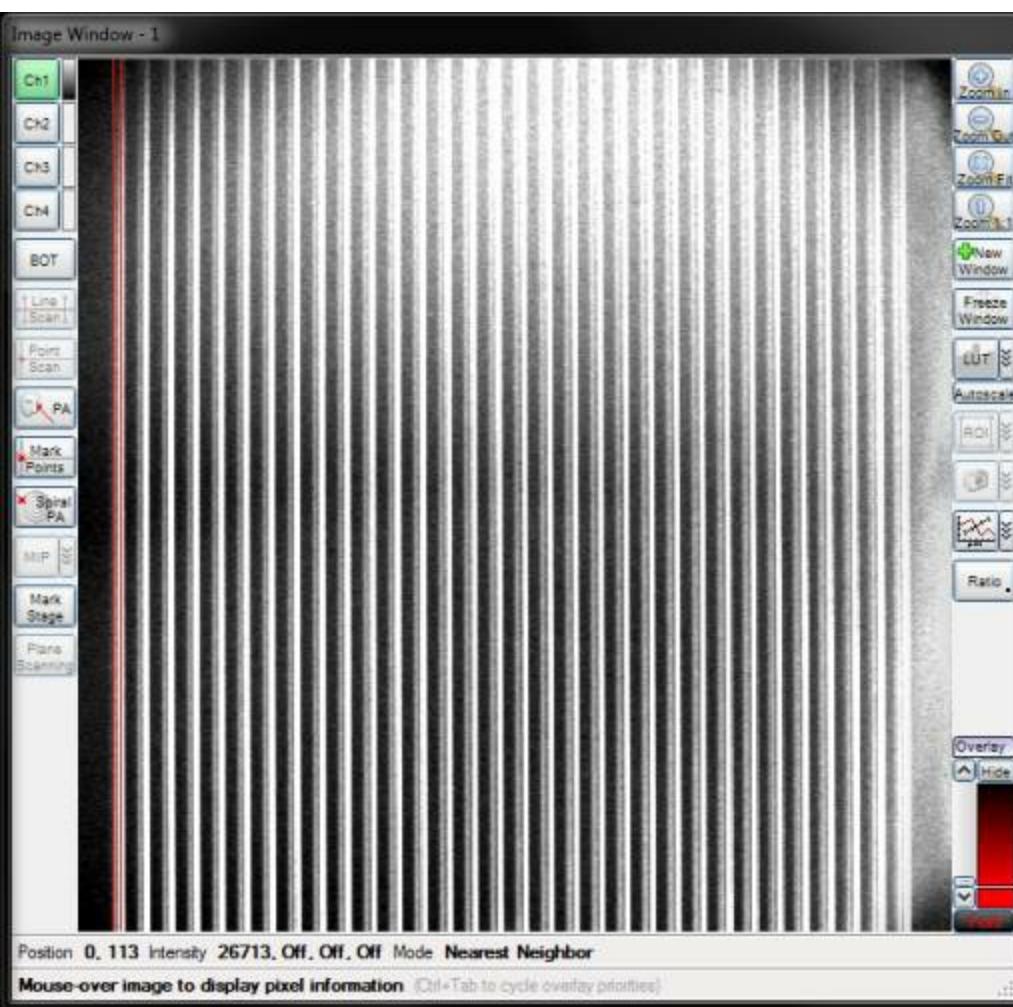
Map unmixing components to image window display channels using the combo box selections. The **Show in image window(s)** checkbox switches between displaying unmixed and regular spectral data. Note that lookup tables will likely need to be adjusted to obtain the same apparent intensity in the image display. The **Fix display colors** will set the colors used by the image window display channels to the colors used by the components.

Spectral Calibration



The spectral calibration procedure is required before spectral imaging is able to be performed. In spectral mode, the spectral calibration utility can be run from the Tools Menu, Calibration/Alignment, Spectral SFC Calibration.





Setup

Before beginning, ensure the system has been setup for spectral imaging as described in the “Setup” section of [Spectral Imaging](#). Use the < and > buttons to step through the calibration guide.

Step 1: Ensure that a pinhole aperture is selected.

Step 2: Zero all lasers.

Step 3: Place prism in beam path (plunger down).

Step 4: Turn on white light source.

Often, the transmitted illumination source on the microscope base may be used. Alternatively, a white LED flashlight may be positioned to shine through the objective lens. The purpose of the white light source is to illuminate the spectral range determined by the spectral bandpass filter in the beam path.

Step 5: “Calibration Mode” is active. In this mode, the bars on the image window correspond to the borders of the spectral spread for each pinhole.

With the white light source turned on, the spectral bandpass filter in place and the prism in the beam path, each pinhole should produce a band of intensity where the left edge of the band corresponds to

the lower wavelength cutoff of the bandpass filter and the right edge corresponds to the higher wavelength cutoff.

Step 6: *Select the desired spectral bandpass filter.*

In filter wheels that only support a single filter in each position, choose the quad notch filter position for the filter wheel and ensure that the spectral bandpass filter is in the beam path.

Step 7: *Live Scan to see the 32 spectral bands from the pinholes.*

Adjust the lookup table, brightness of the light source and the camera gain so that the live-scanned image clearly shows the 32 spectral bands.

Step 8: *Adjust band starting and stopping positions so they correspond to the bright bands in the image.*

Step 9: *“Calibration Mode” has been turned off and the Spectral Mode controls are active.*

Step 10: *Fine tune the calibration by imaging a uniform fluorescent slide. Only have the FIRST spectral bin enabled and start a Live Scan. If there are any bands that are brighter or dimmer than the rest of the field adjust the corresponding BAND START position to achieve uniformity.*

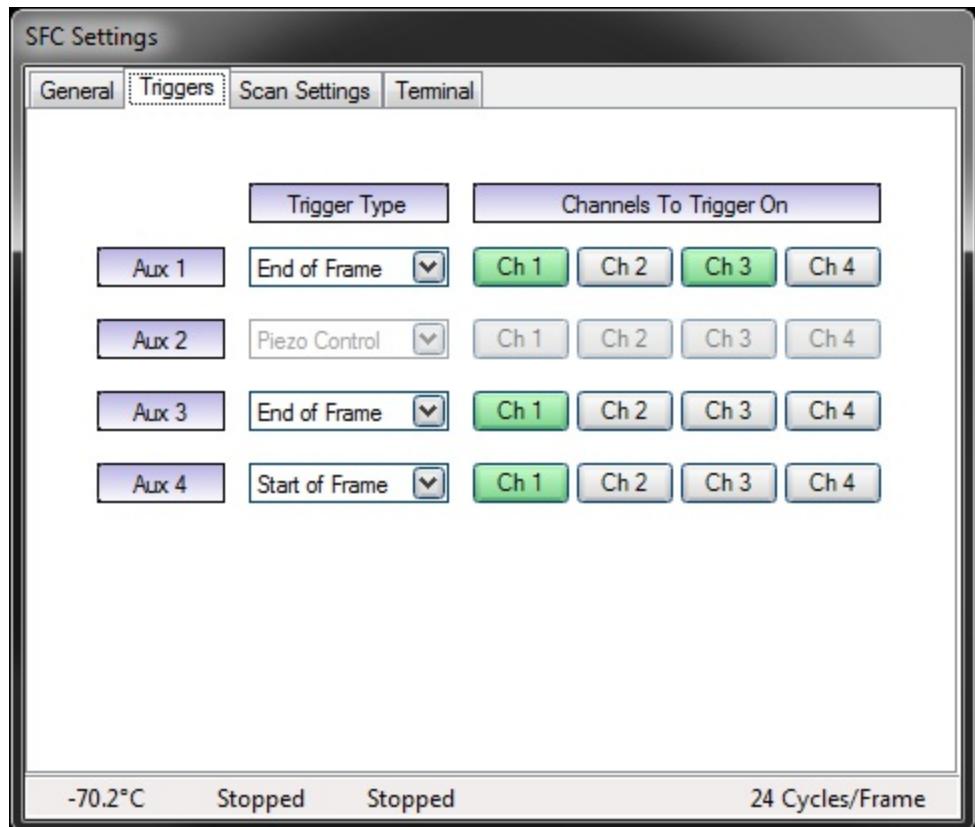
Step 11: *Enable only the LAST spectral bin and start a Live Scan. If there are any bands that are brighter or dimmer than the rest of the field, adjust the corresponding BAND STOP position to achieve uniformity.*

Step 12: *Enter the correct values for the Wavelength Begin(nm) and the Wavelength End (nm) that correspond to the spectral bandpass filter used in the calibration.*

Step 13: *Save Calibration.*

Triggers Tab

The Triggers tab allows the user to enable Start-of-Frame and/or End-of-Frame triggers from the numbered Aux lines on the front of the SFC Scan Control electronics box. For each line, the user can choose the type of trigger and the imaging channel(s) with which the trigger is associated. In the screen shot shown below, triggers will be sent out on Aux lines 1 and 2 at the end of every image on channel 1.



If the system is configured with a piezo device controlled by Prairie View, one of the Aux lines may be used to trigger the piezo. This line is chosen in the SFC/Camera/Filters tab of the Prairie Configuration Utility and then becomes unavailable for use in the Triggers tab.

The user may also designate one Aux line to receive triggers from other equipment. This line is chosen in the SFC/Camera/Filters tab of the Prairie Configuration Utility and then becomes unavailable for use in the Triggers tab. For systems configured with a GPIO box, it is often better to use the numbered Trig In lines on the GPIO box for input triggers; the switching hardware on these GPIO lines allows faster triggering than is possible on the SFC Aux lines. If an SFC Aux line is configured for an input trigger, that is the only input trigger signal the software will monitor. If one is not configured, then the Trigger In connections on the GPIO box would be used as the input trigger. That is, in order to use Trig In lines on the GPIO box, the SFC Aux lines must not be configured to accept input triggers.

Scan Settings Tab

The Scan Settings tab allows much finer control over the movements of the galvanometer and the piezos in the system. These values are set by the installation technician and should only be adjusted under instructions from support personnel at Bruker Fluorescence Microscopy.

System administrators at some sites have been instructed on altering scan settings when putting a different camera on the SFC. Adjustments can be made while following the procedures in the dialog activated by clicking the **Alignment Wizard** button. Consult Bruker Fluorescence Microscopy support personnel before attempting this procedure for the first time.

After changes have been made, the user must select **Update Scan Settings** for the changes to take effect.

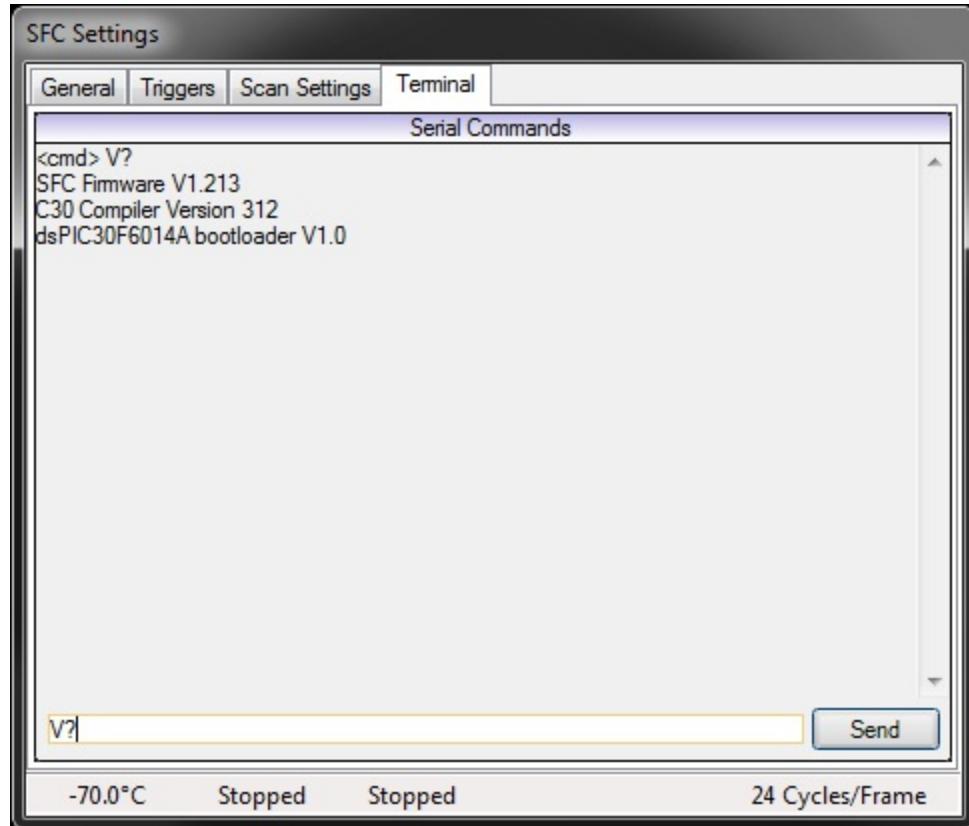


The **Tweak Piezo P2PV** field allows the user to make small changes in the peak-to-peak voltage of the camera piezo. Adjustment may be necessary if bright or dark stripes appear in the image.

The **Synchronization Method** describes the communication between the SFC and the camera. In **Timed** mode, the camera generates the scan trigger for the SFC, and the SFC attempts to match its scan frequency to the frequency of the trigger signal. In **Bulb** mode, each of the camera's exposures is controlled by a signal from the SFC Scan Control Box. In general, **Timed** mode is the preferred mode of operation, but some camera models may require the use of Bulb mode for certain acquisitions.

Terminal Tab

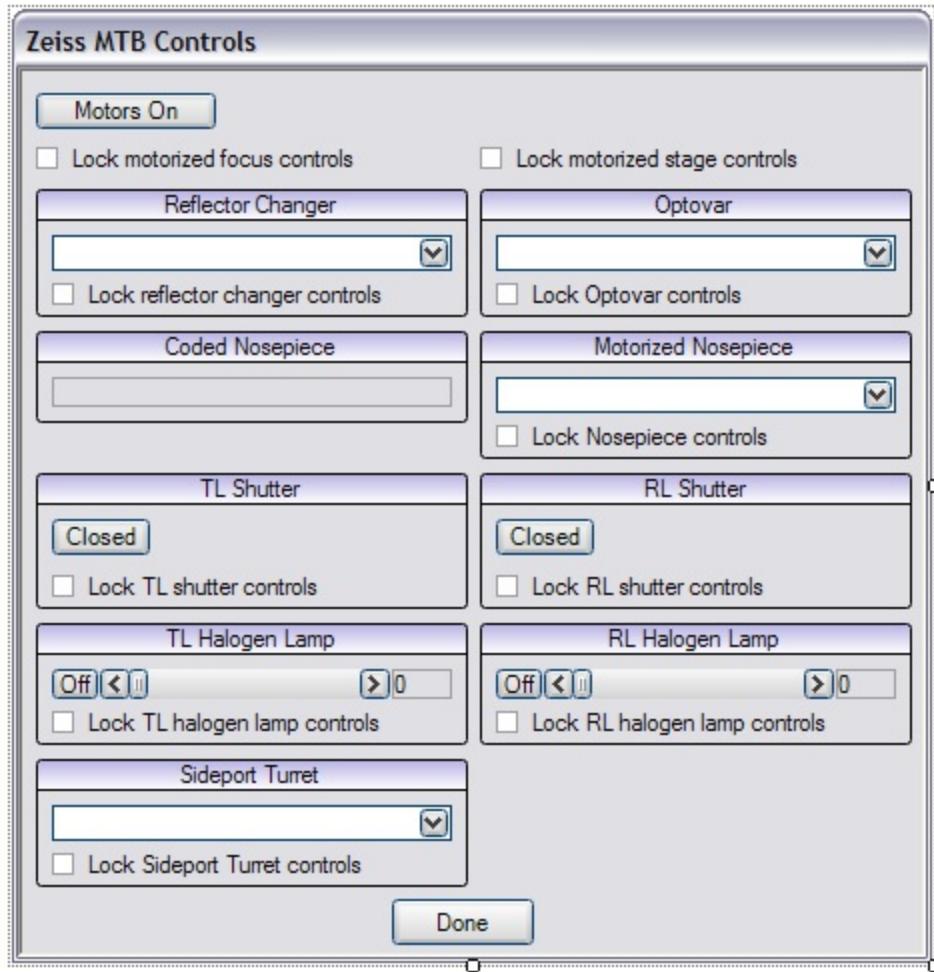
The Terminal tab can be used to send line commands to the SFC control box directly. This can be useful for technicians and support personnel during installation or when troubleshooting problems with hardware and software. Please contact Bruker Fluorescence Microscopy support personnel with questions about these commands.



Microscope Control Overview

Prairie View has support for software control of the automated features of certain Zeiss and Nikon microscopes. If microscope is supported and Prairie View is configured to interface with it, a window will appear which contains software controls for the automated features. If the window has been closed, the user can re-open it from the Tools menu.

Zeiss MTB Controls



This window contains check boxes that allow the user to **Lock** the controls for individual motorized accessories on the microscope. When a motorized accessory is locked, all of the controls for the motor are disabled (software controls, manual knobs, touch-screen interface) and the user will be unable to change the state of the accessory.

The **Motors On/Off** button toggles the motorized functionality of the entire microscope

Reflector Changer displays the current reflected light filter set in the motorized turret and allows the user to rotate the turret to a different filter set

Optovar displays whether the optovar is used and allows the user to change its state

Coded Nosepiece: If an automated nosepiece turret is not present, this displays the objective lens name for the current nosepiece position. If there is a matching objective lens name in Prairie View, the current objective will be changed to this value whenever the user manually changes the nosepiece position.

Motorized Nosepiece displays the current objective lens in the motorized nosepiece and allows the user to change the nosepiece position to a different objective lens. If there is a matching objective lens name in Prairie View, the current objective in the Prairie View main control window will be changed to match this displayed value after the nosepiece position is changed.

TL Shutter opens/closes the transmitted light shutter

RL Shutter opens/closes the reflected light shutter

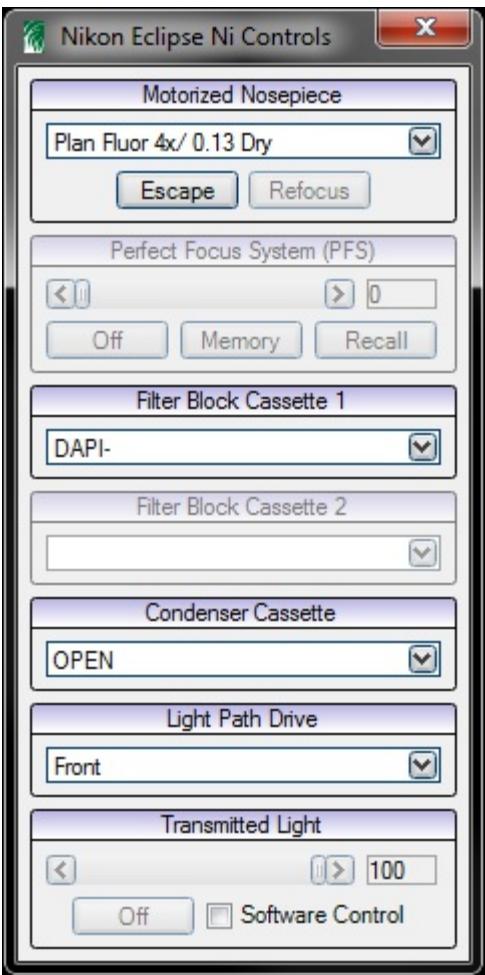
The **TL Halogen Lamp** slider allows the user to set the intensity of the transmitted light halogen lamp. The **On/Off** button toggles its light output on and off.

The **RL Halogen Lamp** slider allows the user to set the intensity of the transmitted light halogen lamp. The **On/Off** button toggles its light output on and off.

Sideport Turret displays the current light path in the microscope and allows the user to select a different light path

The **Done** button closes the window. The user may display the window again by selecting Tools > Zeiss MTB Controls from the Tools menu on the main Prairie View window.

Nikon Eclipse Ni Controls



This dialog will appear automatically at startup if a Nikon Eclipse Ni microscope is configured. If the dialog is closed for any reason it can be reopened using the **Nikon Ni Microscope Controls...** option under the **Tools** menu.

The **Motorized Nosepiece** drop-down menu allows the user to rotate the motorized nosepiece to put different objective lenses into the light path. If the names of objective lenses in Prairie View match the names in this control, Prairie View will automatically change the current objective in the Prairie View main control window when the user changes the objective with **Motorized Nosepiece** control. Objective names must be an exact match for this to occur.

Escape retracts the nosepiece away from the specimen to allow the nosepiece to rotate without risk of running the objective lens into the sample or XY stage

Refocus returns the nosepiece to the previous focal plane prior to using the Escape feature

If the system is configured with a Perfect Focus System (PFS), the **Perfect Focus System (PFS)** slider allows the user to define the offset of the desired focal plane relative to the interface between the slide and the coverslip. **Memory** records the current position of the slider, to which the user can return by clicking **Recall**. The **On/Off** button allows the user to enable/disable the feature. For more information on how this feature is integrated into the software check out the Focus Lock (generic term for manufacturer specific features like PFS)

sections of the [Z-Series](#) and [XY Stage tab](#) documentation. Perfect focus is not yet implemented for this microscope.

Filter Block Cassette 1 displays the current motorized filter cassette turret position for the first cassette and allows the user to rotate the cassette to a new position

Filter Block Cassette 2 displays the current motorized filter cassette turret position for the second cassette and allows the user to rotate the cassette to a new position

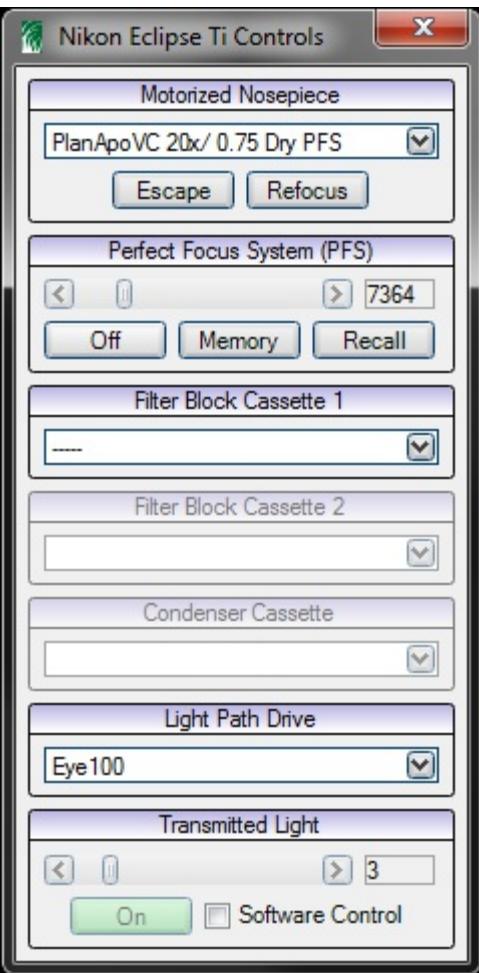
Condenser Cassette displays the current condenser cassette position and allows the user to rotate the cassette to a new position

Light Path Drive displays the current light path configuration and allows the user to change the light path between the available options (e.g. 100% left port, 100% eyepiece observation, 50% left port/50% eyepiece observation, etc.)

Transmitted Light allows the user to control the intensity of the light produced by the transmitted light lamp house. The **On/Off** button controls the powered state of the lamp.

Software Control puts the transmitted light under control of the software rather than the manual controls on the microscope.

Nikon Eclipse Ti Controls



This dialog will appear automatically at startup if a Nikon Eclipse Ti microscope is configured. If the dialog is closed for any reason it can be reopened using the **Nikon Ti Microscope Controls...** option under the **Tools** menu.

The **Motorized Nosepiece** drop-down menu allows the user to rotate the motorized nosepiece to put different objective lenses into the light path. If the names of objective lenses in Prairie View match the names in this control, Prairie View will automatically change the current objective in the Prairie View main control window when the user changes the objective with **Motorized Nosepiece** control. Objective names must be an exact match for this to occur.

Escape retracts the nosepiece away from the specimen to allow the nosepiece to rotate without risk of running the objective lens into the sample or XY stage

Refocus returns the nosepiece to the previous focal plane prior to using the Escape feature

If the system is configured with a Perfect Focus System (PFS), the **Perfect Focus System (PFS)** slider allows the user to define the offset of the desired focal plane relative to the interface between the slide and the coverslip. **Memory** records the current position of the slider, to which the user can return by clicking **Recall**. The **On/Off** button allows the user to enable/disable the feature. For more information on how this feature is integrated into the software check out the Focus Lock (generic term for manufacturer specific features like PFS)

sections of the [Z-Series](#) and [XY Stage tab](#) documentation.

Filter Block Cassette 1 displays the current motorized filter cassette turret position for the first cassette and allows the user to rotate the cassette to a new position

Filter Block Cassette 2 displays the current motorized filter cassette turret position for the second cassette and allows the user to rotate the cassette to a new position

Condenser Cassette displays the current condenser cassette position and allows the user to rotate the cassette to a new position

Light Path Drive displays the current light path configuration and allows the user to change the light path between the available options (e.g. 100% left port, 100% eyepiece observation, 50% left port/50% eyepiece observation, etc.)

Transmitted Light allows the user to control the intensity of the light produced by the transmitted light lamp house. The **On/Off** button controls the powered state of the lamp.

Software Control puts the transmitted light under control of the software rather than the manual controls on the microscope.

Menus Overview

The Prairie View main control window contains several menus containing additional software options and settings. Menu items ending in “...” will open new dialogs when selected. Menu items for features that can be enabled/disabled via the menu will have a check mark next to the item when the feature is enabled. Menu options listed and/or available for selection may vary somewhat based on system configuration.

Load Data Set in Playback mode will open a load file dialog where a data set can be opened and reviewed in [playback mode](#)

Recent Data Sets expands into a submenu containing the last few data sets acquired and/or opened in [playback mode](#), clicking one of the data sets will open the data set to be reviewed in playback mode

Load Environment will open a load file dialog where an [Environment file](#) which is a previously recorded state of the software, loading an Environment file will overwrite everything in the software including the current t-series definition, scripts, actions, etc.

Save Environment will record the current state of the software in an [Environment file](#) so that is can be restored later

Current Calibration File is an informational menu item which cannot be interacted with, but instead displays the name of the current [Uncaging Calibration](#) file, with details of the calibration displayed in its submenu

Load Uncaging Calibration will open a load file dialog where a different [Uncaging Calibration](#) can be selected and applied, this calibration is used by [Mark Points](#) and [Spiral Activation](#)

Load Recent Uncaging Calibration expands into a submenu containing the last few [Uncaging Calibrations](#) which were created and/or applied, clicking one of the calibrations will apply it immediately

Exit will shut down and close Prairie View

Environment Files

An Environment file is a single XML file which contains the complete state of the software as it was when the file was saved. An Environment file is saved with every data set as a reference, and is automatically saved every 5 minutes to help recover in cases where the software isn't shut down properly. When the software isn't shut down properly, and an automatic backup exists, there will be a prompt to load it the next time the software is loaded.

In general an Environment file stores everything and is capable of bringing the software back to the exact same state it was in when the Environment file was created, but there are a few exceptions:

- **Scan Settings** are saved in, but not loaded with an Environment file; these settings are system specific and are not meant to be changed without assistance from a Bruker Fluorescence Microscopy representative
- **XY Stage Positions** and **Z-Series definitions** are excluded because motor positions for many devices are relative, and using positions from a previous session could drive motors to unexpected locations and potentially cause damage
- **Voltage Output**, **Voltage Recording**, and **Seal Test** settings are still stored in separate files since they were developed prior to the introduction of Environment files and were not easily converted due to different architectures
- **Photoactivation Masks** are excluded because the underlying data format is very large, and would increase the file size significantly, decreasing performance
- **Selected Parameter Set** isn't included, but any defined Parameter Sets are; the software always loads with "Current Settings" as the selected Parameter Set on the main form
- **Playback Action to Perform** isn't included and needs to be reselected each session if desired

Since Environment files change so much it may be preferential to use a [Template](#) instead, which affects a much smaller subset of settings.

Preferences Menu

Adjust MAMC Stage Controller vs Optical Zoom: This feature changes the responsiveness of the remote knobs controlling stage movements when an optical zoom is applied. When Fine is selected, then as the optical zoom is increased from 1.0, the number of motor steps generated when the X or Y axis wheels are turned will be decreased to better match the stage movement with the current field of view. When Coarse is selected, a similar affect as with the 'Fine' option will occur, but to a lesser degree. When No Adjustment is selected, then the number of motor steps generated by the X or Y axis wheels will not be modified as the optical zoom is changed.

Automatically Start Playback After Acquisition: When enabled, this option will open the last acquisition in [Playback mode](#) once it has completed.

Automatically Convert Raw Files: Allows the user to choose whether or not data is automatically written to TIFF, CSV, and/or SDT files after an acquisition.

Fluorescence Lifetime Imaging (FLIM): Options in this sub-menu allow the user to determine aspects of file handling during acquisitions made in FLIM mode.

Leave Soft Shutter Open: When available, this option holds the Pockels output at the value determined in the Laser, PMT, DAQ tab at all times, rather than "blanking" the laser power between scans or between lines in within a scan. This option is only supported for particular configurations. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

Zero/Restore PMT Gains With Imaging Shutter: When applicable this option will automatically zero the PMTs when the imaging hard shutter is closed and then restore the previous gains while opening the imaging hard shutter in preparation for the next acquisition. When this option is enabled the software will wait for a minimum of 200ms prior to scanning to allow the PMT gains to ramp up, this wait time is concurrent with any additional wait time specified for any physical shutters.

Preserve User-Modified File Names: Causes Prairie View to retain the file names entered in the fields for saving Z-Series, T-Series, and Single Scan, Voltage Recording, and other types of acquisition files. This option prevents these names from reverting to the default values the next time Prairie View is started.

Use 12-bit Sampling: On a system with a Resonant Scanner, the user may choose to digitize data on a 12-bit scale to match that of systems without a Resonant Scanner. When this option is not enabled, Resonant Scanner system acquire images with 13-bit sampling (in Galvo, Spiral, and Resonant modes).

Nyquist Sampling: Opens a dialog in which users determine preferences for the calculations used in the Nyquist Sampling feature found in the Tools menu.

[Frame Trigger Output Selection](#): Allows the user to determine which frame triggers are generated by the system during acquisition.

Save Images Generated With Photoactivation: When enabled, TIFF files are created for scans that used [Photoactivation](#) Masks.

[T-Series](#): Opens the T-Series Preferences dialog.

[Z-Series](#): Opens the Z-Series Preferences dialog.

Adjust Focus with Mouse Wheel: This option allows the user to move the Z device by scrolling with the mouse wheel. To use this feature, first enable the option in the Preferences menu. Then left click in an Image window to bring software focus to that window; the title bar of that window will change color to reflect its active status. The size of the movement per increment of the mouse wheel is determined by the Z step defined in the [Stage Control](#) section of the Main Control window.

Automatically Convert Raw Files

During acquisition, data is streamed to one or more raw files. This allows the system to devote resources to preserving the integrity of the data stream, while delaying most of the processing until acquisition is complete.

Options in this sub-menu allow the user to choose when the raw files are converted to their final formats (TIFF, CSV, or SDT files, depending on the type of acquisition).

Never (Use Image-Block Ripping Utility): Files will not be converted automatically. The images and other files must be converted later using the stand-alone [Image-Block Ripping Utility](#). This option minimizes the processing overhead at the end of an acquisition, and therefore minimizes the time before the next acquisition can begin.

After Acquisition: This is the most commonly chosen conversion option. All images and other data are kept as raw files until the acquisition (T-Series, Z-Series, other), and then converted at the end of the acquisition. Note that long acquisitions may require several minutes to convert; during this time the user cannot start another acquisition.

During Acquisition (May Add Delays Within Acquisition): Raw files will be converted as the acquisition progresses. This means that users can access newly acquired images while an acquisition is still in progress. T-Series images acquired with the Max Speed option enabled will be converted at the end of the Max Speed cycle. Note that this option will take some time to convert raw data between acquisitions. If minimizing the time between acquisitions is important then one of the other two options is a better choice. If there is a planned gap between acquisitions, for example waiting 5 minutes between repetitions, this option allows that time to be used to convert raw files, instead of having to wait for the entire data set to be converted later.

Users of systems with solid state drives (SSDs) are advised to have their Save Path set to the drive letter of the solid state drive (typically the E: drive) and to have the automatic conversion turned off. The data should be moved onto the system (C:) drive and then converted to TIFF images there using the [Image-Block Ripping Utility](#). This reduces the amount of data being written to the SSD, prolonging its useful lifetime.

Fluorescence Lifetime Imaging (FLIM)

The SDT file format is used by SPCImage (Becker & Hickl's FLIM analysis software) to analyze FLIM data. If the Preferences menu option to [Automatically Convert Raw Files](#) enabled, FLIM data is converted to SDT files immediately after the FLIM acquisition is finished. If this option is not checked, then Prairie View creates a batch file in the same folder as the one containing the FLIM raw data. Running the batch file would convert the raw data into SDT file.

Additional options in the Preferences > FLIM sub-menu provide additional controls for file handling.

Compress SDT files:

SDT files contain multidimensional FLIM data which leads to large file sizes. However, this data can be compressed to save disk space without any loss. If this option is checked, SDT files are compressed as they are created.

Keep Raw Data Files:

When this option is checked, the raw FLIM data is not deleted after SDT file creation. This can be used in cases where the user wants to analyze the FLIM data in different software (from SPCImage).

Photon Counting Only:

When this option is checked, the system operates in the Photon Counting Mode. In this mode, no SDT file is created and the system does not record the fluorescence lifetime data. Instead, the system generates TIFF images, where the intensity of each pixel corresponds to the number of photons detected at that location.

Acquire Phosphorescence Data:

When this option is checked, the system will acquire phosphorescence lifetime (PhLIM) data in addition to fluorescence lifetime (FLIM) data. The dwell times are each pixel are significantly longer in this mode of operation, but the laser is blanked for a significant portion of that time unlike normal scanning operations where the laser is continuously pulsing. Two SDT files are created for each frame of data in this mode, one for FLIM data and one for PhLIM data; FLIM data is acquired while the laser is pulsing and PhLIM data is acquired while the laser is blanked. More detail information can be found in the [phosphorescence lifetime](#) section.

Phosphorescence Lifetime Imaging (PhLIM)

Phosphorescence lifetime (PhLIM) data collection is an extension of fluorescence lifetime collection, to use this feature change the acquisition mode to FLIM in the [Scanning section](#) of the main form and be sure to enable phosphorescence data acquisition in the [Fluorescence Lifetime Imaging \(FLIM\) Preferences](#) submenu of the [Preferences](#) menu.

When PhLIM data collection is enabled two things will happen: one a new image window will appear which will show phosphorescent photon counts (existing image windows will continue to display fluorescent photon counts) and the dwell time controls will be replaced with more advanced controls for controlling laser blanking.

PhLIM Excitation Controls

On Time [μs]	10	-	+
Dwell Time = On Time x Cycles/Pixel			
Cycle Period [μs]	80	-	+
Cycles Per Pixel	10	-	+

PhLIM Laser Blanking Controls

When acquiring PhLIM data the laser will no longer scan across a line without stopping like Galvo or FLIM acquisition mode (without PhLIM enabled), but instead will stop at each pixel for a number of excitation cycles and then proceed to the next pixel. The number of excitation cycles is defined by the **Cycles Per Pixel** parameter.

Each excitation cycle is made up of an **On Time** where the laser is pulsing and FLIM data can be acquired, and an off time where the laser is blanked and PhLIM data can be acquired. The two configurable parameters are **On Time** and **Cycle Period** which is the sum of the on and off times. The equivalent of a dwell time when acquiring PhLIM data is the product of the on time and the number of excitation cycles, where dwell time is thought of as the time the laser is actually pulsing for each pixel.

Since the laser is not blanked/unblanked instantaneously, the Pockels lag time configured on the scan settings dialog is used as padding on either side of the on time to exclude photons which could have been caused by fluorescence as being phosphorescence. This is why the time period represented in the PhLIM SDT file is actually twice the Pockels lag time shorter than the off time.

Phosphorescence Image Window

When PhLIM data acquisition is enabled an additional image window is brought up to display phosphorescent photon counts; the other image windows will continue to show fluorescent photon counts. The phosphorescence image window has its own [lookup tables](#) which only affect the one image window and can be accessed by pressing the **LUT** button on the image window.

Frame Trigger Output Selection

Prairie View can automatically generate frame triggers during an acquisition. These triggers can be used internally to synchronize imaging with other Prairie View modules such as Voltage Output, Mark Points, Voltage Recording, etc. The triggers can also be used externally by hardware from Bruker Fluorescence Microscopy or other companies.

In some system configurations, external frame triggers are required to run particular pieces of hardware. Do not disconnect frame trigger signals that run between electronics boxes on the system. If the frame trigger port is already in use, add a T-connector to split the signal rather than severing the connection between existing control boxes.

Galvo, Spiral, and AOD modes

The frame trigger is a 5 Volt signal. The rising edge of the signal marks the time of the trigger. The signal will remain high until being pulled back to 0 Volts briefly before the next rising edge.

The user can choose what triggers are generated via the **Preferences** menu.

The hardware present for generating and counting frame triggers allows any two of three functions to happen simultaneously. The three possible functions are:

- Generate Start of Frame triggers
- Generate End of Frame triggers
- Count frame triggers internally (for Voltage Output, Mark Points, Voltage Recording, etc.)

To generate Start of Frame triggers and count frames internally, enable the following:

- **Preferences > Frame Trigger Output Selection > Trigger Logic > Generate/Count Start of Frame OR End of Frame Triggers** (the middle option in the sub-menu)
- **Preferences > Frame Trigger Output Selection > Start of Frame Trigger**

Trigger signals are used internally and also sent out via PCI-6713 FTO on the front of the GPIO box.

To generate End of Frame triggers and count frames internally, enable the following:

- **Preferences > Frame Trigger Output Selection > Trigger Logic > Generate/Count Start of Frame OR End of Frame Triggers** (the middle option in the sub-menu)
- **Preferences > Frame Trigger Output Selection > End of Frame Trigger**

Trigger signals are used internally and also sent out via PCI-6713 FTO on the front of the GPIO box.

To generate both Start of Frame and End of Frame triggers and disable internal frame counting, enable the following (note that this means that modules within Prairie View cannot be started on particular frame counts):

- **Preferences > Frame Trigger Output Selection > Trigger Logic > Generate Start of Frame AND End of Frame Triggers** (the last option in the sub-menu)

Start of Frame triggers are sent out via PCI-6052 FTO on the front of the GPIO box.

End of Frame triggers are sent out via PCI-6713 FTO on the front of the GPIO box.

To generate no frame triggers:

- **Preferences > Frame Trigger Output Selection > Trigger Logic > Generate NO Frame Triggers** (the first option in the sub-menu)

Resonant mode

When imaging in Resonant mode, frame triggers are generated by the Resonant Scanner control box rather than the GPIO box.

The signals are held at 0 Volts, and the rising edge of a short pulse of 5 Volt pulse marks the frame trigger.

Both Start of Frame and End of Frame triggers are always generated by this box. The signals are routed out of Start of Frame Out and End of Frame Out on the back of the Resonant Scanner control box.

The End of Frame signal is routed back to the GPIO box (via a BNC cable) for internal counting of frames by Voltage Output, Mark Points, Voltage Recording, and other software modules.

SFC mode

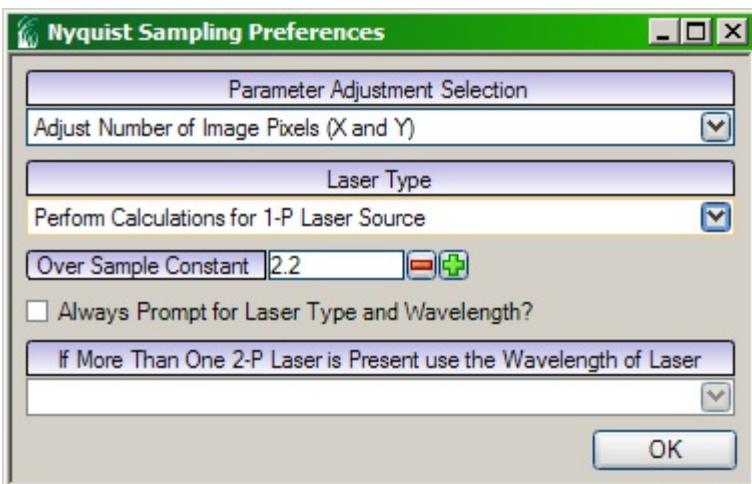
Frame triggers can be generated by the SFC control box and accessed from one or more AUX ports on the front of that box. This is described in the [SFC Settings - Triggers Tab](#) section of this manual.

Some cameras generate trigger signals, which can be accessed via ports on the camera itself.

Camera mode

In this mode, no frame triggers are generated by Prairie View software. Some cameras generate trigger signals, which can be accessed via ports on the camera itself.

Nyquist Sampling Preferences



The [Nyquist Sampling tool](#) will automatically adjust the scan settings so that the XY pixel size and z step size will satisfy the Nyquist sampling theorem. A dialog in the Preferences menu allows the user to configure parameters used by the Nyquist Sampling feature.

Parameter Adjustment Selection allows the operator to choose what changes are made to satisfy the Nyquist sampling theorem. There are two methods for getting the desired pixel size. One is to adjust the number of pixels in the XY image; the other is to adjust the optical zoom. The option specified determines the first parameter to be adjusted when setting the Nyquist sampling values. Depending on the current system configuration, both the number of pixels and the optical zoom may need to be adjusted. For example, if optical zoom was specified, but the computed optical zoom would be less than 1.0 (the system minimum), then the number of image pixels would also be changed to achieve the desired pixel size.

Laser Type specifies the laser source to be used for the Nyquist calculations. There is a difference in the equations used if the imaging is done with 2-P (two photon) versus a 1-P (single photon) excitation.

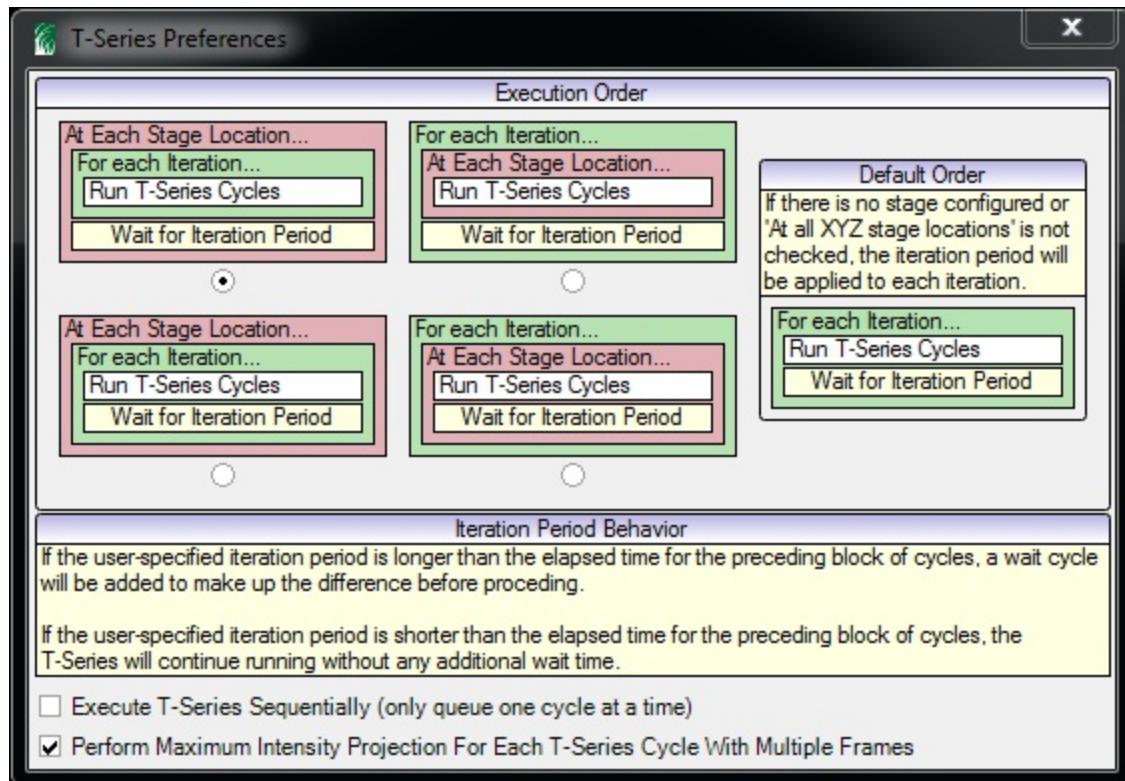
Over Sample Constant specifies the factor by which the pixel density will exceed the theoretical limit of resolution. The Nyquist sampling theorem requires sampling a minimum of two pixels for the smallest resolvable feature. The user can choose an Over Sample Constant from 2.0 to 4.0.

Always Prompt for Laser Type and Wavelength? specifies whether or not a dialog should appear to collect the desired wavelength value and laser type (1-P or 2-P) when the Nyquist option is invoked. This option is particularly useful with system configurations that include both 1-P and 2-P lasers, as the software won't know in advance what wavelength to use. This selection will also be used as the default for the Laser Type in the Tools menu dialog for Nyquist Sampling.

If More Than One 2-P Laser is Present use the Wavelength of Laser is only active if the system has more than one 2-P laser being controlled by Prairie View. If more than one laser is being controlled by *Prairie View*, then this will specify which laser to use for determining the wavelength for the Nyquist sampling calculation.

T-Series Preferences

The T-Series Preferences dialog (Preferences > T-Series) contains options that affect the display update and file saving during acquisitions.



T-Series Execution Order

In cases where the number of T-Series **Iterations** is greater than 1 AND the **Run at all XYZ stage locations** box is checked, the order of operations in a T-Series can be chosen by the user. The dialog contains graphical representations of the T-Series **Execution Order**. The **Iteration Period** designation in the execution order options indicates at which layer of operations the **Period** for iterations is applied.

Consider as an example a T-Series which contains 5 cycles totaling 3 minutes, 2 iterations, the **Run at all XYZ stage locations** box is checked, 4 stage locations are saved, and the Period is set to 3600 seconds. The option selected in the screen shot above is “For each Iteration, At Each Stage Location, Run T-Series Cycles”, with “Wait for Iteration Period” at the Iteration level. This means that the 5 cycles would be performed at the first stage location, then the second stage location, etc, and then the system would drive back to the first stage location. The 3600 second period would be applied at the iteration level, so after a total of 3600 seconds (including acquisition time), the whole cycles/stage locations acquisition would be repeated as the second iteration. The top portion of the T-Series Preferences window contains options for the T-Series Execution Order, described elsewhere in this manual.

Additional Options

The **Execute T-Series Sequentially (only queue one cycle at a time)** option, when enabled, forces the software to prepare each cycle as it is run. Note that enabling this option can result in increased delay time between T-Series cycles. Most experiments are best run with this option

disabled, so the software can prepare the list of cycles before beginning acquisition, and minimize the inter-cycle delay time. This option needs only be checked if very specific types of T-series are being executed. This would primarily pertain to T-series where some aspect of a given cycle is affected by a preceding cycle, such as Script commands for dynamic changes to an existing Z-Series definition. Contact Bruker Fluorescence Microscopy personnel for assistance with complex scripting within a T-Series, and the use of this option.

The Perform Maximum Intensity Projection For Each T-Series Cycle With Multiple Frames

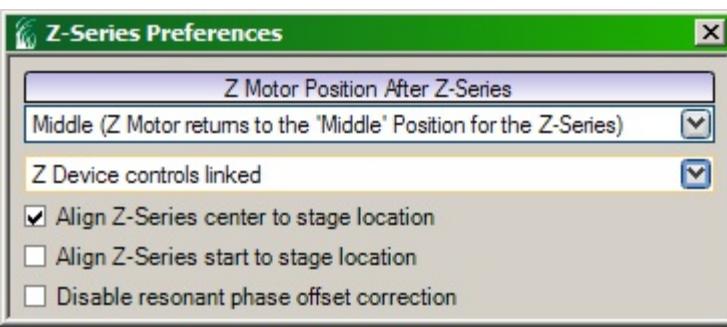
option, when enabled during raw data conversion of T-Series and Z-Series, creates pre-calculated Maximum Intensity Projection tiff files during conversion. The files are located in the "MIP" directory underneath the main data directory. The files can be used in playback mode to play through MIP projections smoothly.

Additional selections in the Preferences menu pertain to T-Series acquisitions.

The user can choose whether or not data is automatically written to TIFFs after a T-Series with the Preferences menu options to [Automatically Convert Raw Files](#). If images are not automatically converted, there is less processing overhead at the end of the T-Series before another image can be acquired. The images can be converted later using the stand-alone [Image-Block Ripping Utility](#). Users of systems with solid state drives (SSDs) are advised to have their Save Path set to the drive letter of the solid state drive (typically the E: drive) and to have the automatic conversion turned off. The data should be moved onto the system (C:) drive and then converted to TIFF images there using the Image-Block Ripping Utility. This reduces the amount of data being written to the SSD, prolonging its useful lifetime.

When files are automatically converted after a T-Series, the user can also choose whether to review T-Series data immediately after conversion. This is accomplished using the **Automatically Start Playback After Acquisition** option in the Preferences menu.

Z-Series Preferences



The Z-Series Preferences dialog is accessed through the Preferences menu in the Prairie View main control window (Preferences > Z-Series).

The **Z Motor Position After Z-Series** dropdown provides options for the position of the Z device after the Z-Series is completed. This setting will also determine the Z device position after executing a Z-Series from within a T-Series. Note that these settings can be useful to users performing Z-Series acquisitions while holding a patch pipette in place – a delicate patch could be severed when a motor makes a large movement, and by choosing to leave the Z device at the Stop position after a Z-Series, the user can prevent the motor from making a large movement at the end of the acquisition.

If a Focus Lock device is present (for example Nikon's Perfect Focus System), and enabled for the Z-Series, its state after the Z-Series will depend on the **Z Motor Position After Z-Series** setting. **Default** will restore the Focus Lock state before the Z-Series started, **Start** will restore the Focus Lock state recorded with the Z-Series start position, **Middle** will only restore a Focus Lock state if the Z-Series was centered on an [XY Stage](#) location (in which case it will restore the Focus Lock state of the XY Stage position), and **Stop** will always disable the Focus Lock.

A second dropdown allows the user to link the Z Device controls on systems configured with multiple Z devices; this is described in the [Using Multiple Z Devices](#) section.

The **Align Z-Series center to stage location** and **Align Z-Series start to stage location** checkboxes allow the user to choose whether saved coordinates are used as the center or start of a Z-Series (when performing a Z-Series at multiple X,Y stage locations).

The **Disable resonant phase offset correction** option determines whether resonant scanning systems automatically adjust phase offset during a Z-Series. When collecting slices above and below the main slices of interest, there may not be enough clearly-defined features in the sample for the automatic offset to function. This can result in image artifacts from automatic correction. See additional information in the Resonant Phase Offset section of this manual.

Tools Menu

[**Nyquist Sampling**](#): Adjusts the scan settings to minimize over-sampling and optimize spatial acquisition parameters.

[**Fluorescence Unmixing**](#): Opens a control panel for adjustment of signal data between the channels.

[**Zeiss MTB Microscope Controls**](#): Opens a dialog for controlling motorized components of a Zeiss microscope stand.

[**Nikon Ti Microscope Controls**](#): Opens a dialog for controlling motorized components of a Nikon Ti microscope stand.

[**Nikon Ni Microscope Controls**](#): Opens a dialog for controlling motorized components of a Nikon Ni microscope stand.

[**Fluorescence Lifetime Imaging \(FLIM\)**](#): Opens a dialog of controls and parameters relevant to acquisition in FLIM mode.

[**Scripts**](#): Scripts are commands that allow the user to customize an operation to perform during an experiment. The items in this sub-menu allow the user to create, run, and abort Scripts.

[**Actions**](#): Brings up access to creating sets of external commands to use within *Prairie View* for the controlling of data import and export.

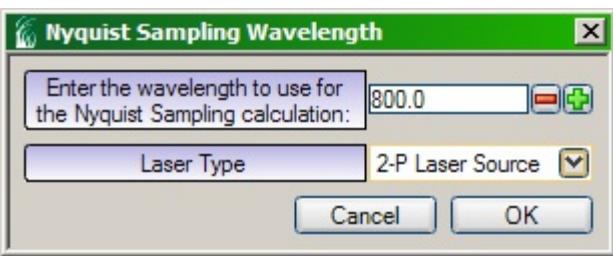
[**Calibration/Alignment**](#): Options in this sub-menu allow users to set up and modify software-controlled calibrations. Individual options are described elsewhere in this manual.

[**Reset**](#): Options in this sub-menu allow the user to reset the connection to listed components in the event of a failure. Components may include a Dott Controller, MAMC, and Preamplifier.

[**Scan Settings**](#): Brings up a panel of settings for the scan parameters of the system. These settings should only be changed by Bruker personnel. These settings are not intended for operator use.

[**Maintenance**](#): Opens a dialog allowing independent control of shutters and galvanometers, for use during installation, alignment, and troubleshooting.

Nyquist Sampling



Nyquist Sampling will automatically adjust the scan settings so that the pixel size will satisfy the Nyquist sampling theorem as well as the z step size for Z-Series acquisitions. The reasoning behind this is to set up the spatial acquisition parameters (x, y, and z) for optimal image data collection. These system settings will help to eliminate unnecessary over sampling of the data and protect against under sampling of the data.

Preferences associated with the Nyquist Sampling tool can be accessed by the user via the Preferences menu ([Preferences > Nyquist Sampling](#)).

The Nyquist sampling algorithm is invoked by selecting Tools > Nyquist Sampling or by pressing **<F9>**.

After invoking Nyquist sampling, if the currently selected objective lens has not been calibrated, a message box will display the necessary corrective action.

If the currently selected objective lens is calibrated and one or more of the following are true, then a dialog will appear to collect the wavelength to use for the Nyquist sampling calculation:

- The laser source is 1-P, **or**
- The laser source is 2-P and not being controlled by Prairie View, **or**
- Always Prompt for Laser Type and Wavelength? is checked in the [Nyquist Sampling Preferences](#) dialog

The dialog will also allow the laser type (1-P or 2-P) to be specified.

If **Cancel** is pressed then no changes will be made.

Once **OK** is pressed, the system settings will change as necessary.

If the currently selected objective lens is calibrated **and** the laser source is 2-P **and** the laser is being controlled by Prairie View, then the system settings will change as necessary without any further interaction.

In addition to adjusting the number of pixels in the x and y dimensions and/or the optical zoom, the Nyquist sampling logic will also set the proper Z-series step size. This adjustment will happen only if the radio box next to the 'Step Size' IS NOT set to 'Calculate' on the [Z-Series tab](#).

Equations

The equations for determining the desired pixel size or z step size are as follows:

For a 1-P laser:

Desired x and y pixel size (in nanometers) = $((0.61 * \lambda) / \text{NA}) / \text{OS}$

Desired z step size (in nanometers) = $((0.61 * \lambda) / \text{NA}) / \text{OS} * (\pi / \text{NA})$

For a 2-P laser:

Desired x and y pixel size (in nanometers) = $((0.61 * (\lambda / \sqrt{2})) / \text{NA}) / \text{OS}$

Desired z step size (in nanometers) = $((0.61 * (\lambda / \sqrt{2})) / \text{NA}) / \text{OS} * (\pi / \text{NA})$

Where **λ** is the specified laser wavelength in nanometers, **NA** is the numerical aperture of the current objective lens, **OS** is the Over Sample Constant specified by the user, and **$\sqrt{2}$** is the square root of two.

Fluorescence Unmixing



When Fluorescence Unmixing is selected, a user-defined percentage of the intensity of one channel is subtracted from that channel and added to another original channel. This enables the user to correct for bleed-through of signals from one channel to another.

By checking **Enable**, the fluorescence unmixing for that line will be applied.

The **Source Channel** is the channel to which the subtracted signal will be added, while the **Channel to Subtract From** will be the channel the signal is removed from.

The **Percentage** slider allows the operator to control the amount of the **Source Channel** signal to be subtracted from the **Channel to Subtract From**.

These controls will work either on a static image (acquired with **Single Scan**) or while in **Live Scan** mode. This altered image cannot be saved.

Consider the following example. There is bleed-through of Channel 1 into Channel 2. The goal is to subtract out the Channel 1 signal from Channel 2 and add it back to Channel 1:

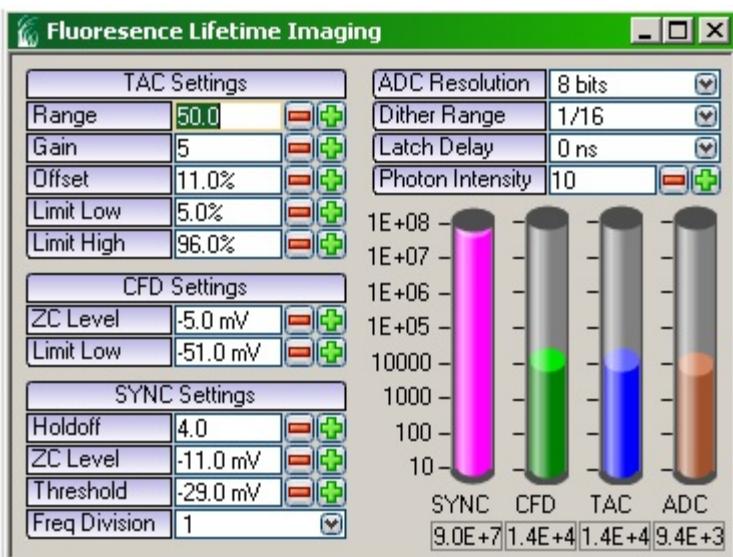
1. Place a check in the box in the **Enable** column for one line of unmixing controls
2. Set the **Source Channel** to Channel 1
3. Set the **Channel to Subtract From** to Channel 2
4. While acquiring a **Live Scan** or after having acquired the images on Channels 1 and 2 with a **Single Scan**, slowly move the **Percentage** control up. The signal from Channel 1 that is present in Channel 2 should diminish. At the same time, the signal in Channel 1 should increase by the same amount as this data is recovered into Channel 1.

One idea on how to do this visually so that not too much signal is subtracted from Channel 2 is to place Channel 2 into Range Check color mode, and then as the **Percentage** slider is increased, some blue (zero intensity) pixels will show in the area where Channel 1 was bleeding into Channel 2. This shows that the right amount of Channel 1 has been subtracted from

Channel 2.

All images acquired while Fluorescence Unmixing is enabled will have the unmixing applied.

Fluorescence Lifetime Imaging (FLIM)



TAC (Time to Amplitude Converter) Settings: This section is used to change different TAC parameters. The default values for Range and Gain are 50 and 10 respectively. The Offset is set to position the TAC range on the TAC characteristics. TAC contains a window discriminator to suppress values outside the range defined by Limit Low and Limit High.

CFD (Constant Fraction Discriminator) settings: This section is used to set the CFD parameters. The signal into CFD comes from the detector. Any pulses below the Limit Low are ignored. ZC Level is set to avoid spurious triggering because of noise; its ideal value is 0 but a practical value is around -10mV.

SYNC (Synchronous) settings: This section is used to set the SYNC parameters. This signal comes from the excitation source. Any pulses below the 'Threshold' are ignored. ZC Level is set to avoid spurious triggering because of noise; its ideal value is 0 but a practical value is around -10mV.

ADC (Analog to Digital Converter) Resolution: This defines the number of time bins per laser pulse period. The higher the ADC resolution, the greater is the number of time bins but the lower is the number of counts per time bin. A default value of 8 bits (256 time bins) is used.

Dither Range: This is used in error correction during the ADC operation. It is set to a default value of 1/16.

Latch Delay: For dual channel FLIM systems, a router is used to indicate the channel generating the data. The router adds a delay to the electronics. This parameter is set to 0nsec for single channel FLIM and 20nsec for dual channel FLIM systems.

Photon Intensity: This is used for live display of fluorescence data during a FLIM acquisition. Intensity at each pixel is given by Photon intensity multiplied by the number of photons at that pixel.

Additional information about FLIM can be found in the discussion of [FLIM preferences](#).

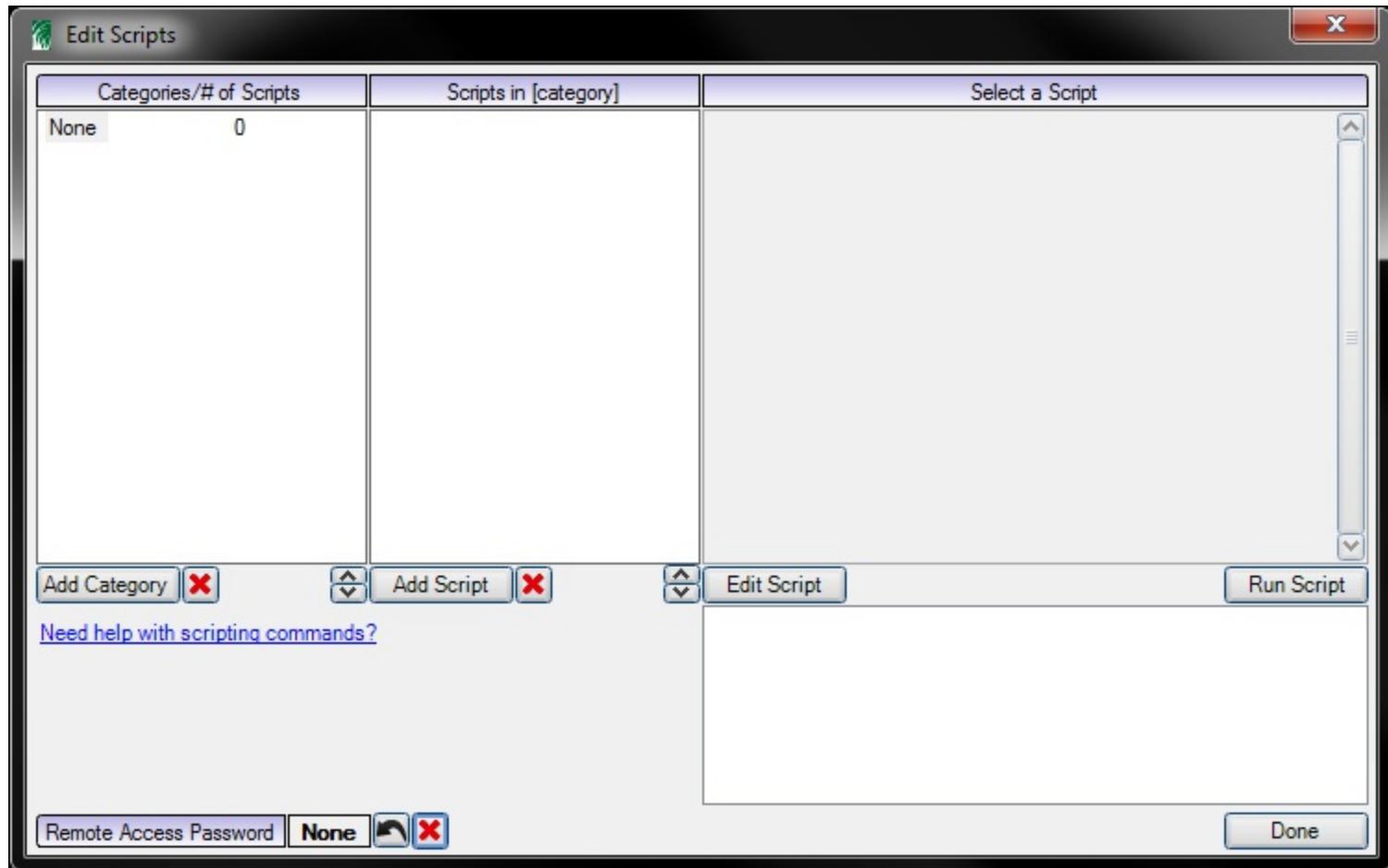
Scripts

Scripts are commands that allow the user to customize an operation to perform during an experiment. They are essentially a programming language embedded in Prairie View to expand functionality without cluttering up the interface. A Script could change laser power, find a particular slice of a Z-Series, or do any number of other things.

Script controls are accessed from the Tools menu. The Scripts sub-menu will expand as scripts are defined, to allow the user to run and abort Scripts through the menu options.

Defining a Script

Scripts are defined in the dialog opened by the **Edit Scripts...** menu option in the main menu under **Tools -> Scripts**.



Script definitions are divided into Categories, which are listed on the left side of the window. A Category could be a particular experiment in which the script would be used, the name of the user who defined the Script, or the type of command used in the Script (PMT, Stage, Laser, etc.). Categories can be added or deleted via buttons below the list of existing Categories.

When a Category has been selected, the Scripts within that category will be displayed in a list in the middle of the window. Scripts can be added or deleted via the buttons below the list of Scripts.

The command(s) within the selected Script are defined in the Select a Script box on the right

side of the window. Clicking the **Edit Script** button allows the user to type in the box to add or change commands. Detailed information about scripting commands can be accessed from within this window by clicking **Need help with scripting commands?** at the bottom of the window.

To test a Script, select it from the list and click the **Run Script** button. Status messages in the box below the editor report the time and status of each Script run from the editor.

After defining or editing Scripts, click the **Accept** button to close the Edit Scripts window. Clicking **Cancel** will close the window without saving the additions and changes made during that session.

For additional security when connecting the system to a network; to set a remote access password, press the button with an arrow to generate a random password. To change the remote password, use the same button. To remove the password, press the button with an X. The password is only required when connecting from another machine (i.e. not using the loopback address on the same computer) and any connections attempting to send commands before providing the password will be ignored and disconnected. The password is user specific for multi user systems to help prevent accidental commands being sent from a computer another user would normally be using for their experiments.

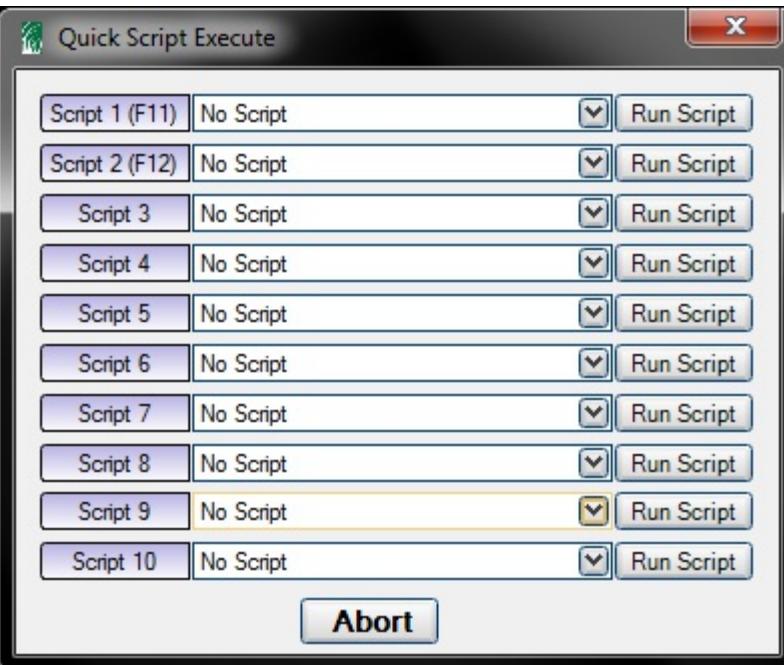
Running a Script

A Script can be run directly from the Edit Scripts window by clicking the **Run Script** button. This can be useful when testing a newly defined script.

A Script can be run from the Tools menu by selecting it from a sub-menu defined by the Category.

A Script can be run during a [T-Series](#) by adding a Script cycle to that T-Series and then selecting the previously-defined Script from the drop-down menu for the cycle.

The **Quick Execute** option in the Scripts menu opens a dialog in which the user can assign up to 10 Scripts to individual **Run Script** buttons. This dialog can be left open for easy access to the chosen Scripts. The **Abort** button at the bottom of the dialog allows the user to stop the Script.



Stopping a Script

If a Script was started from the Tools menu, it can be stopped using the Abort option in the Script sub-menu (Tools > Scripts > Abort). When using the Quick Script Execute dialog to run a Script, use the **Abort** button at the bottom of the dialog. It is also possible to abort a script by pressing the Pause/Break key; either option will immediately abort any acquisition in progress, or anything else in progress, in addition to aborting any scripts currently running.

Script Command Reference

A command is a case insensitive keyword (or abbreviation) prefixed by a hyphen (-), backslash (\) or forward slash (/). Command keywords and abbreviations are listed below along with any associated parameters.

Abbreviations, shown in parenthesis after the command name, can be used to reference commands rather than entire words or phrases.

Each command may take one or more parameters each specific to the command. Some are required and some are optional. Parameters and commands are delimited by spaces, any parameters containing spaces need to be enclosed in double quotations ("") to denote that the spaces are part of the parameter.

Note: **Bold text** is entered as is; *grey italicized text* needs to be replaced with actual values before entered.

When a script is executed from the "Edit Scripts" dialog within Prairie View by pressing the "Run Script" button, the "Run Script" button will be modified to display "Abort Script". If this button is pressed while it is displaying "Abort Script", the currently executing script will be terminated. In certain instances, it will be necessary for the currently executing script command to finish before the remainder of the script is aborted; any scan in progress will be aborted immediately. Likewise, if a script is executed from the 'Tools->Scripts' menu directly, there is an "Abort Script" menu option that will become active when the script is started. Clicking on the "Abort Script" menu option will operate on menu started scripts the same way that the "Abort Script" button operates on scripts started from the "Run Script" button in the "Edit Scripts" dialog.

In addition to sending script commands via the command line, it is possible to connect directly to Prairie View via TCP/IP for a faster, more flexible, interface. Note that this option will require some programming in any environment which supports TCP/IP communication, such as MATLAB or C. Prairie View is always listening for connections on port 1236; once a connection is established send commands as you would type them on a command line, with two notable exceptions: instead of spaces between commands and arguments, use an ASCII character 1 instead (not the number 1 which is ASCII character 49), and instead of pressing enter to run the commands send a carriage return (ASCII character 13) followed by a line feed (ASCII character 10). After Prairie View receives the commands and arguments it will respond with ACK

terminated by a carriage return and line feed character, then proceed to execute the commands. Once the commands have been executed Prairie View will respond DONE terminated by a carriage return and line feed character. Any commands which return a result, such as GetState, will respond after the ACK response and before the DONE response, again terminated by a carriage return and line feed character. When finished with the connection send the -x command (TCP/IP only) to gracefully close the connection with Prairie View.

When running scripts from a different computer it may also be required to provide a password immediately upon connecting before sending any other commands; this password is set on the [Edit Scripts](#) dialog accessible under the 'Tools' -> 'Scripts' menu.

Command Categories

(* denotes a TCP/IP only command)

General Commands

[-AppendNote \(-an\)](#)
[-ClearNotes \(-cn\)](#)
[-ConfigurationFile \(-cf\)](#)
[-DoNotWaitForScans \(-dw\)](#)
[-ExecuteProgram \(-ep\)](#)
[-ExecuteScript \(-es\)](#)
[-Exit \(-x\)](#)
[-MessageToOperator \(-mto\)](#)
[-NoWait \(-nw\)](#)
[-Silent \(-s\)](#)
[-UtilityButton \(-ub\)](#)
[-Wait \(-wt\)](#)
[-WaitForInputTrigger \(-wfit\)](#)

File Commands

[-LoadEnvironment \(-le\)](#)
[-LoadTemplate \(-lt\)](#)
[-LoadImages \(-li\)](#)
[-LoadROIFile \(-lrf\)](#)
[-SaveEnvironment \(-se\)](#)
[-SetSavePath \(-p\)](#)
[-SetFileIteration \(-fi\)](#)
[-SetFileName \(-fn\)](#)

Action Commands

[-ClearBOTs \(-cb\)](#)
[-ClearROIs \(-cr\)](#)
[-GetBOTRegions \(-gb\)](#)
[-GetROIs \(-gr\)](#)

[-Abort \(-stop\)*](#)
[-DroppedData \(-dd\)*](#)
[-GetImage \(-gi\)*](#)
[-LimitGSDMABufferSize \(-lbs\)](#)
[-LiveScan \(-lv\)](#)
[-MarkPoints \(-mp\)](#)
[-PointScan \(-ps\)](#)
[-ReadRawDataStream \(-rrd\)*](#)
[-SingleScan \(-ss\)](#)
[-SingleScanTriggered \(-sst\)](#)
[-StreamRawData \(-srd\)*](#)
[-TSeries \(-ts\)](#)
[-TSeriesLoad \(-tsl\)](#)
[-WaitForScan \(-w\)](#)
[-SetAcquisitionMode \(-sam\)](#)

Line Scan Commands

[-GetFreehandLine \(-gl\)](#)
[-Linescan \(-ls\)](#)
[-LinescanDialog \(-ld\)](#)
[-LinescanLines \(-lsl\)](#)
[-LinescanMode \(-lm\)](#)

Z-Series Commands

[-FindSlice \(-fs\)](#)
[-SetZSeriesNumberOfSlices \(-zsn\)](#)
[-SetZSeriesStart \(-zsb\)](#)
[-SetZSeriesStepSize \(-zs\)](#)
[-SetZSeriesStop \(-zse\)](#)
[-ZSeries \(-zs\)](#)
[-ZSeriesLoad \(-zsl\)](#)
[-ZSeriesMoveTo \(-zsm\)](#)
[-ZSeriesSave \(-zss\)](#)

Hardware Commands

[-Camera \(-ca\)](#)
[-CenterGalvos \(-cg\)](#)
[-GetMotorPosition \(-gmp\)*](#)
[-MoveMotor \(-mr\)](#)
[-NikonNiMicroscope \(-nni\)](#)
[-NikonTiMicroscope \(-nti\)](#)
[-PanGalvo \(-png\)](#)
[-SendGPIOCommand \(-gc\)](#)
[-SendMAMCCommand \(-mc\)](#)
[-SendPiezoCommand \(-pc\)](#)
[-SendResonantCommand \(-rc\)](#)
[-SetMotorPosition \(-ma\)](#)
[-SetObjectiveLens \(-sol\)](#)
[-SFC \(-sfc\)](#)
[-ZDeviceControl \(-zdc\)](#)
[-ZeissMicroscope \(-zm\)](#)

Laser/PMT Commands

[-SecondaryLaserBeamRoute \(-slbr\)](#)
[-SetLaserPower \(-lp\)](#)
[-SetMultiPhotonWavelength \(-mpw\)](#)
[-SetPMTGain \(-pg\)](#)

Shutter Commands

[-OverrideHardShutter \(-ohs\)](#)
[-SetHardShutter \(-hrd\)](#)
[-SetHardShutterSelection \(-shss\)](#)
[-SetSoftShutter \(-sft\)](#)

Stage Position Commands

[-SetActionAfterFrame \(-af\)](#)
[-SetActionAfterScan \(-as\)](#)

Image Commands

[-ImageWindowFit \(-iwf\)](#)
[-ImageWindowLarger \(-iwl\)](#)
[-ImageWindowOriginal \(-iwo\)](#)
[-ImageWindowSmaller \(-iws\)](#)

Scan Setting Commands

[-EnterROI \(-er\)](#)
[-GetState \(-gts\)*](#)
[-ParameterSet \(-pa\)](#)
[-ROILoad \(-roi\)](#)
[-SamplesPerPixel \(-spp\)*](#)
[-SetChannel \(-c\)](#)
[-SetDwellTime \(-dt\)](#)
[-SetFrameAveraging \(-fa\)](#)
[-SetImageSize \(-is\)](#)
[-SetOpticalZoom \(-oz\)](#)
[-SetScanRotation \(-sr\)](#)
[-SetState \(-sts\)](#)

[-AddStagePosition \(-spa\)](#)
[-ClearStagePositions \(-spc\)](#)
[-LoadStagePositionFile \(-lspf\)](#)
[-MoveToStagePosition \(-mtsp\)](#)
[-SaveStagePositionFile \(-sspf\)](#)
[-SetGridLocations \(-sgl\)](#)
[-SetGridOverlap \(-sgo\)](#)
[-SetGridStartLocation \(-sgstartl\)](#)
[-SetGridStopLocation \(-sgstopl\)](#)

-CommandName (CommandAbbreviation) "Parameter 1 (Required)" "Parameter 2 (Optional)"

This is an example command showing the format that the actual commands below will follow.

-Help (-h)

Displays this document.

General Commands

-AppendNote (-an) "note (Required)"

Adds the given text to the notes. Notes are accessible under the "Tools" menu on the main form or by pressing F8. Notes containing specific keywords enclosed in <>'s will be replaced with actual values when the script command is executed. Keywords are case-insensitive and include any of the xml keys used to record Prairie View's state (these include "pixelsperline", "rotation" and "opticalzoom" to name a few) as well as specific keywords such as: "time" which translates to the current time and "linescancount" which translates to the current line number when appending to a line scan.

[Back to Commands](#)

-ClearNotes (-cn)

Clears any notes currently recorded. Notes are accessible under the "Tools" menu on the main form or by pressing F8.

[Back to Commands](#)

-ConfigurationFile (-cf) "filename without path (Required)"

This command has no effect unless Prairie View is launched with it (i.e. it is included as

part of the Prairie View shortcut). This command specifies another configuration file aside from the default ‘configuration.xml’ in the ‘Configuration Files’ folder to load instead. This allows a single version of Prairie View to support two separate configurations (i.e. one with 2P laser control and one without).

[Back to Commands](#)

-DoNotWaitForScans (-dw) "*execute commands during scans (True/False) (Optional, defaults to True)*"

When this flag is set to True script commands will no longer wait for a scan in progress to complete before executing, with the exception of commands which start a new scan which will always wait.

[Back to Commands](#)

-ExecuteProgram (-ep) "*path (Required)*" "*arguments (Optional)*" ...

Runs the program specified by the given path and passes along any additional arguments provided. This command can be used to launch another program automatically as Prairie View starts up.

[Back to Commands](#)

-ExecuteScript (-es) "*category (Required)*" "*script (Required)*" "*iterations (Optional)*" "*iteration interval (Optional)*" "*iteration variable (Optional)*"

Executes the specified *script* within the specified script *category*. If the script being started calls any script before it, a warning message will be displayed and the entire script will be terminated. The optional argument, *iterations*, indicates the number of times that the specified script should be repeated. If this value is an integer (1, 2, 3, ...) that indicates the number of times to execute the specified script. If the value of *iterations* is "allXY" (without the quotes), that indicates to execute the specified script at all of the defined XY stage locations. If the value of *iterations* is "allROI" (without the quotes), that indicates to execute the specified script for all of the ROI definitions. If the value of *iterations* is "allZSlices" (without the quotes), that indicates to execute the specified script at each slice of the currently defined Z-series. The optional argument, *iteration interval*, indicates the amount of time (in seconds) to wait from the start of one iteration to the start of the next iteration of the specified script. If the value for *iterations* is "allXY", then the value of *iteration interval* indicates the amount of time to wait between executing the specified script at each of the XY stage locations. If the value for *iterations* is "allROI", then the value of *iteration interval* indicates the amount of time to wait between executing the specified script for each ROI definition. If the value for *iterations* is "allZSlices", then the value of *iteration interval* indicates the amount of time to wait between executing the

specified script at each slice of the currently defined Z-series. The optional argument, *iteration variable*, is a string that will contain the current iteration number. To use this variable, both of the optional arguments, *iterations* and *iteration interval* must be specified. This *iteration variable* may then be accessed by specific commands such as "ZSeriesLoad".

[Back to Commands](#)

-Exit (-x)

This command is only used when calling commands via TCP/IP port 1236. The exit command lets Prairie View know that you are done sending commands and it can gracefully close the connection. When sending commands via TCP/IP arguments are separated by character 1 instead of spaces and each command, or set of commands, is terminated by a carriage return (character 13) followed by a line feed (character 10). Prairie View will respond in kind after each command is processed with 'DONE'.

[Back to Commands](#)

-MessageToOperator (-mto) "message (Optional)"

Displays the designated *message* to the operator in a dialog box. The script will 'hold' at this point waiting for the operator to hit the 'OK' button to continue the script execution or to hit the 'Cancel' button to abort the script.

[Back to Commands](#)

-NoWait (-nw)

If this command is present anywhere in a string of commands to be executed Prairie View will not wait for the commands to be processed before either returning control to the controlling application if the commands were called from the command line or PrairieLink, or returning a DONE response immediately if the commands were sent directly over a TCP/IP port.

[Back to Commands](#)

-Silent (-s)

If this command is present anywhere in a string of commands to be executed Prairie View will not pop-up any error message boxes caused by invalid commands/parameters.

[Back to Commands](#)

-UtilityButton (-ub) "index (1,2,3,4) (Required)" "state (open/close) (Required)"

Sets the 'Utility Button' (found on the 'Misc' tab) specifiied by *index* to the specified *state*.

[Back to Commands](#)

-Wait (-wt) "time in milliseconds (Required)"

Pause/wait for the specified number of *milliseconds*.

[Back to Commands](#)

-WaitForInputTrigger (-wfit)

Executing this script command will cause the script to pause until a trigger signal (rising edge) is captured on the trigger hardware for Prairie View. Be careful in the use of this command. When this command is executed, the program will hang indefinitely until a trigger signal is received. While waiting for the input trigger, the script will not respond to attempts to 'abort' the script. Without the receipt of a trigger signal, the only way to abort the script would be to terminate Prairie View.

[Back to Commands](#)

File Commands

-LoadEnvironment (-le) "file path and name (Required)"

Load the specified environment file.

[Back to Commands](#)

-LoadTemplate (-lt) "file path and name (Required)"

Load the specified template file.

[Back to Commands](#)

-LoadImages (-li) "file path and name (Required)"

Load the specified xml file and the associated image file(s) in playback mode.

[Back to Commands](#)

-LoadROIFile (-lrf) "file path and name (Required)"

Load the file containing saved ROI definitions.

[Back to Commands](#)

-SaveEnvironment (-se) "file path and name (Required)"

Save the current environment to the file specified.

[Back to Commands](#)

-SetSavePath (-p) "path (Required)" "addDateTime (Optional)"

Changes the directory where scan data is saved.

If there are any spaces in the *path* then it must be enclosed in double quotations(""). If the optional parameter "addDateTime" is included, then the current date and time will be added to the specified *path*.

[Back to Commands](#)

-SetFileIteration (-fi) "type (Zseries/Tseries/Linescan/Singlescan) (Required)" "value (Required)"

Sets the file iteration *value* for the specified acquisition *type*.

[Back to Commands](#)

-SetFileName (-fn) "type (Zseries/Tseries/Linescan/Singlescan) (Required)" "filename (Required)" "addDateTime (Optional)"

Sets the filename *filename* for the specified acquisition *type*. If the optional parameter "addDateTime" is included, then the current date and time will be added to the specified *filename*.

[Back to Commands](#)

Action Commands

-ClearBOTs (-cb)

Removes all saved regions of interest within the BOT option.

[Back to Commands](#)

-ClearROIs (-cr)

Removes all saved regions of interest.

[Back to Commands](#)

-GetBOTRegions (-gb) "actionName (Required)"

Runs the action with the specified *actionName* as already defined in Prairie View under "Tools/Actions...". This command makes it possible to use a 3rd party tool to analyze an image and generate regions to be loaded into Prairie View so that their intensity can be tracked over time.

[Back to Commands](#)

-GetROIs (-gr) "actionName (Required)"

Runs the action with the specified *actionName* as already defined in Prairie View under "Tools/Actions...". This command makes it possible to use a 3rd party tool to analyze an image and generate regions of interest to be loaded into Prairie View.

[Back to Commands](#)

-SetActionAfterFrame (-af) "*actionName (Optional)*" "*filename (Optional)*" "*argument (Optional)*" ...

Sets up an action to run for the next and any subsequent frames acquired. If just an *actionName* is given then the existing action with that name if any will be run. If the *actionName* is not in use then a new one will be created using the *filename* and *arguments* given. If the *actionName* already exists then the action will be updated with the given *filename* and *arguments* (can include as many as desired). If no *actionName* is passed then no action will be performed and any action currently being performed after frames will cease.

[Back to Commands](#)

-SetActionAfterScan (-as) "*actionName (Optional)*" "*filename (Optional)*" "*argument (Optional)*" ...

Sets up an action to run for the next and any subsequent scans. If just an *actionName* is given then the existing action with that name if any will be run. If the *actionName* is not in use then a new one will be created using the *filename* and *arguments* given. If the *actionName* already exists then the action will be updated with the given *filename* and *arguments* (can include as many as desired). If no *actionName* is passed then no action will be performed and any action currently being performed after scans will cease.

[Back to Commands](#)

Image Commands

-ImageWindowFit (-iwf)

Scales the image window(s) up or down to fit within a 512x512 pixel area on the display (this is the default image window size).

[Back to Commands](#)

-ImageWindowLarger (-iwl) "*percentChange (Optional)*"

Scales the image window up by the percent specified, if no percent is specified the image window(s) are scaled up 10%. Percents are based on the size of the original image in decimal format, for example '10%' would be passed in as '.1'.

[Back to Commands](#)

-ImageWindowOriginal (-iwo)

Resizes the image window(s) to a 1:1 scale based on the current imaging resolution.

[Back to Commands](#)

-ImageWindowSmaller (-iws) "*percentChange (Optional)*"

Scales the image window down by the percent specified, if no percent is specified the image window(s) are scaled down 10%. Percents are based on the size of the original image in decimal format, for example '10%' would be passed in as '.1'.

[Back to Commands](#)

Acquisition Commands

-Abort (-stop)

Aborts any script commands in progress. Any script commands sent along with this command will not be run, even commands appearing before the abort; just run them in a separate request if needed. This command will also abort any acquisitions in progress, even those not started with a script. Additionally any voltage output, voltage recording or mark point experiments in progress will also be aborted. This command will have no effect when run from within Prairie View; it is only for use from the command line, through PrairieLink or direct TCP/IP communication.

[Back to Commands](#)

-DroppedData (-dd)

This command responds with 'True' if data has been dropped during the current acquisition, otherwise it responds 'False'. This command is only useful when using either the command prompt or a TCP/IP connection since it only responds with a value and does not actually do anything.

[Back to Commands](#)

-GetImage (-gi) "*channel (1-4, 1-8 for PhLIM) (Required)*" "*process ID (Required)*" "*memory address (Required)*"

This is a low level command to get live image data from Prairie View, basically the contents of the image windows, for use in third party applications. It is primarily used by PrairieLink to provide a two dimensional array of data representing the image. In order to use the command you need to know what channel of data you are interested in getting, the ID of the process requesting the data (i.e. what is displayed in the task manager), and the address of some memory that has already been allocated to hold the data. The values placed into the memory block will be 32-bit unsigned integers.

[Back to Commands](#)

-LimitGSDMABufferSize (-lbs) "true/false (Required)" "minimum buffer time in ms (Optional)"

This command will limit the size of the DMA buffers for General Standards acquisition cards (used on systems with a resonant galvo) to a single frame, or at least 100ms (by default) if a frame period is shorter, in order to get display updates more often, or get raw data with lower latency with the –ReadRawDataStream (-rrd) command. This is automatically set to true when using start/stop triggers to minimize data left in a buffer unprocessed when a stop trigger is received. When planning to abort an acquisition in the middle of a long sequence of frames, setting this to true could also help to ensure all the data that had been acquired thus far is saved. Shrinking the DMA buffers will increase the chance that data will be dropped since it eliminates the safety net of a larger buffer; some testing should be done to insure a specific experiment will run successfully prior to investing in an actual sample; the dropped data flag will indicate if the experiment succeeded or not. Decreasing the minimum buffer time from the default of 100ms will further increase the chance of dropping data.

[Back to Commands](#)

-LiveScan (-lv) "state (on/off) (Optional, defaults to on)"

Begins live scanning as soon the current acquisition ends, or immediately if no acquisition is currently running. If state is specified to be “off” and a live scan is currently running, the live scan will be terminated. If state is specified to be “on” when already live scanning or if state is specified to be “off” when not live scanning, this script is ignored. Other script commands will NOT automatically terminate the live scan as in previous versions of Prairie View.

[Back to Commands](#)

-MarkPoints (-mp) "category name (Optional)" "experiment name (Optional, required if category name is provided)"

Runs a saved Mark Point Series (or experiment) provided the category and name of the saved series/experiment; if no optional parameters are provided then the current series/experiment will be run.

-MarkPoints (-mp) "X Position (% of image 0-1, where 0 is the left side) (Required)" "Y Position (% of image 0-1, where 0 is the top of the image) (Required)" "Duration (ms) (Required)" "Laser Name (as it appears in the UI) (Required)" "Laser Power (same range as UI controls, supports 2P laser calibration) (Required)" "Is Spiral (true/false) (Optional)" "Spiral Size (% of image in the x dimension, spiral will be forced to be a circle) (Optional, required if Is Spiral is provided)" "Spiral Revolutions (Optional,

required if Is Spiral is provided)" "Delay (ms) (Optional, can skip all the spiral parameters if desired)" "repeat all parameters again to mark another location (Optional, required if Delay is provided)"

Marks the specified points on the fly with the laser pulse parameters provided; no need to set up a point location or experiment ahead of time. See [Mark Points](#) documentation for more information on specific parameters.

[Back to Commands](#)

-PointScan (-ps) "time in milliseconds (Required)"

Performs a PointScan acquisition for the specified number of milliseconds.

[Back to Commands](#)

-ReadRawDataStream (-rrd) "process ID (Required)" "memory address (Required)"
"buffer size in samples (Required)"

This is an extremely low level command to get raw live image data from Prairie View, the raw data straight off the acquisition card, for use in third party applications. It is primarily used by PrairieLink, but could be used directly by an advanced user. In order to use the command you need to know the ID of the process requesting the data (i.e. what is displayed in the task manager), the address of some memory that has already been allocated to hold the data, and how large that block of allocated memory is. The values placed into the memory block will be 16-bit signed integers. The command will respond with the number of samples copied into the buffer, it will not wait until the buffer is filled; if no samples were added since last called it will respond with a zero. Consecutive calls of this command will return a continuous stream of whole frames, if more frames are acquired than are read out only the most recent frames are kept and the older frames are skipped (unless the *buffer frames* parameter is passed to specify that all frames should be returned, in which case no more data will be added to the stream if the data isn't read out fast enough). This command will do nothing until the -StreamRawData (-srd) command is sent with a parameter of true; this command starts caching raw data for subsequent acquisitions.

[Back to Commands](#)

-SingleScan (-ss) "autoSave (True/False) (Optional, defaults to True)"

Performs a single scan. Passing the optional parameter *autoSave* as *False* will overwrite the last single scan data.

[Back to Commands](#)

-SingleScanTriggered (-sst) "autoSave (True/False) (Optional, defaults to True)"

Performs a single scan waiting for the scan to start via the input trigger. Passing the

optional parameter *autoSave* as *False* will overwrite the last single scan data.

[Back to Commands](#)

-StreamRawData (-srd) "*true/false (Required)*" "*buffer frames (0-65535) (Optional: Defaults to 0)*"

This is an extremely low level command to enable raw data streaming so that the raw data can be retrieved using the -ReadRawDataStream (-rrd) command. Passing true will enable raw data streaming and passing false will disable raw data streaming. Since streaming raw data is done right as the data is read from the acquisition card, leaving this feature enabled when not in use may affect data integrity. If the data stream is corrupted the dropped data flag will be set.

Not passing the optional *buffer frames* parameter or explicitly passing it as zero, will result in a buffer consisting of three frames used to return the most recent frame data with no possibility of overrunning the buffer. One frame is set aside to return the remainder of any frame which has been partially streamed, and the other two frames are used to double buffer the most recent image data.

Passing any other number for the optional *buffer frames* parameter will set aside a buffer of exactly that many frames and polling for new data will return all the buffered data in the order it was acquired. Prairie View will never overwrite data before it is read out and will stop streaming data when this happens, streaming will resume for the next acquisition. To prevent a buffer overrun: poll for data more often, or increase the number of frames in the buffer.

[Back to Commands](#)

-TSeries (-ts)

Performs a T-series using the current settings.

[Back to Commands](#)

-TSeriesLoad (-tsl) "*path (Optional)*"

Loads the T-Series definition from the ".cfg" file path provided. If no path is provided the T-Series definition will be loaded from the one saved the last time Prairie View was closed.

[Back to Commands](#)

-WaitForScan (-w)

Prairie View will wait for the current scan to complete before moving on to the next command. If there isn't a scan currently in progress this command will do nothing.

[Back to Commands](#)

-SetAcquisitionMode (-sam) "*mode*
(*Galvo/AOD/Spiral/FLIM/SFC/Resonant/Camera*)"

Switches the current acquisition mode to the mode specified by the *mode* parameter.
Depending on the transition, the user may need to place a wait command after this
command to give time for the system to complete the switch

[Back to Commands](#)

Line Scan Commands

-GetFreehandLine (-gl) "*actionName* (*Required*)"

Runs the action with the specified *actionName* as already defined in Prairie View under
"Tools/Actions...". This command makes it possible to use a 3rd party tool to analyze
an image and generate a free hand line scan pattern to be loaded into Prairie View.

[Back to Commands](#)

-Linescan (-ls) "*append* (*true/false*) (*Required*)"

Performs a linescan acquisition. Setting the parameter *append* as *true* will append the
linescan acquisition data to the last linescan acquisition.

[Back to Commands](#)

-LinescanDialog (-ld) "*open/close* (*Required*)"

Opens or closes the line scan dialog depending on whether 'open' or 'close' is passed
as an argument.

[Back to Commands](#)

-LinescanLines (-lsl) "*number of lines* (*Required*)"

Sets the number of lines to scan in the line scan dialog.

[Back to Commands](#)

-LinescanMode (-lm) "*mode* (*Required*)"

Set the desired *mode* for linescan acquisition. Valid options for *mode* are; *Line*, *Circle*,
or *Freehand*.

[Back to Commands](#)

Z-Series Commands

-FindSlice (-fs) "channel (Required)" "sliceThickness (negative to go opposite direction) (Required)" "intensityThreshold (% of previous slice intensity, <100 dimmer, >100 brighter) (Required)" "stopLimit (Required)" "backoff (Required)" "return to start if limit reached (true/false) (Required)"

Steps through a sample slice by slice starting at the current position and moving as determined by the *sliceThickness*. The search process will end when the *stopLimit* is reached or if a slice meets or exceeds appropriate *intensityThreshold* before that. The *intensityThreshold* comparison is based upon the intensity value from the first image and the intensity value from the current image. Warning: Failure to set an appropriate *stopLimit* may cause damage to sample and/or hardware if physical limits are reached and/or exceeded. The *backoff* argument allows the operator to specify an amount for the z motor to move from its current location (in microns) before starting the search. The *return to start if limit reached* argument if true, will have the z motor move back to the position it was at when the search was started (ignoring the value of *backoff*), if the *stopLimit* is reached. If the *return to start if limit reached* argument if false, then if the *stopLimit* is reached, the z motor will remain at that location.

[Back to Commands](#)

-SetZSeriesNumberOfSlices (-zsn) "numberOfSlices (Required)"

Sets the number of slices in the current Z-series definition.

[Back to Commands](#)

-SetZSeriesStart (-zsb) "onlyMotors/allSettings (Optional)" "true/false (resetZSeriesStop Optional)" "true/false (resaveZ-series Optional)"

Sets the starting position for the current Z-series definition. If the optional parameter, *onlyMotors* is used, then only the motor positions related to the Z-series definition will be updated for the start position. If this parameter is missing, or is set to *allSettings*, then all of the "start" position parameters will be defined based upon the current motor positions and control settings (laser and PMT settings). The second optional parameter *resetZSeriesStop*, has a value of either *true* or *false*. If *true*, then the stop position of the Z-series definition will be adjusted by the amount that the start position was adjusted. If this value is *false*, then the stop position of the Z-series will not be adjusted from its current value. The third optional parameter *resaveZ-series* has a value of either *true* or *false*. If *true*, then the Z-series definition will be saved again using the new start (and possibly stop) position settings. If this value is *false*, then the modified start (and possibly stop) position settings will not be permanently saved for the currently selected Z-series. If there is not a currently selected Z-series, then this parameter is ignored. Since all of these parameters are optional, if any are

used, they must appear in the order shown. For example, if the operator wishes to use the third parameter, *resaveZ-series*, then he/she must define the settings for the first two parameters as well.

[Back to Commands](#)

-SetZSeriesStepSize (-zs) "*stepSize (Required)*"

Sets the step size for the current Z-series definition.

[Back to Commands](#)

-SetZSeriesStop (-zse) "*onlyMotors/allSettings (Optional)*" "*true/false (resetZSeriesStart Optional)*" "*true/false (resaveZ-series Optional)*"

Sets the ending position for the current Z-series definition. If the optional parameter, *onlyMotors* is used, then only the motor positions related to the Z-series definition will be updated for the stop position. If this parameter is missing, or is set to *allSettings*, then all of the "stop" position parameters will be defined based upon the current motor positions and control settings (laser and PMT settings). The second optional parameter *resetZSeriesStart*, has a value of either *true* or *false*. If *true*, then the start position of the Z-series definition will be adjusted by the amount that the stop position was adjusted. If this value is *false*, then the start position of the Z-series will not be adjusted from its current value. The third optional parameter *resaveZ-series* has a value of either *true* or *false*. If *true*, then the Z-series definition will be saved again using the new stop (and possibly start) position settings. If this value is *false*, then the modified stop (and possibly start) position settings will not be permanently saved for the currently selected Z-series. If there is not a currently selected Z-series, then this parameter is ignored. Since all of these parameters are optional, if any are used, they must appear in the order shown. For example, if the operator wishes to use the third parameter, *resaveZ-series*, then he/she must define the settings for the first two parameters as well.

[Back to Commands](#)

-ZSeries (-zs)

Performs a Z-series using the current settings.

[Back to Commands](#)

-ZSeriesLoad (-zsl) "*name (Required)*"

Load the Z-series definition with the *name* given as the current Z-series. The variable, *name*, could be either the name of the saved Z-series definition, or a *variable*, such as the *iteration interval* defined in the "ExecuteScript" command. If the *iteration interval*

is used, then the Z-series that will be "loaded" will be based upon the order of the current list of Z-series definitions.

[Back to Commands](#)

-ZSeriesMoveTo (-zsmt) "*start/middle/stop/slice # (Required)*"

Move the focus assembly to the start/middle/stop position or a specified slice number as defined by the current Z-series. In addition to moving the focus assembly to the specified location, if laser power and/or PMT gain compensation is enabled for the current Z-series, then the laser and PMT settings will be set to the values corresponding to the selected location.

[Back to Commands](#)

-ZSeriesSave (-zss) "*name (Required)*"

Saves the current Z-series definition with the *name* given.

[Back to Commands](#)

Scan Setting Commands

-EnterROI (-er) "*X Location (% FOV, Required)" "Y Location (% FOV, Required)" "Width (% FOV, Required)" "Height (% FOV, Required)*"

Enters a region of interest to be scanned based on the location and dimensions provided based on the source image (scan area when not scanning a region of interest).

[Back to Commands](#)

-GetState (-gts) "*key (Required)" "index (Required for indexed keys, omit otherwise)"*

"subindex (Required for subindexed keys, omit otherwise)"

Gets any setting in Prairie View's environment file (see 'master.env' in the 'Configuration' folder for valid keys). The in addition to the *key* there are also two additional required parameters: *index* if the key is index or subindexed and *subindex* if the key is subindexed. This command is only useful when using either the command prompt or a TCP/IP connection since it only responds with a value and does not actually do anything.

[Back to Commands](#)

-ParameterSet (-pa) "*parameter set name (Required)" "track number (Optional)"*

Applies settings from the [Parameter Set](#) which matches the name provided. For multi-track Parameter Sets an optional track number can be specified which defaults to the

first track.

[Back to Commands](#)

-ROILoad (-roi) "name (Required)"

Load the ROI definition with the specified *name*. The variable, *name*, could be either the name of the saved ROI definition, a *variable*, such as the *iteration variable* defined in the "ExecuteScript" command, or "noROI". If the *iteration variable* is used, then the ROI that will be "loaded" will be based upon the order of the current list of ROI definitions. If the "noROI" value is found, then the currently defined ROI will be terminated.

[Back to Commands](#)

-SamplesPerPixel (-spp)

Gets the current number of samples acquired for each pixel in the image. This command is useful in conjunction with the -ReadRawDataStream (-rrd) command to figure out how to parse the raw data stream. This command is only useful when using either the command prompt or a TCP/IP connection since it only responds with a value and does not actually do anything.

[Back to Commands](#)

-SetChannel (-c) "channelNumber (1-4) (Required)" "channelState (On/Off) (Required)" ...

Enables and disables channels on the first image window. Can include as many *channelNumber/channelState* pairs as desired to enable/disable multiple channels with the same command.

[Back to Commands](#)

-SetDwellTime (-dt) "dwellTime (Required)"

Sets the dwell time as close as possible to the given dwell time value. Minimum dwell time and sampling rate limit potential dwell time values. A warning will be displayed if the specified dwell time cannot be achieved.

[Back to Commands](#)

-SetFrameAveraging (-fa) "frame averaging (1,2,4,8,16,32,64, or 128) (Required)"

Sets the frame averaging to the specified value.

[Back to Commands](#)

-SetImageSize (-is) "width (Required)" "height (Optional)"

Sets the image size as close as possible to the given dimensions. Minimum pixels per

line limits potential image widths. A warning will be displayed if the specified image size cannot be achieved. If height is not specified then the width will be used for both dimensions.

[Back to Commands](#)

-SetOpticalZoom (-oz) "*optical zoom (1.0>= optical zoom=<128.0) (Required)*"

Sets the optical zoom to the specified value.

[Back to Commands](#)

-SetScanRotation (-sr) "*rotationAngle (Required)*"

Sets the scan rotation to the specified angle.

[Back to Commands](#)

-SetState (-sts) "*key (Required)*" "*value (Required)*" "*index (Required for indexed keys, omit otherwise)*" "*subindex (Required for subindexed keys, omit otherwise)*" "*forceModified (true/false) (Optional, do not use without a specific reason for doing so)*"

DO NOT USE THIS COMMAND WITHOUT EXPLICIT INSTRUCTION FROM A BRUKER FLUORESENCE MICROSCOPY REPRESENTATIVE, AS IT COULD CRASH THE SOFTWARE AND/OR POTENTIALLY DAMAGE HARDWARE. Sets any setting in Prairie View's environment file (see 'master.env' in the 'Configuration' folder for valid keys). The two required parameters are a *key* and a *value*, there are also two additional required parameters: *index* if the key is index or subindexed and *subindex* if the key is subindexed. The *forceModified* parameter specifies whether or not to mark the value as being changed even if the value is the same, this should always be omitted unless there is a specific reason for passing it.

[Back to Commands](#)

Hardware Commands

-Camera (-ca) "*feature (Required)*" "*value (Required)*"

Change the *value* of the specified *feature*. The list of *features* and their abbreviated form is; ExposureTime (et), ExposureMode (em), BinFactor (bf), Gain (gn), GainMultFactor (gnmf), and ReadOutPort (rop). Each command *feature* is listed below with the corresponding range for *value* or the options for *value*. The particular *values* are going to be dependent upon the actual camera. The quickest way to know the proper *value* or range for a given *feature* for a camera, would be to look at the corresponding camera control within Prairie View.

ExposureTime 1..n

ExposureMode Bulb|Timed|TriggerAll|TriggerFirst

BinFactor 1,2,4,8

Gain 0..100

GainMultFactor 0..4095

ReadOutPort 0,1

CameraFan On|Off

[Back to Commands](#)

-CenterGalvos (-cg) "state (true/false) (Optional)"

This command will center the galvos. Passing optional state parameter as true will also center the galvos where as passing false will return the galvos to their parked positions.

[Back to Commands](#)

-GetMotorPosition (-gmp) "axis (X/Y/Z) (Required)" "device index (optional)"

Returns the current position (as displayed on the main form) for a motor driving the specified *axis*. If there are multiple motors/devices used for a particular axis the optional *device index* parameter (zero indexed) can be used to specify a specific device, the default *device index* will be that of the currently active device. This command is only useful when using either the command prompt or a TCP/IP connection since it only responds with a value and does not actually do anything.

[Back to Commands](#)

-MoveMotor (-mr) "axis (X/Y/Z) (Required)" "relativePosition (Required)" "device index (optional)"

Moves a motor for a specified *axis* by a specified amount (*relativePosition*). If there are multiple motors/devices used for a particular axis the optional *device index* parameter (zero indexed) can be used to specify a specific device, the default *device index* will be that of the currently active device.

[Back to Commands](#)

-NikonNiMicroscope (-nni) "device (Required)" "value (optional based upon device)"

Change the *value* of the specified *device*. The list of *devices* and their abbreviated form is; FilterCassetteBlock1 (ftcb1), FilterCassetteBlock2 (ftcb2), CondenserCassette (cc), and MotorizedNosepiece (mnp). Each command *device* is listed below with the corresponding range for *value* or the options for *value*. For *devices* that have a *value* range of 1..n, n would be replaced by one less than the number of discrete positions for the *device*. For example, if there are 6 reflector changer positions, the valid

options for *value* are 0, 1, 2, 3, 4, and 5.

FilterCassetteBlock1 1..n or fbc1 1..n
FilterCassetteBlock2 1..n or fbc2 1..n
CondenserCassette 1..n or cc 1..n
LightPathDriver 1..n or lpd 1..n
TLHalogenLampSoftwareControl true|false or tlhlsc true|false
TLHalogenLampState true|false or tlhls true|false
TLHalogenLampPower 0..24 or tlhlp 0..24
MotorizedNosepiece 1..n or mnp 1..n
PerfectFocusState true|false or pfs true|false (*Not Yet Implemented*)
PerfectFocusOffset 0..40000 or pfo 0..40000 (*Not Yet Implemented*)
PerfectFocusMemory (*Not Yet Implemented*)
PerfectFocusRecall (*Not Yet Implemented*)

[Back to Commands](#)

-NikonTiMicroscope (-nti) "device (Required)" "value (optional based upon device)"

Change the *value* of the specified *device*. The list of *devices* and their abbreviated form is; FilterCassetteBlock1 (ftcb1), FilterCassetteBlock2 (ftcb2), CondenserCassette (cc), and MotorizedNosepiece (mnp). Each command *device* is listed below with the corresponding range for *value* or the options for *value*. For *devices* that have a *value* range of 1..n, n would be replaced by one less than the number of discrete positions for the *device*. For example, if there are 6 reflector changer positions, the valid options for *value* are 0, 1, 2, 3, 4, and 5.

FilterCassetteBlock1 1..n or fbc1 1..n
FilterCassetteBlock2 1..n or fbc2 1..n
CondenserCassette 1..n or cc 1..n
LightPathDriver 1..n or lpd 1..n
TLHalogenLampSoftwareControl true|false or tlhlsc true|false
TLHalogenLampState true|false or tlhls true|false
TLHalogenLampPower 0..24 or tlhlp 0..24
MotorizedNosepiece 1..n or mnp 1..n
PerfectFocusState true|false or pfs true|false
PerfectFocusOffset 0..40000 or pfo 0..40000
PerfectFocusMemory
PerfectFocusRecall

[Back to Commands](#)

-PanGalvo (-png) "axis (X/Y/center) (Required)" "panValue (coarse/medium/fine/float) (Required)"

Pans the specified amount in either X or Y, if the scan is rotated the panning will occur in both axes such that X will pan horizontally and Y will pan vertically. Pan values can be either coarse (.25 V), medium (.1 V), fine (.01 V) or any floating point number of your choosing for finer or more specific control. Numeric values will be interpreted as values in microns when the selected objective lens has been calibrated, otherwise numeric values will be interpreted as Volts. A negative value will pan up or left and a positive value will pan down or right (i.e. '-png x -coarse' will pan left by a large step). Passing 'center' for the axis will reset the pan offsets back to the center.

[Back to Commands](#)

-SendGPIOCommand (-gc) "command (Required)"

Sends the specified command directly to the GPIO controller. Contact a Bruker Fluorescence Microscopy representative for more details.

[Back to Commands](#)

-SendMAMCCommand (-mc) "command (Required)"

Sends the specified command directly to the multi-axis motor controller. Contact a Bruker Fluorescence Microscopy representative for more details.

[Back to Commands](#)

-SendPiezoCommand (-pc) "command (Required)"

Sends the specified command directly to the prairie piezo controller. Contact a Bruker Fluorescence Microscopy representative for more details.

[Back to Commands](#)

-SendResonantCommand (-rc) "command (Required)"

Sends the specified command directly to the resonant galvo controller. Contact a Bruker Fluorescence Microscopy representative for more details.

[Back to Commands](#)

-SetMotorPosition (-ma) "axis (X/Y/Z) (Required)" "absolutePosition (Required)" "device index (optional)"

Moves a motor for a specified *axis* to a specified location (*absolutePosition*). If there are multiple motors/devices used for a particular axis the optional *device index* parameter (zero indexed) can be used to specify a specific device, the default *device index* will be that of the currently active device.

[Back to Commands](#)

-SetObjectiveLens (-sol) "*objective lens name (Required)*"

Sets the objective lens selection to the specified value.

[Back to Commands](#)

-SFC (-sfc) "*feature (Required)*" "*value (Required)*"

Change the *value* of the specified *feature*. The list of *features* and their abbreviated form is; ExposureTime (et), ExposureMode (em), BinFactor (bf), Gain (gn), GainMultFactor (gnmf), ReadOutPort (rop), EmissionFilter(ef), and Aperture(ap). Each command *feature* is listed below with the corresponding range for *value* or the options for *value*. The particular *values* are going to be dependent upon the actual camera. The quickest way to know the proper *value* or range for a given *feature* for a the SFC, would be to look at the corresponding camera control within Prairie View.

ExposureTime 1..n

ExposureMode Bulb|Timed|TriggerAll|TriggerFirst

BinFactor 1,2,4,8

Gain 0..100

GainMultFactor 0..4095

ReadOutPort 0,1

EmissionFilter 0..n

Aperture 0..n

CameraFan On|Off

[Back to Commands](#)

-ZDeviceControl (-zdc) "*external/software (Required)*"

Sets the current Z device for either *software* control via Prairie View or for *external* control. *External* control means that the Z device will be in a state to be controlled via an analog voltage level. Not all Z devices support this feature.

[Back to Commands](#)

-ZeissMicroscope (-zm) "*device (Required)*" "*value (Required)*"

Change the *value* of the specified *device*. The list of *devices* and their abbreviated form is; ReflectorChanger (rc), TLShutter (tls), TLHalogenLampState (tlhls), TLHalogenLampPower (tlhlp), RLShutter (rls), RLHalogenLampState (rlhls), RLHalogenLampPower (rlhlp), SideportTurret (spt), Optovar (op), and MotorizedNosepiece (mnp). Each command *device* is listed below with the corresponding range for *value* or the options for *value*. For *devices* that have a *value* range of 0..n, n would be replaced by one less than the number of discrete positions for the *device*. For example, if there are 6 reflector changer positions, the valid

options for *value* are 0, 1, 2, 3, 4, and 5.

ReflectorChanger 0..n or rc 0..n
TLShutter true|false or tls true|false
TLHalogenLampState true|false or tlhls true|false
TLHalogenLampPower 0..100 or tlhlp 0..100
RLShutter true|false or rls true|false
RLHalogenLampState true|false or rlhls true|false
RLHalogenLampPower 0.100 or rlhlp 0..100
SideportTurret 0..n or spt 0..n
Optovar 0..n or op 0..n
MotorizedNosepiece 0..n or mnp 1..n

[Back to Commands](#)

Laser/PMT Commands

-SecondaryLaserBeamRoute (-slbr) "state (true/false) (Required)"

Sets the 'Secondary Laser Beam Route' button (found on the 'Misc' tab) to the specified *state*.

[Back to Commands](#)

-SetLaserPower (-lp) "*laserName* or *laserNumber* (Required)" "BY/*laserPower* (Required)"

Sets the power for a specified laser. If *BY* is specified, then the laser power will be adjusted from its current value by the amount (positive or negative) specified by the *laserPower*.

[Back to Commands](#)

-SetMultiPhotonWavelength (-mpw) "*wavelength* (e.g. 840)(Required)" "index (1..4) (Required)"

Sets the operating wavelength for the multi-photon laser indicated by *index*. If only one laser is present, *index* = 1.

[Back to Commands](#)

-SetPMTGain (-pg) "*PMTNumber* (Required)" "ZERO/PREVIOUS/BY/*PMTGain* (Required)"

Sets the gain (*PMTGain*) for a specified PMT (*PMTNumber*). If *ZERO* is specified, then the designated PMT HV will be set to 0. If *PREVIOUS* is specified, the PMT HV will be

set back to the value that was last used before the *ZERO* command had been issued. If *BY* is specified, then the PMT HV will be adjusted from its current value by the amount (positive or negative) specified by the *PMTGain*.

[Back to Commands](#)

Shutter Commands

-OverrideHardShutter (-ohs) "*shutter name/number (Required)*" "*state (open/close/auto) (Required)*"

Forces a specific shutter, specified by a zero indexed number or by name, to either open or close until instructed otherwise. Setting the state to *auto* will resume normal operation. This command can be used to keep a shutter open for the duration of a scripted experiment, otherwise the default behavior is to open and close the shutter for each separate acquisition.

[Back to Commands](#)

-SetHardShutter (-hrd) "*state (open/close) (Required)*"

Opens or closes the hard shutter.

[Back to Commands](#)

-SetHardShutterSelection (-shss) "*shutter number (1..n/all) (Required)*"

Select the specified *shutter number* to be used for the hard shutter. If *all* is specified, then all hard shutters will be selected.

[Back to Commands](#)

-SetSoftShutter (-sft) "*state (open/close) (Required)*"

Opens or closes the soft shutter.

[Back to Commands](#)

Stage Position Commands

-AddStagePosition (-spa) "*absoluteXPosition (Optional)*"

"*absoluteYPosition (Optional)*" "*absoluteZPosition (Optional)*" ...

Adds one or more stage locations passed in sets of 3 coordinates X, Y and Z. In case of multiple Z devices the active Z device denoted in the stage control section of the main form will be used, other Z devices will retain their current positions. If no parameters are passed then the current stage location will be added.

[Back to Commands](#)

-ClearStagePositions (-spc)

Clears all existing saved stage locations.

[Back to Commands](#)

-LoadStagePositionFile (-lspf) "file path and name (Required)"

Load the file containing saved XYZ stage positions.

[Back to Commands](#)

-MoveToStagePosition (-mtsp) "index (1..n) (Required)"

Moves the stage to the specified entry (*index*) in the table of stage locations found on the 'XY-Stage' tab. The variable, *index*, could be either the number of the index in the table of stage locations, or a *variable*, such as the *iteration interval* defined in the "ExecuteScript" command.

[Back to Commands](#)

-SaveStagePositionFile (-sspf) "file path and name (Required)"

Save a file containing saved XYZ stage positions.

[Back to Commands](#)

-SetGridLocations (-sgl) "replace/add (Required)"

Generates the grid of XY-Stage locations based upon the 'Start' and 'Stop' locations for the XY-Stage grid feature found on the 'XY-Stage' tab. If *replace* is specified, then the current table of XY-Stage locations will be deleted before the grid locations are generated. If *add* is specified, then the current table of XY-Stage locations will be retained when the grid locations are generated.

[Back to Commands](#)

-SetGridOverlap (-sgo) "overlap % (Required)"

Defines the amount of overlap (as a percentage (%)) of the field of view based upon the current objective lens selection) for the XY-Stage grid feature found on the 'XY-Stage' tab. The variable, *overlap*, must be between 0 and 50 (inclusive).

[Back to Commands](#)

-SetGridStartLocation (-sgstartl) "absoluteXPosition (Optional)"

"absoluteYPosition (Optional)" "absoluteZPosition (Optional)" ...

Defines the XY-Stage grid start location. In case of multiple Z devices the active Z device denoted in the stage control section of the main form will be used, other Z devices will retain their current positions. If no parameters are defined, then the current stage location will be used for the XY-Stage grid start location.

[Back to Commands](#)

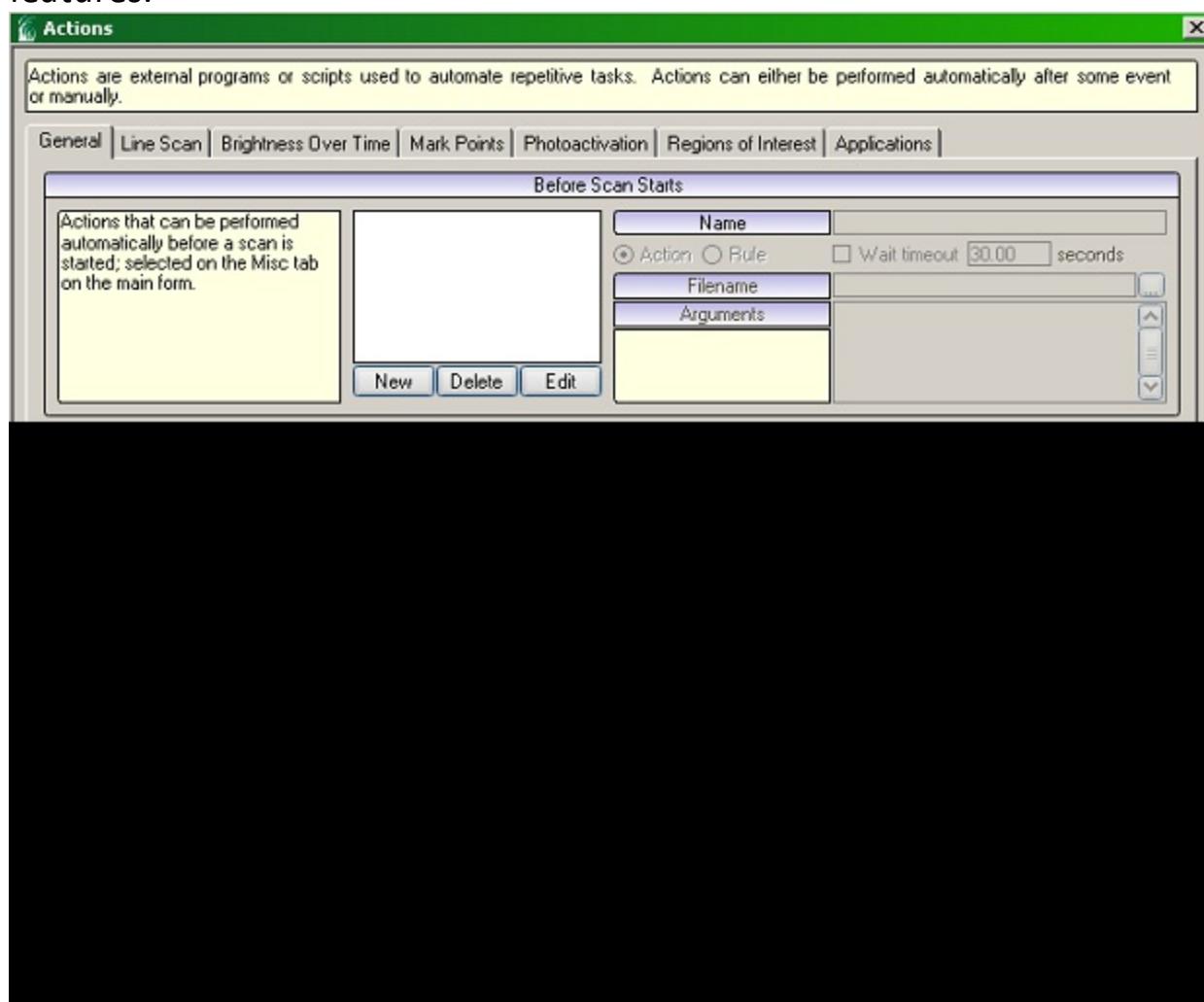
-SetGridStopLocation (-sgstopl) "*absoluteXPosition (Optional)*"
"*absoluteYPosition (Optional)*" "*absoluteZPosition (Optional)*" ...

Defines the XY-Stage grid stop location. In case of multiple Z devices the active Z device denoted in the stage control section of the main form will be used, other Z devices will retain thier current positions. If no parameters are defined, then the current stage location will be used for the XY-Stage grid stop location.

[Back to Commands](#)

Actions

This section of the Help file is under construction and has not been updated recently. The information below is from a previous version of the manual and may be somewhat out-dated. Please contact Bruker Fluorescence Microscopy support personnel with questions about these features.



Actions are external programs or scripts used to automate repetitive tasks. There are two types of actions: actions and rules. Actions perform a task; rules determine another action to perform based on some criterion.

An Action consists of a **Name**, a **Filename**, and set of **Arguments**. The **Name** is used to identify the action from others and give some indication of its behavior. Actions will be referenced by their names throughout the application. The **Filename** is the path to the program or script which will be executed. For example "notepad" would launch a text editor. **Arguments** make up a list of arguments passed to the program or script specified. The arguments can contain tokens specified to the left of the text box; for example in the argument "<image:ch#> = .TIF File", "<image:ch1>" would be replaced by the path and filename for the image containing the data for channel one.

A Rule consists of a list of criteria with corresponding actions to perform if the criterion for a given line is met. For example, a rule may define that a particular action be performed each

time a **Single Scan** is executed. A rule can point to another rule, but if a rule references itself directly or indirectly it will terminate rather than continue in an infinite loop. When generating a rule, buttons to the right of the grid allow lines in the rule to be moved up or down or removed.

Types of Actions

There are a number of different types of actions available in different places throughout the application. Some of these types are described below:

After Frame Complete actions are run automatically after a frame is acquired with the exception of max speed acquisitions. In order for an After Frame Complete action to be performed it must first be specified on the [Misc tab](#) of the Prairie View main control window. The only token available for use in the arguments field is '<image:ch#>' where '#' is replaced by a channel number 1-4. This token will be replaced by the path and filename of the image containing the channel data for the frame that just completed.

After Scan Complete/Playback actions are run automatically after a scan has completed. In order for an After Scan Complete action to be performed it must first be specified on the [Misc tab](#) of the Prairie View main control window. After scan complete actions are also available to be performed manually in playback mode from the image window. Rule type actions are disallowed in this context. The only token available for use in the arguments field is "<metadata>". This token will be replaced by the path and filename of the metadata file associated with the scan which has just completed (or the scan which is currently open in playback mode). The metadata file is in an XML format and contains all information about the scan. The program or script specified must be able to parse the metadata file in order to retrieve scan data.

Freehand Linescan Path Generation actions are run manually from the [Linescan Control](#) dialog and are used to automatically generate freehand line scan paths based on the current image data.

There are two tokens available for use in the arguments field:

'<image:ch#>' where '#' is replaced by a channel number 1-4. This token will be replaced by the path and filename of the image containing the current image data for the channel specified.

'<outfile>' which replaced by the path and filename of a temporary file created to pass line scan path data from the program or script specified into Prairie View.

The format of the output file is a comma delimited list of points that make up the path to line scan. Each point is made up of an X and Y coordinate again separated by a comma that range from 0.0,0.0 (upper left corner) to 1.0,1.0 (lower right corner).

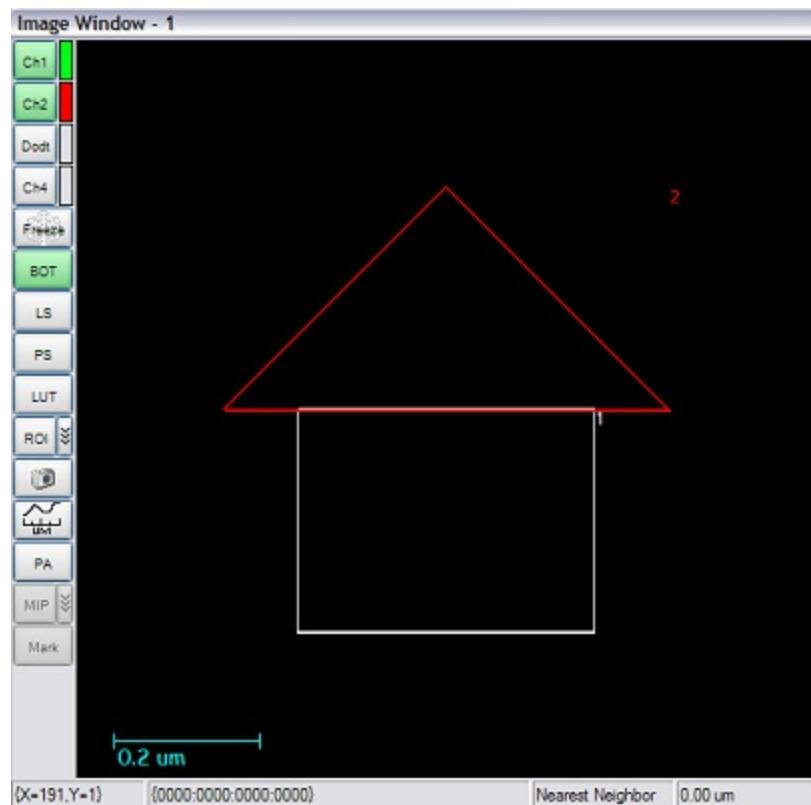
Brightness Over Time Region Generation actions are run manually from the [Brightness Over Time](#) dialog and are used to automatically generate brightness over time regions based on the current image data. There are two tokens available for use in the arguments field:

'<image:ch#>' where '#' is replaced by a channel number 1-4. This token will be replaced by the path and filename of the image containing the current image data for the channel specified.

'<outfile>' which replaced by the path and filename of a temporary file created to pass region data from the program or script specified into Prairie View.

The format of the output file is a comma delimited list of vertices that make up polygon based regions. Each vertex is made up of an X and Y coordinate again separated by a comma that range from 0.0,0.0 (upper left corner) to 1.0,1.0 (lower right corner). Each polygon is terminated by the channel number the region should be created for prefixed by a '-' (minus sign).

For example an output file which would create a square region in the center for channel two and a triangle above it for channel one would look something like
.3,.5,.7,.5,.7,.8,.3,.8,-2,.2,.5,.5,.2,.8,.5,-1 and will look something like the following when loaded:



Mark Points Point Generation actions are run manually from the [Mark Points](#) dialog and are used to automatically generate a set of points to mark based on the current image data. There are two tokens available for use in the arguments field:

'<image:ch#>' where '#' is replaced by a channel number 1-4. This token will be replaced by the path and filename of the image containing the current image data for the channel specified.

'<outfile>' which replaced by the path and filename of a temporary file created to pass point data from the program or script specified into Prairie View.

The format of the output file is a comma delimited list of points that make up the path to line scan. Each point is made up of an X and Y coordinate again separated by a comma that range from 0.0,0.0 (upper left corner) to 1.0,1.0 (lower right corner).

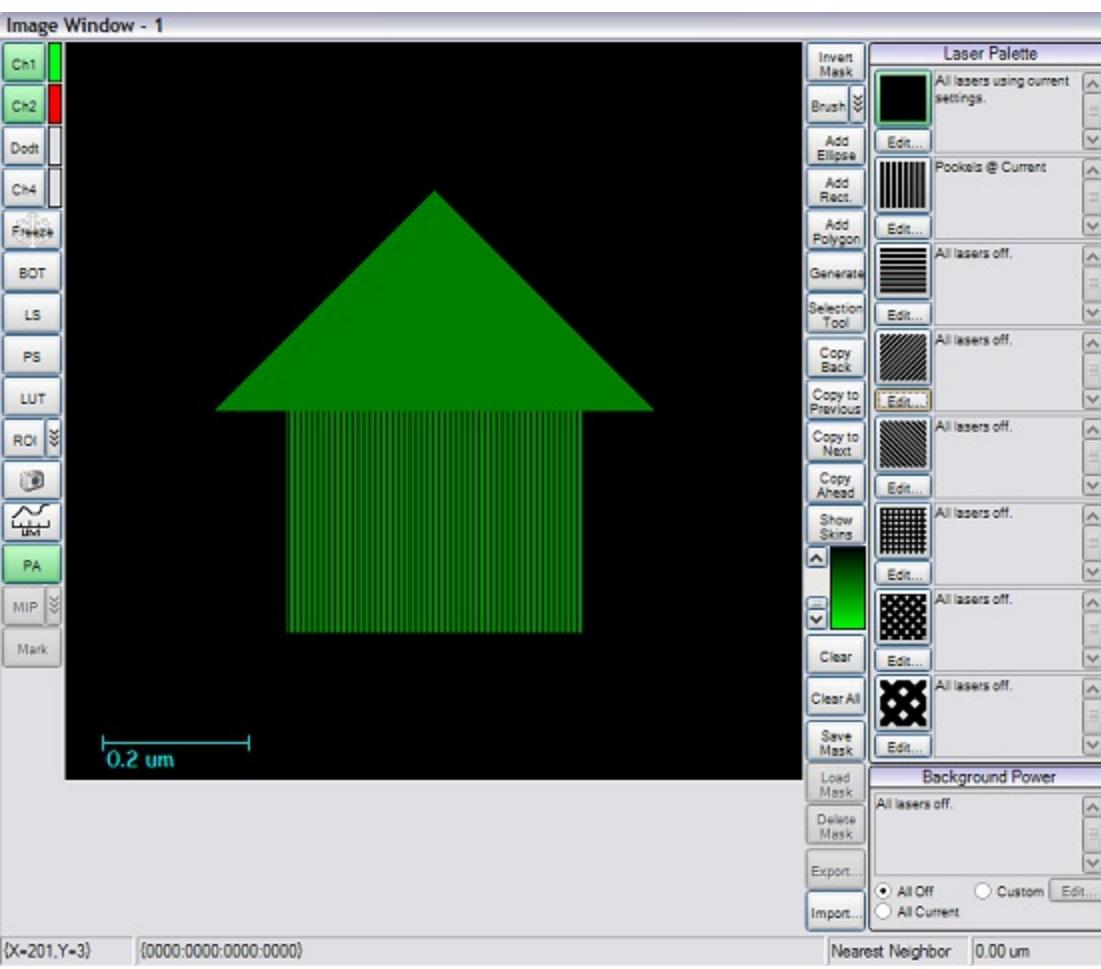
Photoactivation Mask Generation actions are run manually from the image window when the [Photoactivation](#) mask editor is enabled and are used to automatically generate photo activation masks based on the current image data. There are two tokens available for use in the arguments field:

'<image:ch#>' where '#' is replaced by a channel number 1-4. This token will be replaced by the path and filename of the image containing the current image data for the channel specified.

'<outfile>' which replaced by the path and filename of a temporary file created to pass mask data from the program or script specified into Prairie View.

The format of the output file is a comma delimited list of vertices that make up polygon based regions. Each vertex is made up of an X and Y coordinate again separated by a comma that range from 0.0,0.0 (upper left corner) to 1.0,1.0 (lower right corner). Each polygon is terminated by the palette number the polygon should use when added to the current mask prefixed by a '-' (minus sign).

For example an output file which would create a square region in the center using palette two and a triangle above it using palette one would look something like
.3,.5,.7,.5,.7,.8,.3,.8,-2,.2,.5,.5,.2,.8,.5,-1 and will look something like the following when loaded:



Application Menu Extensions actions are run manually from the Applications menu on the Prairie View main control window. Actions defined here will be added to the menu and add the ability to launch other applications directly from Prairie View.

Actions Using MATLAB

Actions can make use of any number of third party tools one of which is MATLAB. Unfortunately, MATLAB does not provide command line support so an intermediary program has been provided, PrairieMLL.exe (Prairie MATLAB Link). To use this program in an action, select PrairieMLL.exe as the **Filename** (should be found in the same directory as Prairie View.exe) and add any number of MATLAB commands in double quotation marks in the **Arguments** field.

For example, to display channel one in MATLAB after each frame, the arguments would be something like: "image = imread('<image:ch1>')" "imagesc(image)" "colormap(gray)" "axis('square')" "axis('off')". It is also possible to reference M files by changing to the directory where the M file is found. For example, if the command in the previous example were found in "C:\ShowImage.m" then the arguments would be something like: "cd c:\\\" "ShowImage('<image:ch1>')"

Once run, PrairieMLL.exe continues to run until explicitly terminated. This allows it to continue

to use the same MATLAB command window, saving the time required to load a new command window each time as well as preserving the state in the command window allowing variables to be saved and referenced again between actions. To terminate PrairieMLL.exe, pass \x as an argument. Once the \x argument is reached PrairieMLL.exe will stop running any other commands passed after the \x and will close the MATLAB command window it was using. Calling PrairieMLL.exe again afterwards will create a new MATLAB command window and execute commands normally.

Calibration/Alignment

[Uncaging Galvo Calibration](#): Opens a dialog with step-by-step instructions for calibrating photo-stimulation galvo positions to locations in the image. Note that all beam alignment must be done before performing this software calibration.

[Objective Lens/FOV Calibration](#): Opens a dialog for calibrating the field of view for objectives.

[1-P Pinhole](#): If a 1-P Confocal pinhole detector is present on the system, this option opens a control dialog for the pinhole motors.

[2-P Laser Power Calibration](#): Opens a dialog box for calibrating the power output from the Pockels cell to the power output from the laser cavity for mW control of laser power at the sample.

[Photoactivation Alignment](#): If the system is configured with a Camera or SFC and a photoactivation module, this option opens a dialog to adjust the scan rotation of the photoactivation scan relative to the imaging scan.

Manually Tune Piezo: This option opens a dialog with step-by-step instructions for tuning a Z-piezo device manufactured by Bruker Fluorescence Microscopy. It is not frequently used after initial installation.

Autocalibrate Piezo Focus: This option automatically maps the limits of the piezo for the current conditions. Calibration should be performed when switching between objectives of significantly different mass, or when switching between samples of very low and very high viscosity. Note that this calibration is different from that performed in the Z-Series tab, which uses a feedback algorithm to determine the drive signal needed to perform a specific Z-Series.

Center Galvos: When selected, **Center Galvos** will apply a zero voltage to the imaging and uncaging galvos for the purpose of checking alignment. Clicking again to de-activate this option will return the galvos to their park positions.

Uncaging Galvo Calibration

The uncaging calibration wizard provides a series of steps to calibrate the galvanometers used for uncaging to accurately point at specific locations in the imaging field of view.

The result of the calibration process is a map that relates voltage values for the photo-stimulation galvanometers to pixel locations on the image. This map is then used by the [Mark Points](#) and [Spiral Activation](#) features for a fast method by which the user can specify a location on the image at which to direct the photo-stimulation.

Note that all beam alignment must be done before performing this software calibration.

Step One: Select the Calibration Method

Multiple methods of calibration are available in the software. The best method to use will depend on the system configuration.

The **Burn Spots** method involves burning visible spots onto a uniform sample. Any system with uncaging capabilities can use this method. This method is also very robust in that it can be performed on almost any sample that can be visibly affected by the uncaging beam.

The **Spot Detector** method involves looking at a reflection of the uncaging beam, which requires a specific light path and hardware be present on the system. This method is only possible for Ultima systems with an IR laser on the uncaging path. The benefit of this method over other methods is that it does not require changing the sample, although it is a good idea to push the trinoc plunger in, as if to use the eyepieces, to block any uncaging light from getting to the sample. The drawback of this method is that the calibration is only as good at the spot detector alignment. To account for any alignment offset, there is an optional step at the end to burn one spot and determine how much the calibration needs to be offset when compared to where the uncaging beam actually hits the sample.

The **Fluorescence** method involves imaging the uncaging beam on a uniform sample. This method is not available for 2P imaging; only Opterra (SFC), camera, and confocal imaging modes are supported. This method works a lot like the Burn Spots method, but instead of physically marking the sample to image it afterwards, the emission caused by the uncaging beam is imaged in real-time. This method should be used above all others when available.

The **Quick Offset** method isn't a true calibration, but rather a quick fix to an existing calibration. It is useful in cases where the uncaging beam alignment has shifted and only a fixed offset is needed to correct for the change. This method attempts to burn one spot in the center of the image, and after the actual burn spot location is marked, will compute the difference between the predicted location and the actual location and apply that offset to the calibration file.

Step Two: Select Which File to Update, or Create a New Calibration

Updating the **Current** calibration will update or extend the currently loaded calibration file. If no calibration file is currently loaded, this option isn't available. If this option is available, it is typically the best choice; one calibration file should be enough for most systems, as it will take into account any settings known to alter the calibration: laser/light path, acquisition mode, and zoom.

Updating an **Existing** calibration will update or extend any calibration file saved on the system. When selecting an existing calibration file, or the current calibration file, the details of which calibrations were performed and saved to that file, and when it was saved, will be displayed.

Creating a **New** calibration file will result in a new file containing one calibration. This is the only option available if no calibration files were created previously.

Step Three: Calibrate Center

The first actual calibration step in the calibration wizard is to get a sense of where the uncaging galvanometers point relative to the imaging field of view; this is the only step for a quick offset calibration. Depending on the type of calibration, different instructions will be displayed on how to proceed. In general, there will be a crosshair overlay drawn on the Image window which will need to be dragged onto the spot indicating the location of the uncaging beam. The crosshair can be moved more accurately by using the cursor keys.

During this step there are instructions on how to get the uncaging beam spot to display in the image window by specifying which laser to use, at what power, and for burn spots how long to leave the laser on. Once these settings are determined they will be used throughout the calibration process.

Step Four: Calibrate X

The second actual calibration step is to get a rough idea of how the X uncaging galvanometer moves relative to the imaging field of view. This step, combined with the step before, allows the software to point the X uncaging galvo with some degree of accuracy. Much like the step before, an uncaging beam location will be marked by dragging crosshair over it, or by using the cursor keys.

Step Five: Calibrate Y

The third actual calibration step is to get a rough idea of how the Y uncaging galvanometer moves relative to the imaging field of view. This step, combined with the two before, allows the software to point both uncaging galvos with some degree of accuracy, which will be useful

for the next step. Just like the step before, an uncaging beam location will be marked by dragging crosshair over it, or by using the cursor keys.

Step Six: Calibrate Grid

The final actual calibration step is to mark a grid of uncaging beam locations by dragging a set or series of crosshairs over them. The number of points in the grid help will determine how accurate the calibration can be. At lower optical zooms having more points can result in higher accuracy. At higher zooms, a smaller calibration grid is sufficient.

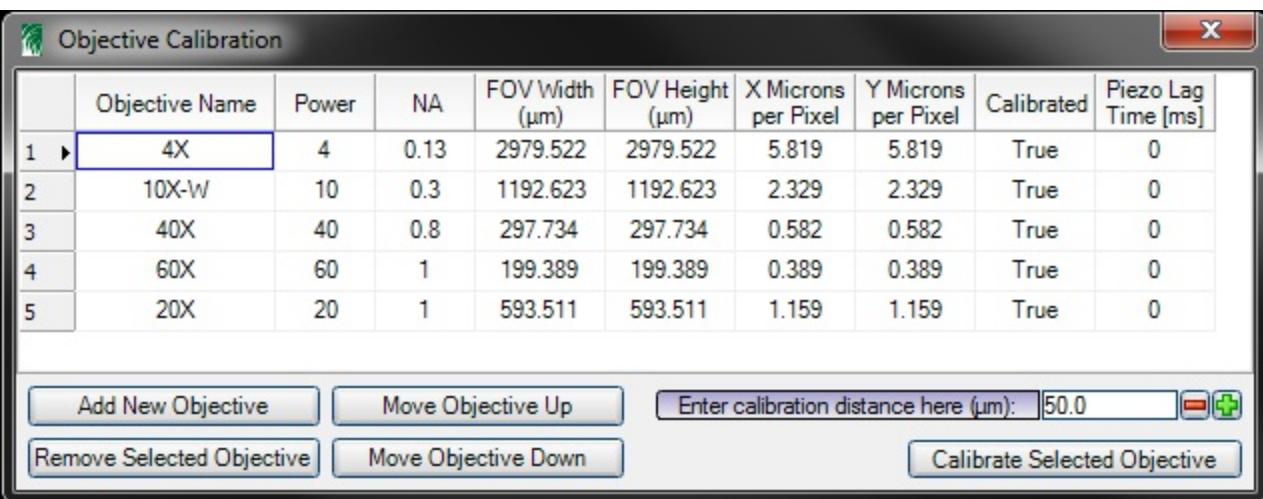
When using the burn spots or fluorescence calibration methods there may be an area of the sample in which it is difficult to tell where the uncaging beam is pointed. In these cases, it is possible to move the stage and/or sample during the calibration process without negatively impacting the calibration. Specifically with grids it is possible to mark the positions of a subset of grid points and then move the sample to mark the remaining points.

Most calibration methods can only show one point at a time, and for these modes you can use the cursor keys to move the cursor by small amounts, but for a burn spot calibration all of the grid points are burnt in a single pass and the cursor keys will have no effect with multiple crosshairs on the image.

Additional Steps

Some calibration methods will have additional steps between or after the steps mentioned described here. These steps are explained in the on-screen instructions within the wizard. For example, when performing a quick offset calibration it is important to pick which portions of the calibration file should be updated. If only one laser was realigned it makes sense to only update offsets using that laser.

Objective Lens/FOV Calibration



To correctly use the measurement tools and have accurate field of view calculations in the metadata, the objective lenses used need to be calibrated. Objectives delivered with the system are calibrated upon installation. Any other objectives purchased later will have to have their own calibration performed. A marked calibration slide is necessary to complete this calibration. The calibration steps are as follows:

1. Move the objective lens to be calibrated into position on the microscope
2. Focus on a slide containing an object of known width (a calibration slide is recommended for this purpose)
3. Select Tools > Calibration/Alignment > Objective Lens/FOV Calibration to open the Objective Calibration dialog
4. Click **Add New Objective**
5. Highlight the new objective in the table and fill in the objective name, power, and NA
6. In the **Enter calibration distance here** field, enter the width in microns of the object being used for calibration. Generally, longer calibration distances result in more accurate calibrations.
7. Click **Calibrate Selected Objective**.
8. In the Image window, move the endpoints of the line to the edges of the object used for calibration (the distance specified in step 6). Expanding the Image window may allow for more accurate calibration.
9. To accept the calibration, click the **Accept** button in the Image window. To abort the calibration, click the **Cancel** button in the Image window.
10. The FOV Width and Height field will now contain values and the Calibrated field will say 'True'.

11. If the objective will be used for 3D line scans (described [here](#)), enter its calculated Piezo Lag Time. If this feature will not be used, leave the parameter set to 0.
12. Close this window. The newly calibrated objective lens will appear in the Objective Lens pull-down list in the [Scanning Controls](#) section on the main control window.

During calibration, the **Calibrate Selected Objective** button becomes the **Abort Calibration** button, which can be used to end the calibration process.

Additional buttons in this dialog allow the user to change the order of objectives in the list and to add/remove objectives from the list.

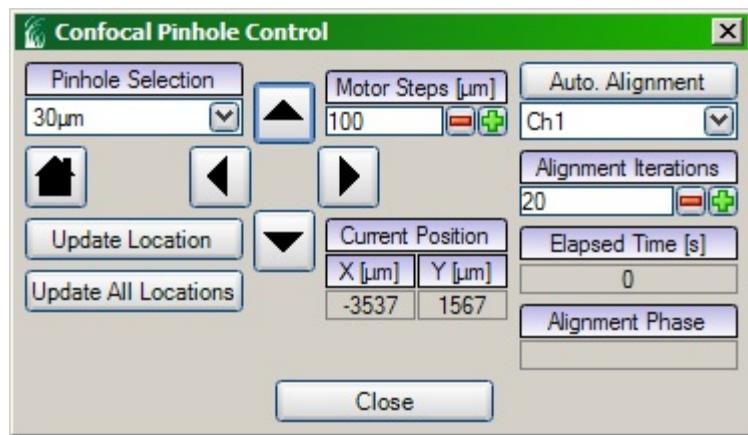
An objective must be calibrated in each Acquisition Mode in which it will be used. For example, the 40x objective will need to be calibrated in both Galvo mode and SFC mode, if both scanners are present on the system. If the objective is calibrated only in Galvo mode, the field of view and pixel size information may be incorrect for SFC mode. Also, the Calibrated field will display 'False' for that objective in SFC mode.

Note that objectives calibrated in earlier versions of Prairie View software may have a Calibrated field that displays 'False'. This occurs because earlier versions of software did not keep track of the Acquisition Mode in use when the objective was calibrated. However, the calibration will continue to work as it did before the software was updated.

1-P Pinhole

This section of the Help file is under construction and has not been updated recently. The information below is from a previous version of the manual and may be somewhat out-dated. Please contact Bruker Fluorescence Microscopy support personnel with questions about these features.

If a 1-P Confocal pinhole detector is present on the system, this option opens a control dialog for the pinhole motors.



The 3-channel confocal system has a pinhole plate with 9 differently sized apertures. The user selects an aperture in the software, and motors drive the pinhole plate to put the selected aperture in the light path. Older versions of this scanner save a total of 9 positions - one for each pinhole. Newer versions of this scanner save the matrix of 9 positions for each objective calibrated on the system, up to 8 objectives.

The 2-channel confocal system has a pinhole plate with one aperture. To change to a different aperture size, the user manually replaces the pinhole plate.

Choose a pinhole from the Pinhole Selection drop-down menu to move the pinhole plate to the saved position of that pinhole.

The Current Position fields display the positions of the motors driving the pinhole plate.

The user can make adjustments to the pinhole position by defining a number of Motor Steps and using the arrow buttons to move the motor(s) by that number of steps.

Update Location updates the selected pinhole position to the current motor positions. When clicked, the currently selected pinhole definition will be modified to have its coordinates match the current position of the pinhole plate. To save a new location for the currently selected pinhole, move to the desired position and click the **Update Location** button.

Update All Locations updates the selected pinhole position based on current stage location, as described above. In addition, it updates all other pinhole positions by the difference between the old and new position for the current pinhole.



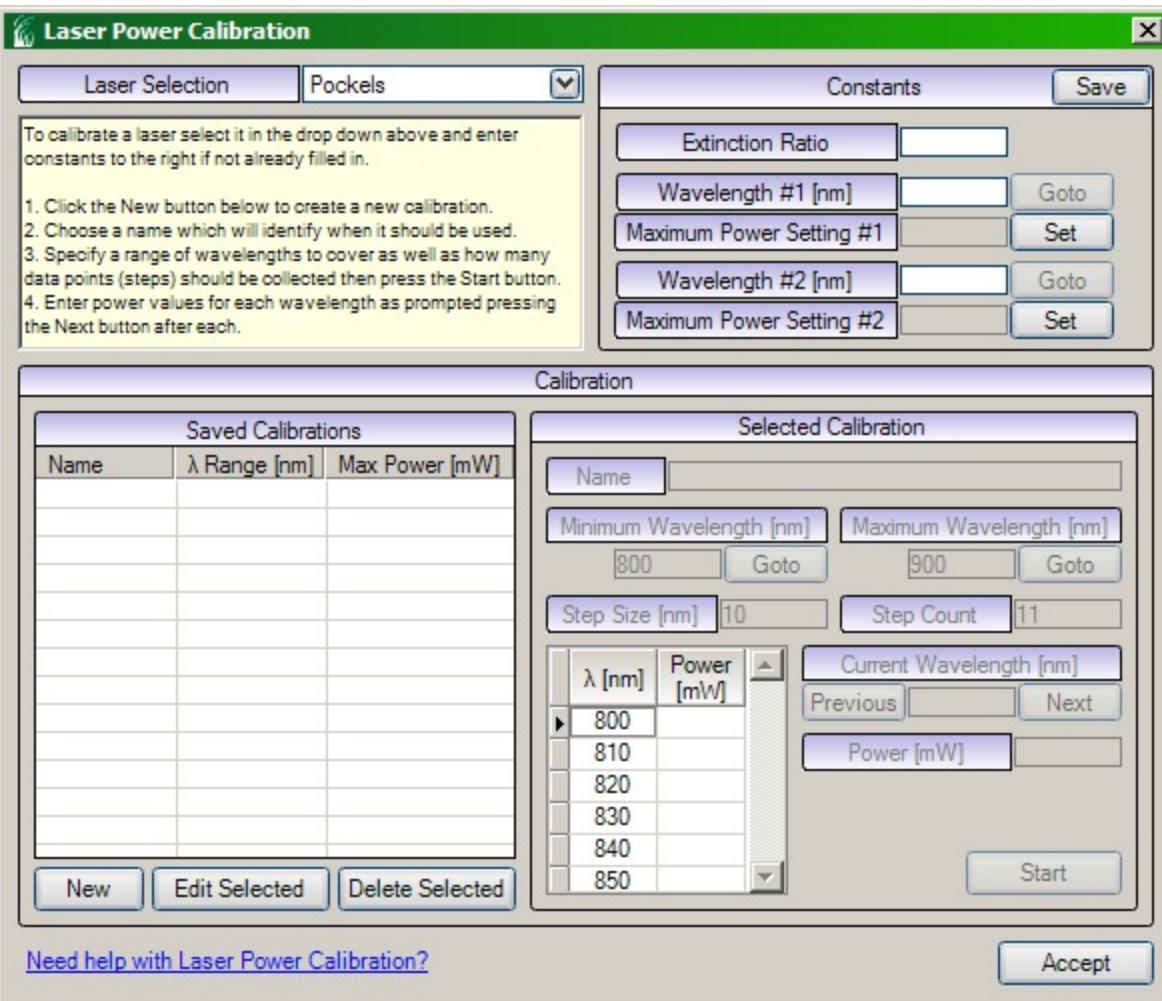
The **Home** button drives the pinhole plate motors to their home positions. This can be used for troubleshooting/diagnostic purposes when working with Bruker Fluorescence Microscopy support personnel. Note that there is unlikely to be a pinhole at the home position.

The **Auto. Alignment** algorithm works for systems with pinholes on separate plates, such as most 2-channel confocal systems. It is not designed to work for systems with an 8-position pinhole plate, such as most 3-channel confocal systems. The algorithm can be used during a Live Scan to find the pinhole position that results in the brightest signal. Select the channel to use during auto-alignment, based on the sample and detection glass in the system. Define the number of Alignment Iterations to use when optimizing the position. The Elapsed Time and Alignment Phase fields display information about the progress of the alignment algorithm. The algorithm is useful for finding the pinhole in a newly-installed plate. After the auto-alignment is complete, the user can use the arrow buttons to fine-tune the position for best signal.

Click **Close** to exit the dialog.

2-P Laser Power Calibration

This section of the Help file is under construction and has not been updated recently. The information below is from a previous version of the manual and may be somewhat out-dated. Please contact Bruker Fluorescence Microscopy support personnel with questions about these features.



Laser Power Calibration applies only to 2P lasers using a Pockels cell for power modulation. The formulas used in this software feature will not apply properly to visible lasers controlled directly or with an AOTF.

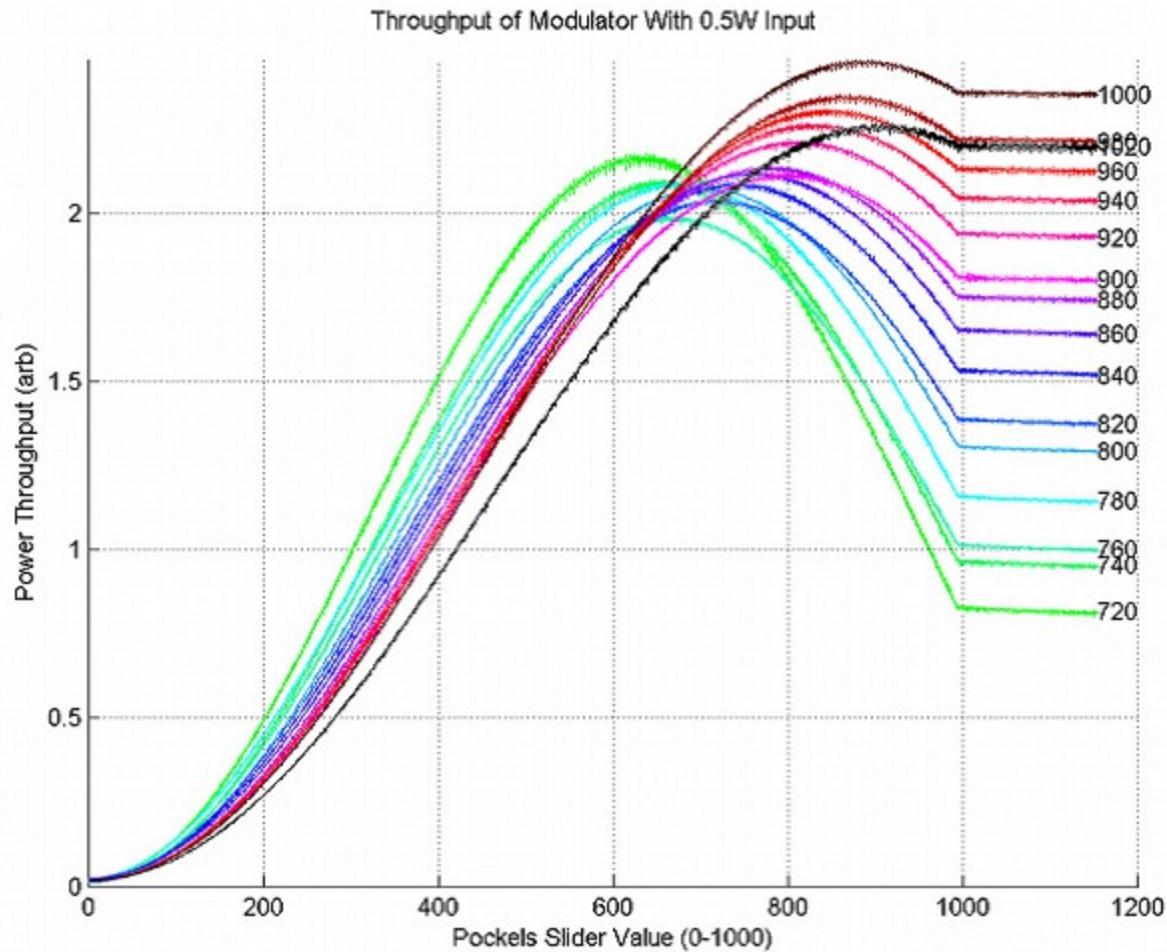
Detailed information about laser calibration can be accessed from within Prairie View by clicking the **Need help with Laser Power Calibration?** link at the bottom of the Laser Power Calibration dialog.

Operating Modes of the Laser Power Slider

In addition to the Default mode of laser power control, two levels of calibration are possible - Attenuation and Calibrated modes. Available modes can be selected from a drop-down menu below each laser power slider in the [Laser, PMT, DAQ tab](#) of the Main Control window.

Default Mode

In Default mode, laser slider values are arbitrary numbers (usually from 0 to 100 or 0 to 1000). Increasing the slider value increases the voltage signal to the Pockels cell. However, higher voltage does not necessarily mean higher laser power. At some point a maximum laser output is reached, after which increases in slider value (voltage) result in lower laser power. This maximum point is different for each laser wavelength. A typical response curve is shown below, with laser slider value on the x axis and normalized laser power throughput on the y axis. Note that due to differences in power produced by the laser, the absolute maximum power at each wavelength will also differ; this effect is not shown in the graph below.



Attenuation Mode

In Attenuation mode, laser slider values become percentages of maximum power at the given wavelength. This prevents the user from going past the voltage providing the maximum laser power for the defined wavelength. If the laser is configured in Prairie View software, the wavelength will be detected automatically. If the laser is not configured in Prairie View, the user will need to select the wavelength from a slider below the power slider.

By filling in the values listed in the Constants section of the Laser Power Calibration dialog, the user enables Attenuation mode. These measurements are all relative values and can be taken with a power meter anywhere in the light path downstream of the Pockels cell.

The Pockels cell should be allowed to warm up for at least 20 minutes after the laser cavity shutter has been opened before taking these measurements.

The bias knob on the Pockels controller should be left at the value determined by Bruker Fluorescence Microscopy personnel during installation. This value is usually determined by tuning the laser to an intermediate wavelength such as 820nm, setting the laser power slider to 0, opening the shutter, and adjusting the knob to get the lowest possible power output (based on a power meter reading or image intensity). Changes to the bias require repeating the measurements described below.

Extinction Ratio (measure at an intermediate wavelength, such as 830nm)

- Minimum power: Set the laser power slider to 0 and open the shutter(s) needed to get light to the power meter.
- Maximum power: Increase the laser power slider in the [Laser, PMT, DAQ tab](#) to get the maximum laser power at the meter. Note that this will likely happen before reaching the maximum slider value.
- Extinction Ratio = Maximum power / Minimum power; enter this value in the Extinction Ratio field in the Laser Power Calibration dialog

Maximum drive voltage across the range of wavelengths (used to map a linear approximation of the maximum drive voltage at any wavelength)

1. Choose two different wavelengths, such as 780nm and 1020nm
2. Enter these two wavelengths in the Wavelength #1 and Wavelength #2 fields
3. Tune the laser to one of the desired wavelengths
 - If Prairie View has the 2-P Laser control integrated into its controls, then pressing the **GoTo** button will tune the laser
 - If Prairie View does not have integrated 2-P Laser control, then the user must tune the laser manually
4. Adjust the laser power slider in the [Laser, PMT, DAQ tab](#) to find the value that results in the highest laser power reading on a power meter in the light path
5. Click the **Set** button next the the Maximum Power Setting field for that wavelength in the Laser Power Calibration dialog
6. Repeat steps 3-5 for the other wavelength selected
7. Click the **Save** button at the top of the Constants section of the Laser Power Calibration dialog

There will now be an additional control associated with the laser power slider in Prairie View for the laser(s) that were just configured in the previous steps. This new control is labeled 'Mode' and the associated combo box has two options Default and Attenuation.

When this control is set to Default the laser slider will operate like it always has. This means that potentially the operator could adjust the drive voltage for the pockels cell past its maximum output and the actual laser power output from the pockels cell will not be

necessarily higher as the laser slider is increased.

When the control is set to Attenuation the laser slider is internally rescaled such that based on the current operating wavelength for the associated 2-P laser the drive voltage for the pockels cell will go between 0 volts and the calibrated voltage that will generate the maximum pockels cell output for the wavelength. The laser control label will include the symbol '[%]' after the laser line label when Attenuation mode is selected. The laser slider value will go from 0.00% to 100.0% (regardless of the scale used when in 'Default' mode). If the laser is configured in Prairie View software, the wavelength will be detected automatically. If the laser is not configured in Prairie View, the user will need to select the wavelength from a slider below the power slider.

Calibrated Mode

When a full Calibration File has been created for a given set of operating parameters, the laser slider will reflect laser power in milliwatts. This is a very useful mode of laser operation, but is also very sensitive to changes over time that can affect the accuracy of the power readings. Keep the following in mind when preparing to use Laser Power Calibration:

- All optical components in the light path between the Pockels cell and the light meter (placed to measure the light output at the objective lens) will have an impact on the power delivered to the sample. Therefore it is necessary to perform a calibration for at least each objective lens used on the system to take proper advantage of this calibration process. Additional calibration files may be needed for systems with exchangeable dichroic mirrors, beam expander lenses, etc.
- Since the maximum sample power for a given calibration is limited to the 'lowest' measured power through the calibration wavelength range, the operator might wish to make several calibrations for a given objective lens at various wavelength ranges.

Laser Power Calibration extends the operating capabilities of the multiphoton imaging system. Once the proper system measurements have been made and the laser power calibration procedure completed, the operator will have the ability to specify the desired laser power to be delivered to the sample in mW units. In addition, once the laser power setting has been made, if the operator changes the wavelength of the 2-P laser, the drive voltage to the Pockels cell will be automatically adjusted to maintain a constant laser power to be delivered to the sample.

Warning: The proper implementation of the Laser Power Calibration is dependent upon the initial Pockels cell installation as well as software during installation. Any adjustments to the light path after the setup and calibration(s) have been performed would most likely result in the necessity of having the calibrations performed again.

Power Calibration

The purpose of the Laser Power Calibration is to provide one or more calibration files that will allow the operator to set the desired laser power in mW at the sample via the laser slider control(s) in *PrairieView*. This option will be in addition to the two operating modes already available; Default and Attenuation.

The actual power calibration is performed by stepping through a range of wavelengths and setting the drive voltage to the proper V_{π} at each wavelength and after the system has had a few seconds to settle at the new wavelength and drive voltage, the laser power at the objective lens is recorded. This creates a table like the following:

Wavelength(nm) Power(mW)

700	200
...	...
800	400
...	...
1050	50

Using the information in the table, when in the ‘power’ mode, the laser power to the sample can be controlled between 0mW and 50mW from 700nm to 1050nm.

The actual calibration process is conducted with the Laser Power Calibration dialog within *PrairieView*.

1. Place a power meter below the objective lens, slightly above or below the focal plane. The meter will probably deliver better results if the laser light isn’t focused to such a fine spot size.
2. Fill in the various fields in the Constants section as outlined above.
3. Press **New**.
4. Enter a name for the calibration file in the **Name** field of the Selected Calibration Field. The calibration name should reflect the objective lens being used as well as the range of wavelengths that will be used in the calibration.
5. Enter the **Minimum Wavelength [nm]**, **Maximum Wavelength [nm]**, and **Step Size [nm]** values to be used for the calibration.
6. Press **Start**. If 2-P laser control is integrated for the selected laser, the software will automatically tune the laser to the **Minimum Wavelength** value for the calibration. If a 2-P laser control is not integrated for the selected laser, the operator must manually tune the laser to the **Minimum Wavelength** value for the calibration.
7. After the laser has finished tuning, wait a couple of seconds for the reading on the power meter to stabilize and then enter the power meter reading (in milliwatts) in the **Power** field and hit **Enter**. The entered value will now appear in the calibration table across from the current 2-P laser wavelength.
8. After hitting **Enter**, if 2-P laser control is integrated for the selected laser, the software will

automatically change the laser wavelength to the next value in the calibration table. If a 2-P laser control is not integrated, then the operator will need to manually tune the laser to the appropriate wavelength.

9. Repeat last step until the calibration has been completed.

10. Press **Accept** to utilize the calibration file.

After at least one calibration file has been generated, then the ‘Mode’ control associated with the calibrated laser line will include the names of the calibration file(s) (in addition to ‘Default’ and ‘Attenuation’ as outlined above).

When one of the calibration files is selected the laser control label will include the symbol ‘[mW]’ after the laser line label.

When one of the calibration files is selected then the laser slider is internally rescaled such that based on the current operating wavelength for the associated 2-P laser the drive voltage for the Pockels cell will go between the minimum achievable output power (not necessarily 0mW) and the maximum output power that can be attained at all wavelengths within the calibration range. For example, if the calibration wavelength range was 780nm to 950nm and the lowest maximum power measured across that range was 50mW, then when this calibration file is used, the maximum output power at any of the calibrated wavelengths will be 50mW.

If the association between the 2-P laser and the laser control was set in ‘PrairieConfigUtility.exe’, then when the operating wavelength is changed, the laser setting will be automatically adjusted to maintain the desired sample power.

Additional Information

The selected laser mode (Default, Attenuation, or Power Calibration file) and laser setting will be retained when a ‘label’ is created/used in Prairie View. The laser setting displayed in the ‘Laser Power(s)’ column will include ‘mW’ or ‘%’ if the label was created when a calibration file or the Attenuation mode was in effect.

The selected laser mode and laser setting will be retained for the ‘Interlaced scan pattern’. The selected laser mode will also be reflected in the laser sliders for the setup dialog for the ‘Interlaced scan pattern’.

The selected laser mode and laser setting will be retained for the Photo Activation masks and settings. The selected laser mode will be reflected in the laser sliders for the Photo Activation palette edit dialog.

As mentioned previously, all optical components in the light path between the Pockels cell and the light meter (placed to measure the light output at the objective lens) will have an impact on the power delivered to the sample. Therefore it is necessary to perform a calibration for at least each objective lens used on the system to take proper advantage of this calibration process.

Since the maximum sample power for a given calibration is limited to the ‘lowest’ measured power through the calibration wavelength range, the operator might wish to make several calibrations for a given objective lens at various wavelength ranges.

If a laser control is configured to use a calibration file and there is a 2-P laser integrated into Prairie View and associated with the particular laser control, if the operator attempts to tune the laser to a wavelength outside of the range of the calibration file, the operator will be warned that that is an illegal operation and the wavelength change will not take place.

If the system is equipped with the high-speed optics option (AOD), it might not be necessary to perform the Pockels cell calibration. This is due to the fact that the AOD system is set up and optimized for a single wavelength. It might be better to simply take a couple of power measurements at the objective lens in 'Attenuation' mode and then manually determine which setting in Attenuation mode will provide the desired sample power.

Laser Power Calibration extends the operating capabilities of the Bruker Fluorescence Microscopy Ultima system. Once the proper system measurements have been made and the laser power calibration procedure completed, the operator will have the ability to specify the desired laser power to be delivered to the sample in milliwatts. In addition, once the laser power setting has been made, if the operator changes the wavelength of the 2-P laser, the drive voltage to the pockels cell will be automatically adjusted to maintain a constant laser power to be delivered to the sample.

There are several aspects to the Laser Power Calibration process. The first step; [**Software Configuration**](#) need only be performed once, and should be performed by the Bruker Fluorescence Microscopy representative who installed the system. The second step; [**Pockels Cell Installation/Setup**](#) should only need to be performed once (when the system is installed) but if a pockels cell is replaced or any changes are made to the optical alignment of the laser beam before it reaches the pockels cell would probably necessitate performing this step again. The final step; [**Laser Power Calibration**](#) can be performed by the operator.

The actual power calibration is performed by stepping through a range of wavelengths and at each wavelength, after the system has had a few seconds to settle at the new wavelength and drive voltage, record the laser power at the objective lens. This would create a table like the following:

Wavelength(nm)	Power(mW)
700	200
...	...
800	400
...	...
1050	50

Using the information in the table, when in the 'power' mode, we can control the laser power to the sample between 0mW and 50mW from 700nm to 1050nm.

[**Software Configuration \(Top\)**](#)

1. Start the program 'PrairieConfigUtility.exe'.

2. Load the appropriate 'configuration.xml' file (starting with Prairie View version 3.1.0.0 the configuration.xml file should be loaded automatically when the program is started).
 3. To obtain finer control over the output laser power, it is recommended that the 'Display Max' setting for the laser controls on the 'Laser' tab be set to 1000 (instead of the default value of 100).
 4. On the 'Laser' tab, there is a new 'Device Type' option 'PVAnalogOutputDAQBuffered'. For maximum flexibility, it is recommended that this 'Device Type' be used. However, the default option of 'PVAnalogOutputDeviceControlBox' will work.
 1. Please pay attention to the 'note' regarding the 'PVAnalogOutputDAQBuffered' type at the bottom of the 'Laser' tab. This note provides information on the desired setting for the 'OUT Max' parameter based upon the 'Device' selection.
 5. If one or more 2-P lasers are integrated into the control of Prairie View (e.g. Coherent Chameleon or Spectra Physics Mai Tai), then the control in the '2-P Laser' column will be active. Use this control to specify the association between the laser control (pockels cell) and 2-P laser.
-

Pockels Cell Installation/Setup ([Top](#))

If you are starting with a new system installation, then continue with the following section, [New System Installation](#). If you are starting with a system that has already been installed, then skip the [New System Installation](#) section and continue with the [Existing System Installation](#) section. After completing either the [New System Installation](#) section or the [Existing System Installation](#) section, continue to the [Laser Power Calibration](#) section.

New System Installation ([Top](#))

1. **Always exercise extreme caution when working with the 2-P laser beam. Safety goggles are recommended.**
2. **Extreme caution should be exercised when working with a pockels cell. Misalignment of the laser beam into the pockels cell could result in permanent damage to the device and render it unusable. Always consult with Bruker Fluorescence Microscopy before attempting to make any changes to the 2-P laser light path (pockels cell rotation, etc.).**

3. If more than one 2-P laser is integrated for imaging on the system, then the calibration process needs to be carried out for each laser/pockels cell combination independently.
4. For initial setup, the pockels cell is NOT yet rotated 90 degrees to the incoming laser. This means that with **no** drive voltage applied, all of the laser light will pass through the pockels cell. Also, the drive voltage (usually a line from the Device Control Box or a line from one of the National Instruments DAQ boards) should **not** even be connected to the pockels cell controller.
 1. Preferably there are no optical components between the laser and the 1/2 wave plate (this includes mirrors). This is important since any optical movement of the beam before it reaches the pockels cell would basically invalidate any previous calibration.
 2. Modify the position (rotation) of the 1/2 wave plate to get 200mW of laser power at the input side of the pockels cell.
 3. If 200mW of laser power is going into the pockels cell, we should get about 95% of that power on the backside (output) of the pockels cell, this would be 190mW.
 4. If you are getting much less than 95% out based upon the input power, then something is either improperly aligned or not working properly.
5. Pockels Cell bias adjustment.
 1. Turn off Con Optics box.
 2. Remove laser trap from the pockels cell (the pockels cell extinction port should be parallel to the table surface).
 3. Place an iris in front of the pockels cell to get the proper beam height setting for the iris.
 4. Place the iris as far away as possible from the pockels cell on the rejected light path (where the beam trap was removed) and make sure that the pockels cell is properly rotated to get the laser parallel to the table and oriented 90 degrees to the incoming laser light.
 5. Measure the laser power on the output path of the pockels cell. With this orientation and no power applied to the drive voltage, approximately 95% of the incoming laser power should be measured at the output.
 6. Make certain that the rejected light from the polarizing beam splitter is at the same height as the rejected light from the pockels cell in the previous step.
 7. Turn on the Con Optics box.
 8. Adjust the bias on the pockels cell controller to get minimum output power. The bias should be between +/- 100. If it is outside of this range, either the

alignment is wrong or something else is wrong.

9. **Bias should basically not be adjusted ever again** (even when the operating wavelength is changed). A good idea would be to label the Con Optics box near the bias control knob with the calibrated bias setting. In this manner, the proper bias setting will not be forgotten and may be quickly reset to the proper value.

6. Determining the extinction ratio.

1. Connect the drive voltage to the pockels modulator (usually either a line from the Device Control Box or a line from an analog output from one of the National Instruments DAQ boards (via a BNC-2090 or BNC-2110 box)).
 2. Adjust the half wave plate until we have 200mW of power going into the pockels cell.
 3. Increase the drive voltage until we have minimum power at the output of the pockels cell. This might not be exactly 0mW, but it should be close.
 4. Compare the output power at 0V drive (max power) and at the min power settings and calculate the ratio.
 5. This ratio should be at least 400:1 or something close. Higher is better here. Record this value in the 'Laser Power Calibration' dialog within Prairie View. This dialog is activated when the operator selects the 'Tools' menu option and then selects 'Calibrate Laser Power...'. The extinction ratio is recorded in the 'Constants' area of the dialog in the field labeled 'Extinction Ratio'. If the calculated extinction ratio is 250:1 for example, the value to enter in this field is 250.
 1. If more than one 2-P laser is being used for imaging on the system, be sure to specify the desired laser line that is being calibrated within the 'Laser Power Calibration' dialog. There is a control labeled 'Laser Selection' at the top left hand corner of the dialog with an associated control that will be active if more than one 2-P laser is being used. Use this control to select the laser that is being calibrated.
7. Replace the laser trap on the pockels cell.
 8. Now the pockels cell can be rotated 90 degrees and with the drive voltage set to the value in step 'c' above, we should now have the maximum laser power at the output of the pockels cell.
 9. At the typical imaging wavelengths of 800nm or so, there is so much energy coming out of the lasers that we should use the 1/2 wave plate to reduce the power coming into the pockels cell to less than or equal to 2W (maybe something closer to 1.5W would work for most needs).

1. The main purpose for this is that we can bring our minimum pockels cell output power down closer to 0 since there is a certain amount of 'bleed-through' with the pockels cell since it is not a perfect device for extinguishing the beam.
10. Determine the maximum drive voltage across the wavelength range.
 1. Adjust the half wave plate until we have 1.5 to 2 W of power going into the pockels cell.
 2. Choose two different wavelengths, e.g. 780nm and 1050nm.
 1. In the 'Laser Power Calibration' dialog, in the 'Constants' field, enter these two wavelengths in the 'Wavelength #1[nm]' and 'Wavelength #2[nm]' fields.
 3. With the setup that has been performed to this time, 0.0V for the drive voltage should result in the minimum output power from the pockels cell at all wavelengths.
 4. Tune the laser to one of the desired wavelengths.
 1. If Prairie View has the 2-P laser control integrated into its controls, then the operator may press the 'Goto' button and have the software automatically tune the laser to the specified wavelength
 2. If Prairie View does not have the 2-P laser control integrated into its controls, then the operator must manually tune the laser to the specified wavelength.
 5. Adjust the drive voltage to get the maximum power, measured at the back of the pockels cell. The drive voltage is adjusted via the appropriate Laser slider control on the 'Laser, PMT,DAQ' tab in Prairie View. Record this value for the appropriate wavelength in the 'Laser Power Calibration' dialog by pressing the 'Set' button.
 6. Repeat steps 'd' and 'e' for the other wavelength.
 7. These two drive voltages and their respective wavelength settings will allow us to map (based on a linear approximation), the necessary drive voltage to achieve maximum output at the pockels cell at any wavelength. We'll refer to this value as V_p .
 8. Press the 'Save' button located in the 'Constants' label in the 'Laser Power Calibration' dialog.
 9. Press the 'Accept' button and the 'Laser Power Calibration' dialog will close.
 1. You will now notice an additional control associated with the laser power slider in Prairie View for the laser(s) that were just configured in the previous steps.

2. This new control is labeled 'Mode' and the associated combo box has two options; 'Default' and 'Attenuation'.
 1. When this control is set to 'Default' the laser slider will operate like it always has. This means that potentially the operator could adjust the drive voltage for the pockels cell past its maximum output and the actual laser power output from the pockels cell will not be necessarily higher as the laser slider is increased.
 2. When the control is set to 'Attenuation' the laser slider is internally rescaled such that based on the current operating wavelength for the associated 2-P laser the drive voltage for the pockels cell will go between 0 volts and the calibrated voltage that will generate the maximum pockels cell output for the wavelength.
 1. The laser control label will include the symbol '[%]' after the laser line label when 'Attenuation' mode is selected.
 2. The laser slider value will go from 0.00% to 100.0% (regardless of the scale used when in 'Default' mode).
 3. By just having set the values for the extinction ratio and the drive voltage values across the wavelength range we've improved the usability of the system with the addition of the 'Attenuation' mode.

Existing System Installation ([Top](#))

1. **Always exercise extreme caution when working with the 2-P laser beam. Safety goggles are recommended.**
2. **Extreme caution should be exercised when working with a pockels cell. Misalignment of the laser beam into the pockels cell could result in permanent damage to the device and render it unusable. Always consult with Bruker Fluorescence Microscopy before attempting to make any changes to the 2-P laser light path (pockels cell rotation, etc.).**
3. If more than one 2-P laser is integrated for imaging on the system, then the calibration process needs to be carried out for each laser/pockels cell combination independently.
4. Pockels Cell bias adjustment.
 1. If the installed system contains a laser power meter that is placed after the Pockels cell (usually standard), then this power meter can be referenced for the bias adjustment process. If the system does not contain a power meter, then the operator will have to place a power meter in the light path at the

output of the Pockels cell. If the operator must place a light meter in the light path, exercise extreme caution with regards to the laser light as outlined earlier.

2. Tune the laser to a nominal operating wavelength around 840nm.
3. Adjust the bias on the pockels cell controller to get minimum output power. The bias should be between +/- 100. If it is outside of this range, either the alignment is wrong or something else is wrong.
4. **Bias should basically not be adjusted ever again** (even when the operating wavelength is changed). A good idea would be to label the Con Optics box near the bias control knob with the calibrated bias setting. In this manner, the proper bias setting will not be forgotten and may be quickly reset to the proper value.
5. Determining the extinction ratio.
 1. Leave the laser wavelength at the value set in the previous step.
 2. If the installed system contains a laser power meter that is placed after the Pockels cell (usually standard), then this power meter can be referenced for the bias adjustment process. If the system does not contain a power meter, then the operator will have to place a power meter in the light path at the output of the Pockels cell. If the operator must place a light meter in the light path, exercise extreme caution with regards to the laser light as outlined earlier.
 3. Record the value on the laser power meter when the 'laser control' within Prairie View is at '0'.
 4. Start increasing the 'laser control' value within Prairie View and watch the laser power meter for the maximum output power value. This value will probably be reached before the laser power control reaches its maximum value (e.g. 1000).
 5. Take the laser power meter reading in step (4) and divide it by the laser power meter reading in step (3), this is the extinction ratio.
 6. This ratio should be at least 400:1 or something close. Higher is better here. Record this value in the 'Laser Power Calibration' dialog within Prairie View. This dialog is activated when the operator selects the 'Tools' menu option and then selects 'Calibrate Laser Power...'. The extinction ratio is recorded in the 'Constants' area of the dialog in the field labeled 'Extinction Ratio'. If the calculated extinction ratio is 250:1 for example, the value to enter in this field is 250.
 1. If more than one 2-P laser is being used for imaging on the system, be

sure to specify the desired laser line that is being calibrated within the 'Laser Power Calibration' dialog. There is a control labeled 'Laser Selection' at the top left hand corner of the dialog with an associated control that will be active if more than one 2-P laser is being used. Use this control to select the laser that is being calibrated.

6. Determine the maximum drive voltage across the wavelength range.

1. If the installed system contains a laser power meter that is placed after the Pockels cell (usually standard), then this power meter can be referenced for the bias adjustment process. If the system does not contain a power meter, then the operator will have to place a power meter in the light path at the output of the Pockels cell. If the operator must place a light meter in the light path, exercise extreme caution with regards to the laser light as outlined earlier.
2. Choose two different wavelengths, e.g. 780nm and 1050nm.
 1. In the 'Laser Power Calibration' dialog, in the 'Constants' field, enter these two wavelengths in the 'Wavelength #1[nm]' and 'Wavelength #2[nm]' fields.
3. With the setup that has been performed to this time, 0.0V for the drive voltage ('laser control' set to 0 in Prairie View) should result in the minimum output power from the pockels cell at all wavelengths.
4. Tune the laser to one of the desired wavelengths.
 1. If Prairie View has the 2-P laser control integrated into its controls, then the operator may press the 'Goto' button in the 'Laser Power Calibration' dialog, in the 'Constants' field and have the software automatically tune the laser to the specified wavelength.
 2. If Prairie View does not have the 2-P laser control integrated into its controls, then the operator must manually tune the laser to the specified wavelength.
5. Adjust the drive voltage to get the maximum power, measured at the back of the pockels cell. The drive voltage is adjusted via the appropriate Laser slider control on the 'Laser, PMT,DAQ' tab in Prairie View. Record this value for the appropriate wavelength in the 'Laser Power Calibration' dialog by pressing the 'Set' button.
6. Repeat steps '4' and '5' for the other wavelength.
7. These two drive voltages and their respective wavelength settings will allow us to map (based on a linear approximation), the necessary drive voltage to achieve maximum output at the pockels cell at any wavelength. We'll refer to

this value as V_π .

8. Press the 'Save' button located in the 'Constants' label in the 'Laser Power Calibration' dialog.
9. Press the 'Accept' button and the 'Laser Power Calibration' dialog will close.
 1. You will now notice an additional control associated with the laser power slider in Prairie View for the laser(s) that were just configured in the previous steps.
 2. This new control is labeled 'Mode' and the associated combo box has two options; 'Default' and 'Attenuation'.
 1. When this control is set to 'Default' the laser slider will operate like it always has. This means that potentially the operator could adjust the drive voltage for the pockels cell past its maximum output and the actual laser power output from the pockels cell will not be necessarily higher as the laser slider is increased.
 2. When the control is set to 'Attenuation' the laser slider is internally rescaled such that based on the current operating wavelength for the associated 2-P laser the drive voltage for the pockels cell will go between 0 volts and the calibrated voltage that will generate the maximum pockels cell output for the wavelength.
 1. The laser control label will include the symbol '[%]' after the laser line label when 'Attenuation' mode is selected.
 2. The laser slider value will go from 0.00% to 100.0% (regardless of the scale used when in 'Default' mode).
 3. By just having set the values for the extinction ratio and the drive voltage values across the wavelength range we've improved the usability of the system with the addition of the 'Attenuation' mode.

Laser Power Calibration ([Top](#))

1. The calibration process is conducted with the 'Laser Power Calibration' dialog within Prairie View.
 1. Place a power meter below the objective lens.
 1. Rather than place the power meter at the focal plane for the objective, place it slightly above or below the focal plane. The meter will probably deliver better results if the laser light isn't focused to such a fine spot size.
 2. To perform a calibration, first the various fields in the 'Constants' section must be properly filled in. This is outlined above in the [Pockels Cell](#)

Installation/Setup section.

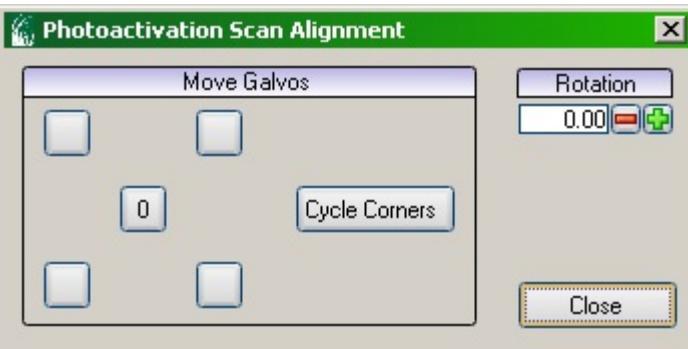
3. Press the 'New' button and in the 'Selected Calibration' area, enter the name of the calibration file in the 'Name' field in the 'Selected Calibration' section.
 1. The calibration name should reflect the objective lens being used as well as the range of wavelengths that will be used in the calibration.
4. Enter the values for the 'Minimum Wavelength [nm]', 'Maximum Wavelength [nm]', and 'Step Size [nm]' fields to be used for the calibration.
5. Press the 'Start' button.
 1. If 2-P laser control is integrated for the selected laser, the software will automatically tune the laser to the 'Minimum Wavelength' value for the calibration. If a 2-P laser control is not integrated for the selected laser, the operator must manually tune the laser to the 'Minimum Wavelength' value for the calibration.
 2. After the laser has finished tuning (changing wavelengths), wait a couple of seconds for the reading on the power meter to stabilize and then enter the power meter reading (in milliWatts) in the 'Power' field and hit the 'Enter' key. The entered value will now appear in the calibration table across from the current 2-P laser wavelength.
 3. After hitting the 'Enter' key, if 2-P laser control is integrated for the selected laser, the software will automatically change the laser wavelength to the next value in the calibration table. If a 2-P laser control is not integrated, then the operator will need to manually tune the laser to the appropriate wavelength.
 4. Repeat steps 1 and 2 until the calibration has been completed.
 5. Press the 'Accept' button to utilize the calibration file.
6. After at least one calibration file has been generated, then the 'Mode' control associated with the calibrated laser line will include the names of the calibration file(s) (in addition to 'Default' and 'Attenuation' as outlined above).
 1. When one of the calibration files is selected the laser control label will include the symbol '[mW]' after the laser line label.
 2. When one of the calibration files is selected then the laser slider is internally rescaled such that based on the current operating wavelength for the associated 2-P laser the drive voltage for the pockels cell will go between the minimum achievable output power (not necessarily 0mW) and the maximum output power that can be attained at all wavelengths within the calibration range. For example, if the calibration wavelength range was 780nm to 950nm and the lowest maximum power measured

across that range was 50mW, then when this calibration file is used, the maximum output power at any of the calibrated wavelengths will be 50mW.

3. If the association between the 2-P laser and the laser control was set in 'PrairieConfigUtility.exe', then when the operating wavelength is changed, the laser setting will be automatically adjusted to maintain the desired sample power.

Photoactivation Alignment

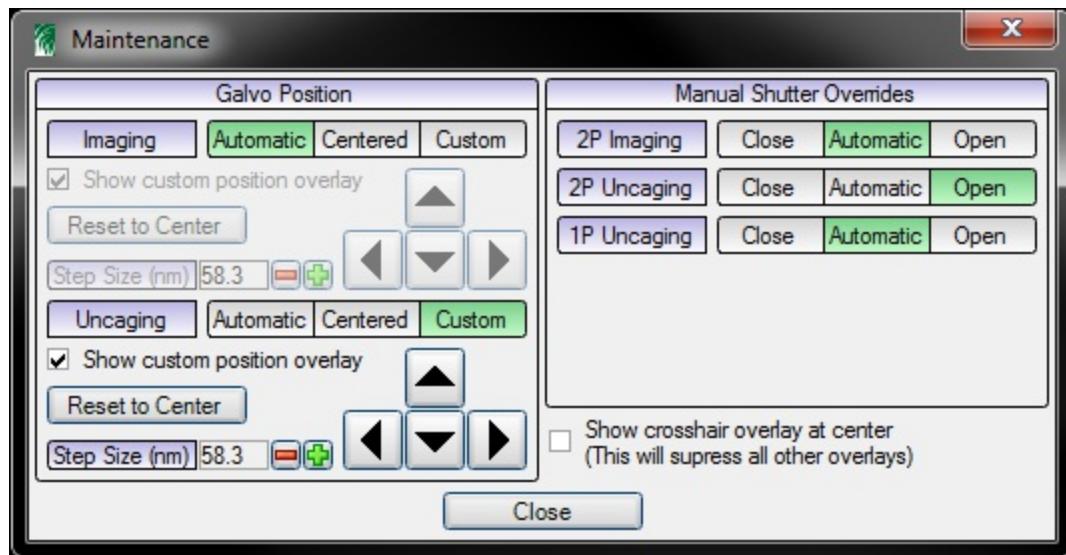
If the system is configured with a Camera or SFC and a photoactivation module, this option opens a dialog to adjust the scan rotation of the photoactivation scan relative to the imaging scan. No adjustments should be made until the user is certain that the camera is properly aligned with the SFC.



A good way to determine this rotation is to set up a [Photoactivation](#) mask to burn a large "+" symbol covering the entire image of a thin film sample (such as a dry erase marker slide). After using the mask to burn away the "+" pattern, compare the image of the burned sample to the mask overlay on the Image window. If the pattern is rotated, adjust the value in the **Rotation** field and repeat the test on a fresh area of the sample. The buttons in the Move Galvos section of this dialog are for use by Bruker Fluorescence Microscopy personnel during installation.

Maintenance

Controls in the Maintenance dialog are useful during installation, trouble-shooting, and maintenance tasks. This dialog gives the user the ability to over-ride usual software control of each shutter and galvanometer set in the system.



Center Galvos

In the Center Galvos section, the user can center the galvo mirrors for the imaging and/or uncaging light paths. The galvo sets present in this section depend on system configuration.

Bringing the galvos to center is useful when checking alignment of the laser beam(s) through the scanning system, as galvos must be centered to use the fluorescent objective target as a reference point. When aligning an uncaging path and/or uncaging spot detector, it can also be useful to center the uncaging galvos via this dialog while scanning with the imaging galvos via the Main Control window.

These buttons will be disabled with the galvanometers are in use. If the software needs to control the galvanometers for any reason (e.g. the user presses the **Single Scan** button on the Main Control window), the galvanometers position will be set to **Automatic** and the buttons will be disabled until the software returns the galvanometers to their park positions.

Click the **Centered** button for one set of galvanometers to bring the galvanometers to center.

Click the **Automatic** button to return the galvanometers to their park positions.

Click the **Custom** button to bring the galvanometers to a custom location. The custom position can be changed by dragging the position overlay in an image window, or by clicking the directional buttons on the Maintenance dialog. The amount moved by the directional buttons can be changed using the step size control to the left, which can be adjusted in discrete increments determined by the limitations of the electronics driving the galvanometers. To

reset the custom position to be the center of the field of view, press the **Reset to Center** button; note that this is not necessarily the same as centering the galvos if the current field of view doesn't share the same center (for example scanning an off centered ROI).

Manual Shutter Overrides

In this section, the user can close or open the hard and soft shutter controls associated with a specific light path. This can be useful during alignment and trouble-shooting. Additionally, this is the only way for the user to open a hard shutter while the laser power sliders are set to 0; this is useful when setting the bias adjustment on the Con-Optics controller for the Pockels cell.

The names and number of controls visible depend on system configuration and the shutters defined in the Prairie Configuration Utility.

Click the **Open** button to force the hard shutter to stay open (even if it would normally be closed).

Click the **Closed** button to force the hard shutter to stay closed (even if it would normally be open).

Click the **Automatic** button to return shutter control to the software.

When the user closes the Maintenance dialog, all shutter controls are returned to the **Automatic** state.

Show crosshair overlay at center

Enabling this option will display a crosshair on the Image window and hide all other overlays. The crosshair marks the center pixel of the image. This is useful for some alignment procedures.

When the user closes the Maintenance dialog, the crosshair overlay will disappear and the previous overlays will be displayed on the image.

Display Menu

New Image Window: Opens a new Image window. It functions the same as the **New** button in the [Image Windows](#) section of the Prairie View main control window.

Ghost Mode Options: Expands to reveal the **Ghost Mode** option. This option toggles Ghost mode on and off. In Ghost mode, all Prairie View windows become appear translucent; images displayed in Image windows do not become translucent. This can be useful in minimizing the noise from external light sources in a darkened room. The user can choose the opacity of the windows during Ghost mode by selecting a value from the **Opacity** submenu.

Interpolation Mode: Specifies a method to use to extrapolate/interpolate intensity values when stretching/shrinking the acquired image. The selection made here is also displayed in the information bar at the bottom of the Image window.

Z-Position Display: For systems configured with multiple Z-devices, this option allows the user to choose whether the Z position displayed at the bottom of the Prairie View main control window is the **Sum of all positions**, **All positions (comma delimited)**, or **Current device position**.

Scale Bar Options: Allows user to enable the **Show Scale Bar** option, to select the position of the scale bar in the Image window, and to choose the units of scale bar.

Frame Rate: Adds an overlay to the Image window to display the actual average frame rate that occurred during the most recent scan. This is often a more accurate representation of the frame rate than the value displayed at the bottom of the main window in Prairie View. However, this displayed value is only updated after a multi-frame scan has finished. This feature applies to Camera and SFC imaging.

Use Classic Pseudocolor: Allows the user to choose between two color mapping schemes for any channels displayed in Pseudocolor. The most noticeable difference between the two options is that Classic Pseudocolor displays its lowest values as black, which Visible Spectrum Pseudocoloring displays its lowest values as purple.

Electrophysiology Menu Overview

The Electrophysiology Menu contains a list of software modules that can be opened and run within Prairie View. Clicking an option will open the dialog of controls for that activity. Each activity is described in its own section of this manual.

- [**Voltage Output**](#) allows the user to define pulses, ramps, and other output waveforms for the analog output connections on the GPIO box
- [**Mark Points**](#) provides photo-stimulation at one or more individual points
- [**Voltage Recording**](#) provides recording of signals through the analog input connections on the GPIO box
- [**Seal Test**](#) allows the user to monitor and send signals to and from a patch amplifier
- [**Spiral Activation**](#) provides photo-stimulation of regions using a spiral over the area of interest
- [**Functional Mapping**](#) generates an overlay on an image to denote the magnitude of an electrical response to photo-stimulation

Photo-stimulation protocols can be carried out in a variety of ways with Prairie View software.

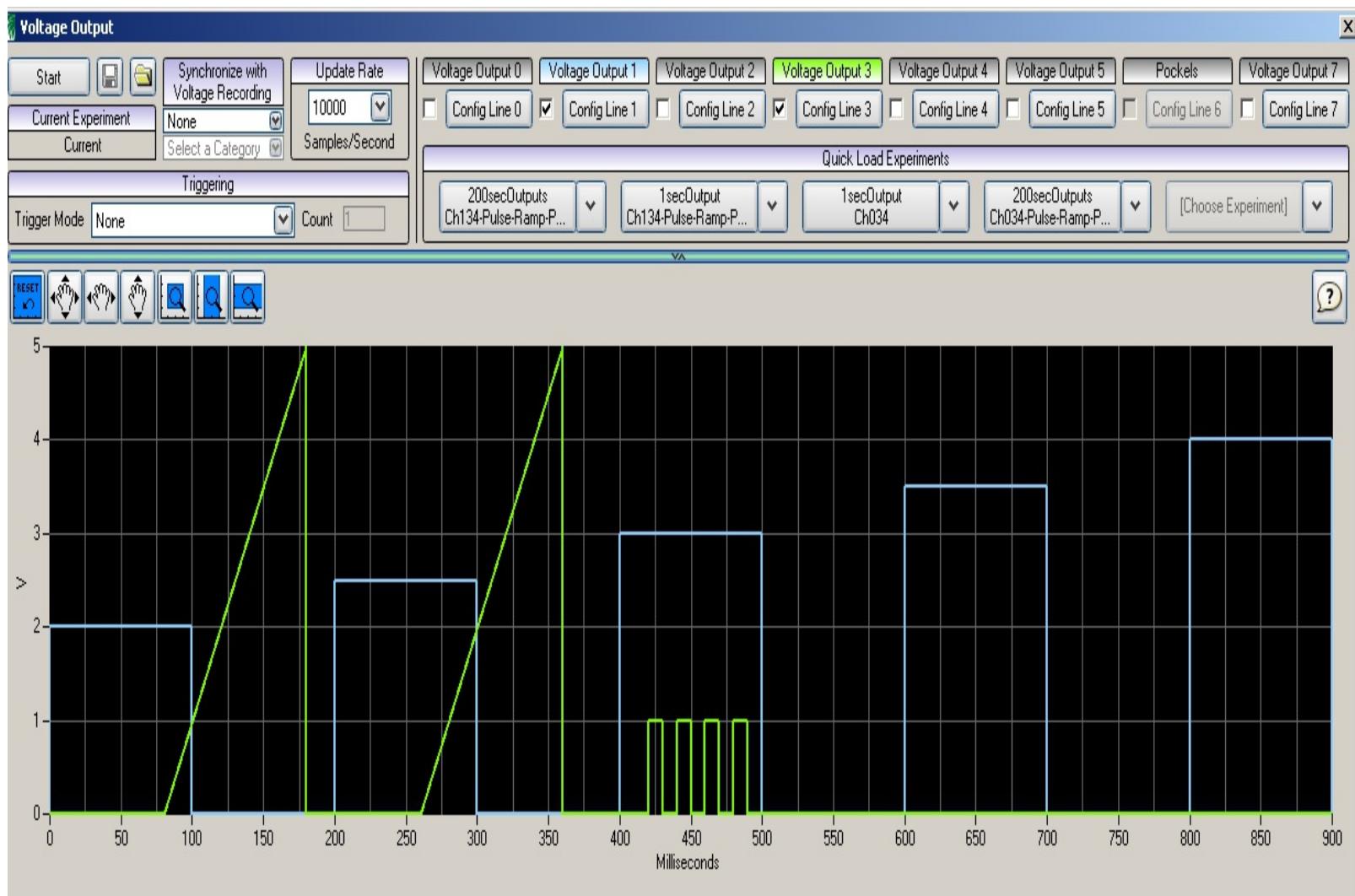
- [**Mark Points**](#) provides photo-stimulation at one or more individual points.
 - On systems with only one set of galvanometers (Ultima with one set, SGS, Arcturus), Mark Points can be performed with the imaging galvos between imaging sequences, in a sequential manner.
 - On systems with a second set of galvanometers (Ultima with two galvo sets, Sagittarius, SFC with Photoactivation/FRAP module), Mark Points can be performed with the second set of galvos during or between imaging sequences, in either a simultaneous or a sequential manner.
- [**Photoactivation Masks**](#) allow the user to define arbitrary regions to raster scan with one or more lasers using the imaging galvos. The galvos scan across the sample as if acquiring an image, but laser power is applied only as defined by the mask.
- [**Spiral Activation**](#) allows the user to define regions to be photo-stimulated using the uncaging galvanometers by driving the galvos in a spiral pattern. This type of activation cannot currently be embedded in a T-Series, but can be synchronized with Voltage Output and/or Voltage Recording.

Please refer to other sections of this manual for details about these software features.

Voltage Output

The Voltage Output feature allows the user to define waveforms to be generated on analog output BNC connections. These waveforms may be run independently of other tasks, or they can be synchronized with imaging, Voltage Recording, or photo-stimulation with Mark Points, Photoactivation masks, or Spiral Photoactivation.

To begin using Voltage Output, open the main interface window by selecting Voltage Output from the Applications menu.



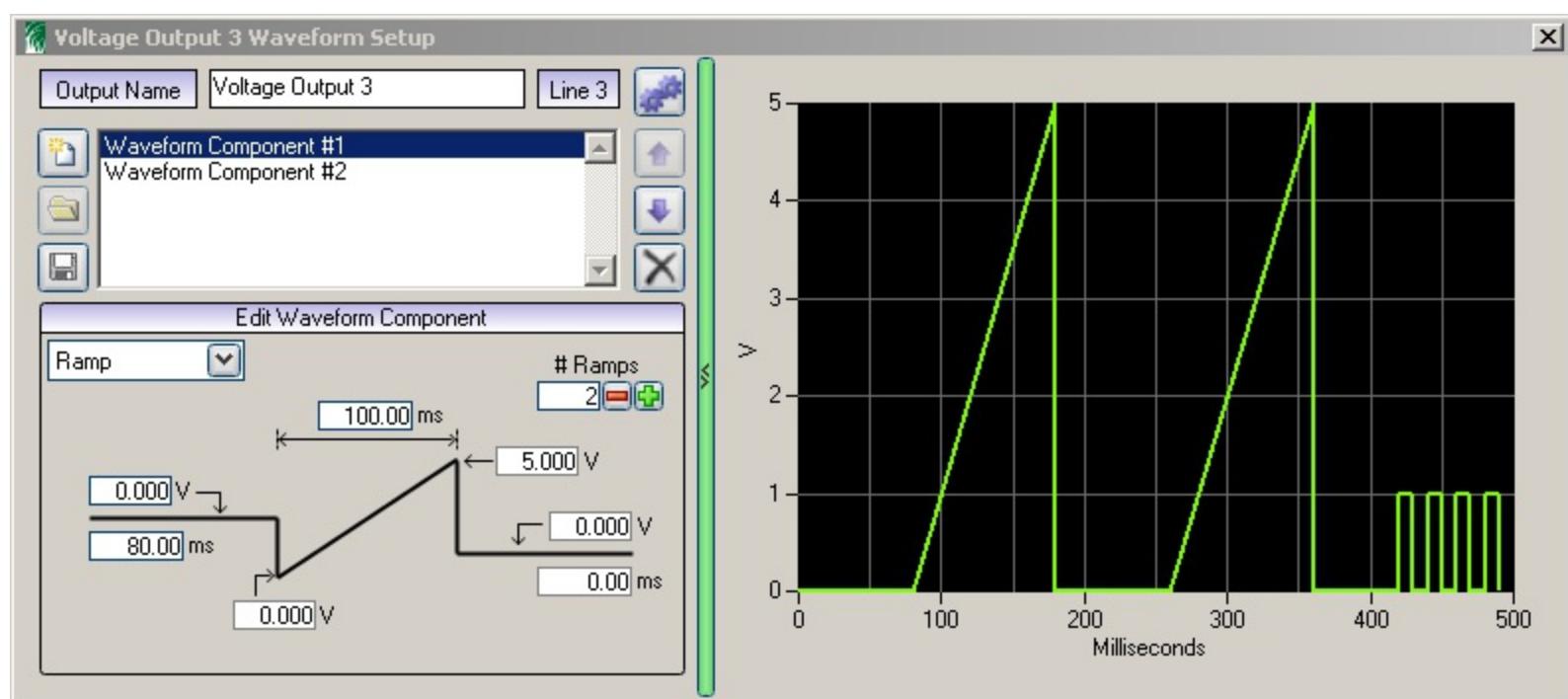
Across the top of the Voltage Output window is a set of checkboxes to enable/disable waveforms along with buttons to configure them. Some of the waveforms may be unavailable if their analog output lines are reserved for other purposes, such as galvanometer control or laser modulation.

At the bottom of the main interface is a graph that displays a summary of the current Voltage Output experiment. An experiment is a set of one or more waveform definitions that will be generated simultaneously with common triggering. The graph may be made visible or hidden by clicking on the green bar that separates the graph from the controls at the top of the

Voltage Output window. Buttons above the graph allow the user to zoom and pan in the horizontal, vertical, or both dimensions within the graph display.

Defining a Waveform

Every waveform has a **Config Line** button. The button text contains a reference to the analog output line on which that waveform will be produced. Clicking this button will open a new Waveform Setup window. This window is where the user defines the waveform.



A waveform is composed of one or more waveform components. Each waveform component has unique characteristics that are used to define the voltage level of the waveform at different time points in a structured manner. To add a waveform component to the waveform, click the **New Waveform Component** button on the left side of the Waveform Component list box.

The name of the waveform component can be changed by clicking the default name in the list box. Individual components can be saved with the button and loaded at a later time using the button. The order of individual waveform components in the list can be changed using the arrow buttons to the right of the list, and a component can be deleted using the button.

Types of Waveform Components

After the waveform component has been added to the list box, it is automatically selected.

Below the list box is the waveform component settings panel. There the user must choose the type of waveform component and set its properties. There are five waveform component types: Pulse Train, Patch Clamp, Ramp, Fixed Voltage, and Custom.

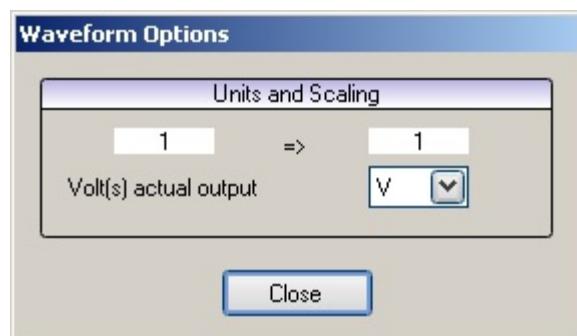
- Pulse Train - A single pulse or set of pulses which share similar characteristics. Every pulse within a pulse train has the same pulse width and inter-pulse delay. The initial delay occurs before the first repetition of a pulse train, while the time between the last pulse of one repetition and the first pulse of the next is defined by the repetition delay. Within one repetition, all pulses may have the same pulse potential or the potential may change (up or down) by a fixed voltage amount, referred to as the pulse potential delta. The initial potential and the potential between pulses can be set as well, and may be nonzero
- Patch Clamp - This is almost identical to the Pulse Train type except that the pulse potentials are defined relative to the initial rest potential rather than relative to zero. The definition also has an “amplitude multiplier” value that can be used to quickly scale the amplitude of the pulse up or down relative to the rest potential. If the rest potential is -1 Volt, the pulse potential is 1 Volt, and the amplitude multiplier is 1, the potential of each pulse is 0 Volts. If the amplitude multiplier is changed to 2, then the pulse potential is doubled but still relative to the rest potential. This would produce a pulse voltage of 1 Volt
- Ramp - This type is also similar to a pulse train, except that instead of producing pulses of a fixed potential it instead produces a continuous ramp of voltage between two values. The ramp does not have to start or stop at the same potential that it holds between ramps.
- Fixed Voltage - This simple component type allows you to define a period of time over which the output should be a constant (DC) voltage value. In the special case where this component is the final component in the waveform there is the additional option to define it to either maintain the set voltage output value indefinitely or transition to a different potential at the end of its duration.
- Custom - This is the most flexible component type since it allows the user to import a file containing any waveform definition. The file must be an ASCII text file with a file extension of .txt or .csv. Each line of the file defines one voltage preceded by one time duration (with units of milliseconds) to hold that voltage for. The two values should be separated by a comma. Below is a simple example:

100, 2.5
400, 0
2000, 5
1, 0

Importing a file with these contents would produce a waveform at 2.5 V for 100 ms, then change to 0 V for 400 ms, then 5 V for 2 seconds, and finally go back to 0 V for 1 ms. If this were the last component in the waveform, the output would remain at the last voltage defined (in this case, 0 V).

When a waveform is changed by adding, removing, or changing one of its components, the graph on the waveform setup window is updated accordingly. If the waveform is enabled on the main interface (its checkbox state is checked) its graph will also be displayed on the summary graph, overlaid with the other enabled waveforms. The user can change the plot color on the summary graph by hovering over the waveform name in the main interface and using the color wheel that pops up.

Units and Scaling of Waveforms



The units of the output can be specified by clicking the button in the Waveform Setup dialog. This will open the Waveform Options dialog where the user can choose the unit displayed and the value of that unit per volt of actual output from the Bruker electronics. This feature is useful whenever a scale factor is applied by external equipment, such as a patch amplifier converting the voltage control signal to a current applied to a cell, or an auditory stimulator converting the voltage control signal to decibels of sound produced.

Update Rate

The update rate for an experiment determines how often the samples for each waveform are generated. The lower the update rate, the faster an experiment will prepare and the smaller its memory footprint will be. However, a low update rate will also cause aliasing of higher frequency components of waveforms. If all of the waveform components in an experiment contain simple fixed voltage levels or pulses whose component time values are multiples of one millisecond, an update rate of 1000 samples per second is sufficient. However, when used with any waveform component whose time values are not whole milliseconds the output will be aliased and the signal may be too irregular for the intended application. In these cases, a larger update rate is needed.

Both the summary graph on the main interface and the waveform preview graphs in the waveform setup windows will show the theoretical plots as the waveforms are designed, and will not illustrate any aliasing problems that may result due to an update rate which is too low.

Note that both Mark Points experiments and Spiral Photoactivation experiments use a fixed update rate of 100 KHz. If a Voltage Output experiment is synchronized with either of these, the Voltage Output experiment will also run at 100 KHz, regardless of the update rate set in the Voltage Output window.

Saving and Loading an Experiment

The entire experiment definition, including all enabled waveforms and the defined update rate, can be saved by clicking the  button on the main Voltage Output window, next to the **Start** button. A saved experiment can be recalled using the  button.

Frequently-used experiments can be assigned to the Quick Load Experiments buttons.



To assign a saved experiment to a button, click the down arrow next to a Quick Load button and choose the desired experiment. The name of the chosen experiment will be displayed on the button, and the experiment can be loaded by clicking the button.

Note that triggering selections are not saved as part of an experiment, and must be defined each time an experiment is loaded.

Running an Experiment

The current Voltage Output experiment can be run by clicking the **Start** button on the Voltage Output main interface. Alternatively, the Voltage Output experiment can be embedded in a [T-Series](#), [Line Scan](#), [Point Scan](#), [Voltage Recording](#), [Spiral Activation](#), or [Mark Points](#) experiment and run from those interfaces.

The **Synchornize with Voltage Recording** selection allows the user to start a Voltage Recording experiment along with the Voltage Output experiment. This selection applies only to experiments started from the Voltage Output interface; experiments embedded in other interfaces (T-Series, Line Scan, etc.) will ignore this setting.

Starting with a Trigger

The **Trigger Mode** selection allows the user to start the Voltage Output experiment upon receipt of one of several available triggers.

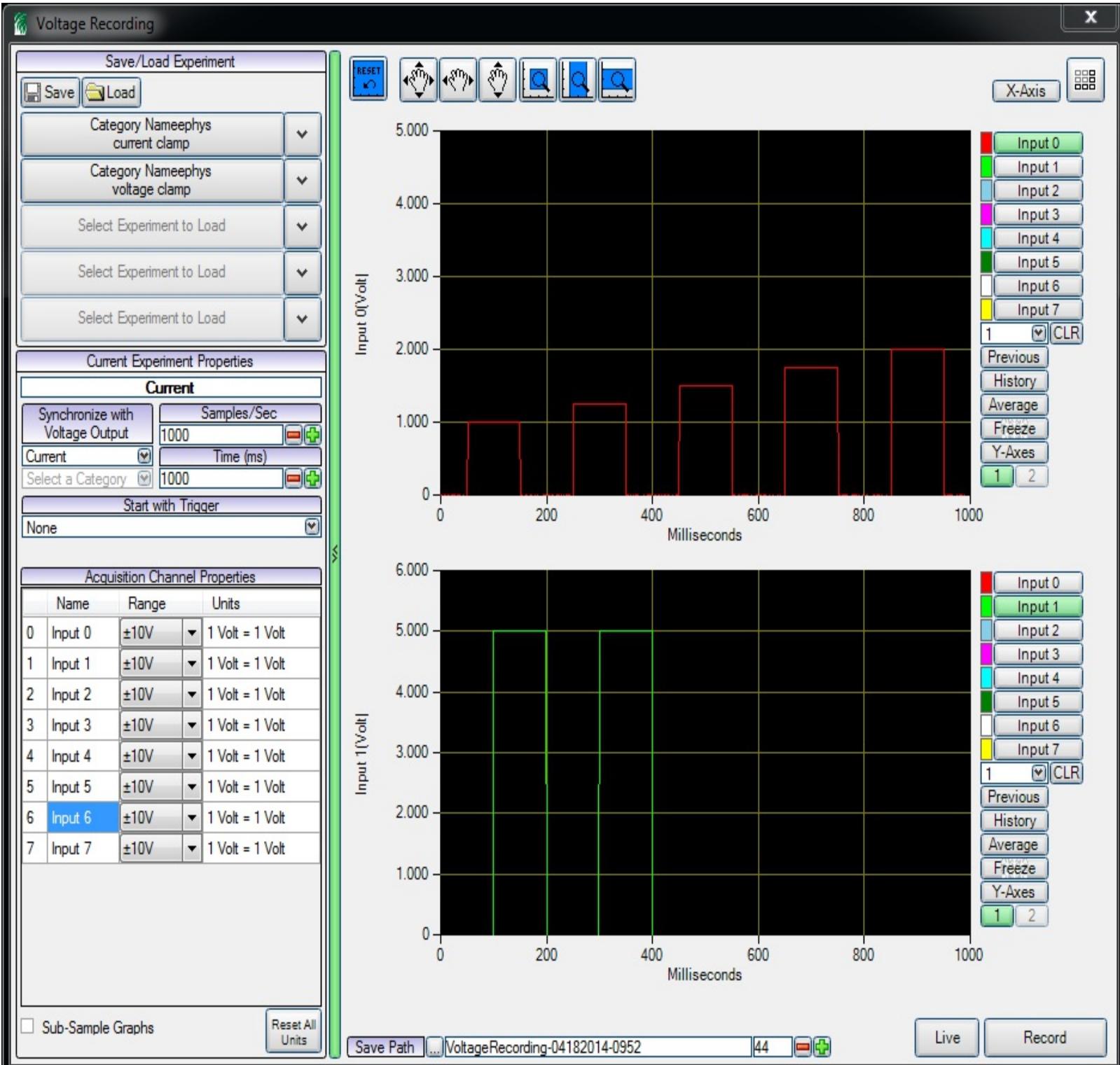
Trigger types include:

- **Start with next scan (PFI0)** - an internal signal generated at the start of an imaging event (Single Scan, T-Series, Z-Series, etc.)
- **Start with external trigger (PFI1)** - provide a 5V signal to the PFI1 connection on the front of the GPIO box
- **Start after N frame triggers (PFI8)** - define a number of frame triggers after which to start an experiment; frame triggers are generated internally and discussed in more detail [here](#)
- **Start with external trigger (Trig In)** - provide a 5V signal to any one of the Trig In connections on the front of the GPIO box

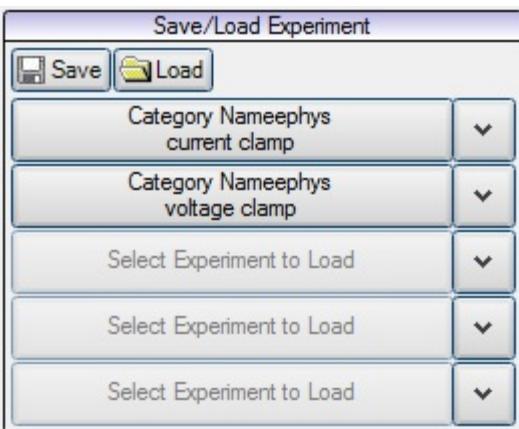
Select the desired trigger and then click the Voltage Output **Start** button. The experiment will prepare, but will not begin to send out waveforms until the selected trigger is received. This selection applies only to experiments started from the Voltage Output interface; experiments embedded in other interfaces (T-Series, Line Scan, etc.) will ignore this setting.

Voltage Recording

The Voltage Recording interface allows the user to record electrical signals over time. Recordings may be run independently of other tasks, or they can be synchronized with imaging, Voltage Output waveforms, or photo-stimulation with Mark Points, Photoactivation masks, or Spiral Photoactivation.



Save/Load Experiment

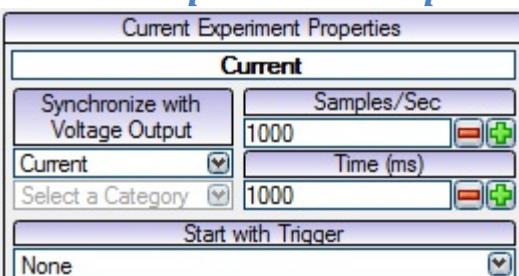


The currently defined experiment can be saved by clicking **Save**. A saved experiment can be recalled using the **Load** button.

Frequently-used experiments can be assigned to the Quick Load Experiments buttons. To assign a saved experiment to a button, click the down arrow next to a Quick Load button and choose the desired experiment. The name of the chosen experiment will be displayed on the button, and the experiment can be loaded by clicking the button.

Note that triggering selections are saved as part of an experiment, and will be updated when a saved experiment is loaded.

Current Experiment Properties



The name field at the top of this section displays the name of the currently defined experiment. This can be the name of a saved experiment, or "Current" if the current settings have not been saved.

Samples/Sec allows the user to define the number of samples to record per second. This is limited by the number of channels being recorded.

Time is the number of milliseconds to record.

Synchronize with Voltage Output allows the user to start a Voltage Outputs experiment at the same time as the Voltage Recording experiment. This selection applies only to experiments started from the Voltage Recording window start button; recordings embedded in other experiments (T-Series, Line Scan, etc.) will ignore this setting.

Start With Trigger allows the user to start the Voltage Recording with one of several available triggers. Select the desired trigger, and then click the **Record** or **Live** button. The Voltage Recording experiment will then prepare, but not begin recording until the selected trigger is

received. This selection applies only to experiments started from the Voltage Recording window start button; recordings embedded in other experiments (T-Series, Line Scan, etc.) will ignore this setting. Trigger types include:

- **Start with next scan (PFI0)** - an internal signal generated at the start of an imaging event (Single Scan, T-Series, Z-Series, etc.)
- **Start with external trigger (PFI1)** - provide a 5V signal to the PFI1 connection on the front of the GPIO box
- **Start after N frame triggers (PFI8)** - define a number of frame triggers after which to start an experiment; frame triggers are generated internally and discussed in more detail here
- **Start with external trigger (Trig In)** - provide a 5V signal to any one of the Trig In connections on the front of the GPIO box

Acquisition Channel Properties

Acquisition Channel Properties			
	Name	Range	Units
0	Input 0	±10V	1 Volt = 1 Volt
1	Input 1	±10V	1 Volt = 1 Volt
2	Input 2	±10V	1 Volt = 1 Volt
3	Input 3	±10V	1 Volt = 1 Volt
4	Input 4	±10V	1 Volt = 1 Volt
5	Input 5	±10V	1 Volt = 1 Volt
6	Input 6	±10V	1 Volt = 1 Volt
7	Input 7	±10V	1 Volt = 1 Volt

Name defines a custom name for an acquisition channel

Range defines an input sensitivity for an acquisition channel. Smaller ranges lead to better sensitivity and less noise but increase the risk of clipping.

Units define a scaling factor for data display. Clicking in the Units column will bring up a dialog to define a unit conversion for the given channel. This is for display purposes only and will not affect saved data. Information about the applied unit conversions is stored in the voltage recording XML file so it can be manually applied to the raw data by the user if necessary.

Reset All Units  button resets unit conversions for all channels to the default of no conversion.



The Units dialog allows the user to define a scale factor for the display of recorded voltages. The appearance of the dialog varies based on system configuration. Configuring a patch amplifier for use in Prairie View enables additional selections in the dialog.

When not reading units from a supported patch amplifier: Use the text boxes to define the number of custom units corresponding to a defined number of volts recorded by the acquisition card. For example, 1.0 Amp = 1 Volt signifies that for each volt recorded, the display will be scaled to show 0.1 Amp.

When an Axopatch 200B amplifier is configured: Check the **Read from** checkbox to obtain unit scaling from the telegraphing output on the Axopatch 200B. Select the analog input to which the telegraph signal is connected, and whether it signifies a scaling for current or potential.

When a Multiclamp 700B amplifier is configured: Check the **Read from** checkbox to obtain unit name and scaling from the Multiclamp Commander Software. Select whether scaling should be read from Multiclamp Ch1 or Ch2, corresponding to the two amplifiers contained in the Multiclamp device. Then select the Channel corresponding to the primary or secondary output for that amplifier.

The **Reset All Units** button at the bottom of the window will reset all channels to display 1 volt of signal per 1 volt recorded by the acquisition card.

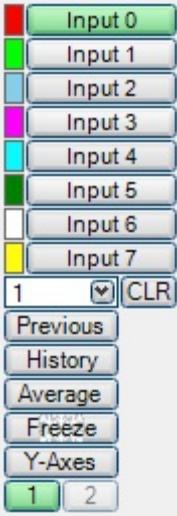
Plots

Buttons above the plots allow the user to zoom and pan within the displayed data in the horizontal, vertical, or both dimensions.



The **Plot Number Button** in the upper right corner of the window allows the user to select the number and layout of plots displayed in the window.

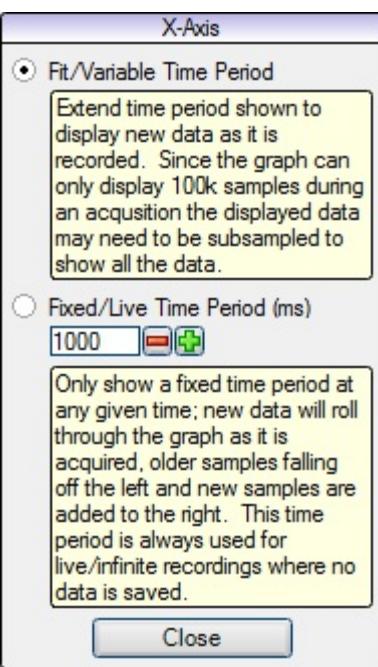
Buttons alongside each individual plot allow the user to adjust the data displayed in that plot.



- **Channel** buttons enable/disable channels to be recorded and displayed in this plot; only those channels enabled in one or more plots will be recorded during an experiment
- Colored rectangles next to the channel buttons allow the user to change the display color of the trace recorded from that channel
- **Number combo box (n)** allows selection of the number of previous recordings used for the Previous, History, and Average features. This selection applies only to recordings with identical settings taken in sequence during the current session.
- Clicking **CLR** (clear) will cause the software to “forget” and stop displaying previous recordings
- **Previous** displays the data recorded n recordings prior to the most recent recording (one trace displayed)
- **History** displays the last n previous recordings (n traces displayed)
- **Average** displays an average of the last n previous recordings (one trace displayed)
- **Y-Axes** opens the Y-Axes properties dialog, described below
- **1** and **2** buttons allow the user to choose which Y axis is displayed, if more than one Y axis is available

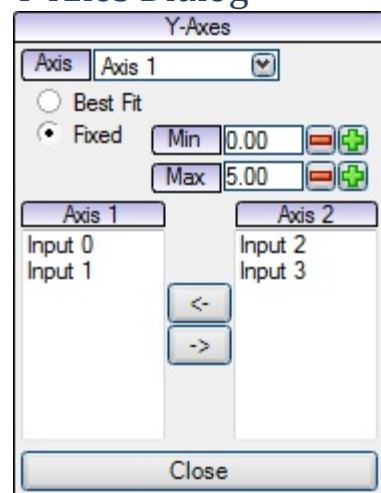
X-Axis Dialog

This dialog is displayed when pressing the **X-Axis** button in the upper right-hand corner.



The X-Axis can either display all data acquired as it is recorded, and the X scale will constantly change as new data is appended and existing data is compressed to make room, or leave the X scale constant and just display the most recent data recorded. To improve performance when showing all data during a recording the data may be subsampled so that no more than 100,000 points will be shown for any channel; opening the same dataset in playback mode after it is recorded will show all the points without subsampling.

Y-Axes Dialog



The Axes dialog allows the user to define two different Y axes for the plot display.

Choose an **Axis** for which to define the display properties.

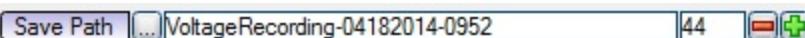
Define a range for the axis using the radio buttons:

- **Best Fit** will auto-scale the range such that all values in the recorded traces are visible in the plot
- **Fixed** allows the user to define a fixed minimum and maximum value for the selected axis

Assign the active input channels to one or the other Y axis using the arrow buttons to move selected channels in the Axis 1 and Axis 2 lists.

Click the **Close** button to return to the main Voltage Recording dialog.

Save Path

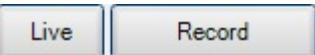


Each Voltage Recording is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the , followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition, Voltage Recording, and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, etc.) can cause data to be overwritten if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each recording. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data.

Start Buttons



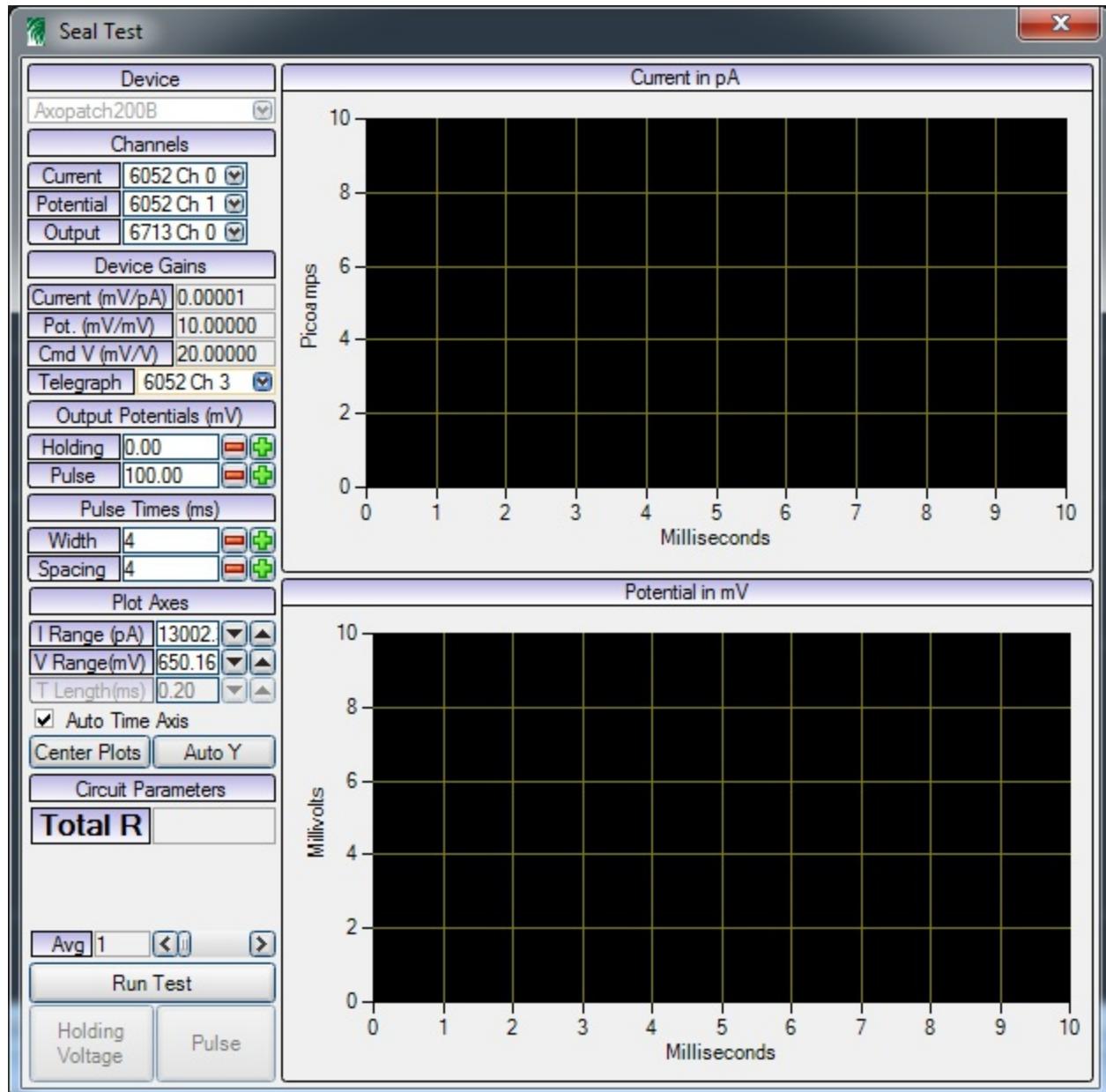
Live starts a live voltage recording of indeterminate length, similar to the traces displayed by an oscilloscope. The plots will display a segment of data of the duration defined in the **Time** field of the Current Experiment Properties. The display will continuously update until the user clicks **Stop**. No data file is saved from this recording.

Record begins a single, finite length recording of the length specified in the **Time** field of the Current Experiment Properties. This type of recording generates a data file that is saved in the directory specified by the user.

Alternatively, the Voltage Recording experiment can be embedded in a [T-Series](#), [Line Scan](#), [Point Scan](#), [Voltage Output](#), [Spiral Activation](#), or [Mark Points](#) experiment and run from those interfaces.

Seal Test

The Seal Test module enables the user to perform a seal test using a patch amplifier, to determine whether electrical contact has been established with a cell by applying a pulsed potential and measuring the response.



The current response is shown in the top plot, while the applied potential is shown in the bottom plot. Controls on the left side of the plots allow the user to define the inputs and outputs to be used, and circuit parameters are calculated and displayed in the lower left portion of the window.

Device Properties

Prairie View can be used to send and receive signals from a variety of patch amplifier devices. Integrated support is provided for reading the scale factors of the MultiClamp 700B and the Axopatch 200B; other amplifiers can be used by manually entering the scale factors. Other

sections of this manual provide information on configuring a MultiClamp 700B or Axopatch 200B amplifier for use with Prairie View software.

MultiClamp 700B

Device	
Multiclamp 700B Ch1	
Channels	
Current	6052 Ch 0
Potential	6052 Ch 1
Output	6713 Ch 0
Device Gains	
Current (mV/pA)	0.00001
Pot. (mV/mV)	10.00000
Cmd V (mV/V)	20.00000

MultiClamp Commander software must be running before Prairie View can connect to the amplifier.

In the drop-down menu under Device, select the desired channel of the MultiClamp 700B amplifier.

Define the **Channels** to correspond to connections between the amplifier and the GPIO box.

- **Current** is the analog input channel connected to the current output of the amplifier.
- **Potential** is the analog input channel connected to the potential output of the amplifier.
- **Output** is the analog output channel connected to the commanding potential input of the amplifier.
 - Commanding potential input must be set to 20mV/V in the MultiClamp Commander software.

Device Gains are automatically obtained from the MultiClamp Commander software.

Axopatch 200B

Device	
Axopatch200B	
Channels	
Current	6052 Ch 0
Potential	6052 Ch 1
Output	6713 Ch 0
Device Gains	
Current (mV/pA)	0.00001
Pot. (mV/mV)	10.00000
Cmd V (mV/V)	20.00000
Telegraph	6052 Ch 3

Define the **Channels** to correspond to connections between the amplifier and the GPIO box.

- **Current** is the analog input channel connected to the current output of the amplifier.

- **Potential** is the analog input channel connected to the potential output of the amplifier.
- **Output** is the analog output channel connected to the 20mV/V commanding potential input of the amplifier.
- **Telegraph** is the analog input channel connected to the telegraphing output of the amplifier.

Device Gains must be read from the amplifier or entered by the user.

- **Current (mV/pA)** is automatically determined if a telegraph input was defined. If no telegraph input was defined, then the user must enter the scaling. To find the scaling manually, divide the Potential reading of the current channel (in mV) by the actual current (in pA).
- **Pot. (mV/mV)** is fixed at 10mV recorded voltage per 1mV displayed and actually applied.
- **Cmd V (mv/V)** is fixed at 20mV/V, corresponding to the 20mV/V command input on the amplifier; this is the only command input supported. For every 1V applied to the command input, the amplifier will apply 20mV to the cell. The Cmd scaling factor allows Prairie View software to calculate the command signal that must be generated to direct the amplifier to apply the correct voltage to the cell.

Generic Patch Clamp Device

Device	
Patch Clamp	
Channels	
Current	6052 Ch 0
Potential	6052 Ch 1
Output	6713 Ch 0
Device Gains	
Current (mV/pA)	0.00001
Pot. (mV/mV)	10.00000
Cmd V (mV/V)	20.00000

Define the **Channels** to correspond to connections between the amplifier and the GPIO box.

- **Current** is the analog input channel connected to the current output of the amplifier.
- **Potential** is the analog input channel connected to the potential output of the amplifier.
- **Output** is the analog output channel connected to the 20mV/V commanding potential input of the amplifier.

Device Gains must be entered by the user.

- **Current (mV/pA)** is determined by dividing the Potential reading of the Current channel (in mV) by the actual current (in pA).
- **Pot. (mV/mV)** is determined by dividing the Potential reading of the Potential channel (in

mV) by the actual potential applied to the cell (in mV).

- **Cmd V (mV/V)** is the scale factor between the signal sent from the GPIO box to the amplifier and the signal sent from the amplifier to the cell. For every 1V applied to the command input, the amplifier must apply the appropriate number of mV to the cell. The Cmd scaling factor allows Prairie View software to calculate the command signal that must be generated to direct the amplifier to apply the correct voltage to the cell.

Stimulus Properties

Output Potentials (mV)	
Holding	0.00
Pulse	100.00
Pulse Times (ms)	
Width	4
Spacing	4

These controls enable the user to define the stimuli applied to the cell when the Seal Test is running.

- **Holding** is the average potential (in mV) seen by the cell over a long period of time while holding or pulsing.
 - In Holding Voltage mode, this is the number of mV applied to the cell.
 - In Pulse mode, this can be seen as the DC component of the pulse signal and corresponds to the average of the top and bottom voltages weighted by their respective durations.
- **Pulse** is the pulse height (in mV) applied in Pulse mode.
- **Width** is the length of each pulse (in milliseconds).
- **Spacing** is the time between pulses (in milliseconds).

To obtain a 50% duty cycle pulse, the Spacing and Width values should be equal. A Holding potential value of 0mV and Pulse height of 100mV would result in a pulse jumping between -50mV and +50mV.

Plot Axes Properties

Plot Axes	
I Range (pA)	13002
V Range(mV)	650.16
T Length(ms)	0.20
<input checked="" type="checkbox"/> Auto Time Axis	
Center Plots	Auto Y

These controls enable the user to adjust the display of the recorded data.

- **I Range (pA)** is the number of pA to display on the Y axis in the Current plot. Arrows allow for quick zooming of the display.
- **V Range (mV)** is the number of mV to display on the Y axis in the Potential plot. Arrows allow for quick zooming of the display.
- **T Length (ms)** is the number of ms to display on the X axis in the two plots. Arrow allow for quick zooming of the display.
- The **Auto Time Axis** check box adjusts the X axis to display one pulse.
- The Y axes can be adjusted based on signal level plotted at that time
 - **Center Plots** adjusts the Y axis of each plot so that the average signal value is in the center of the displayed range.
 - **Auto Y** adjusts the range of the Y axes to show the full range of signals plotted at that time.

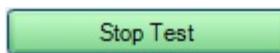
Circuit Parameters

Circuit Parameters	
Total R	50MΩ
Access R	0fΩ
Membrane R	0fΩ
Membrane C	0fF
Avg	1   

Several parameters of the circuit are calculated during a seal test. These parameters are estimated by fitting the transient decay superimposed on the pulse response of the circuit. When not pulsing the amplifier, only Total R is calculated.

- **Total R** is the average total resistance.
- **Access R** is the access resistance.
- **Membrane R** is the membrane resistance.
- **Membrane C** is the membrane capacitance.
- The **Avg** slider enables the user to average multiple measurements of the circuit parameters.

Running a Seal Test

	
Holding Voltage	Pulse
Holding Voltage	Pulse

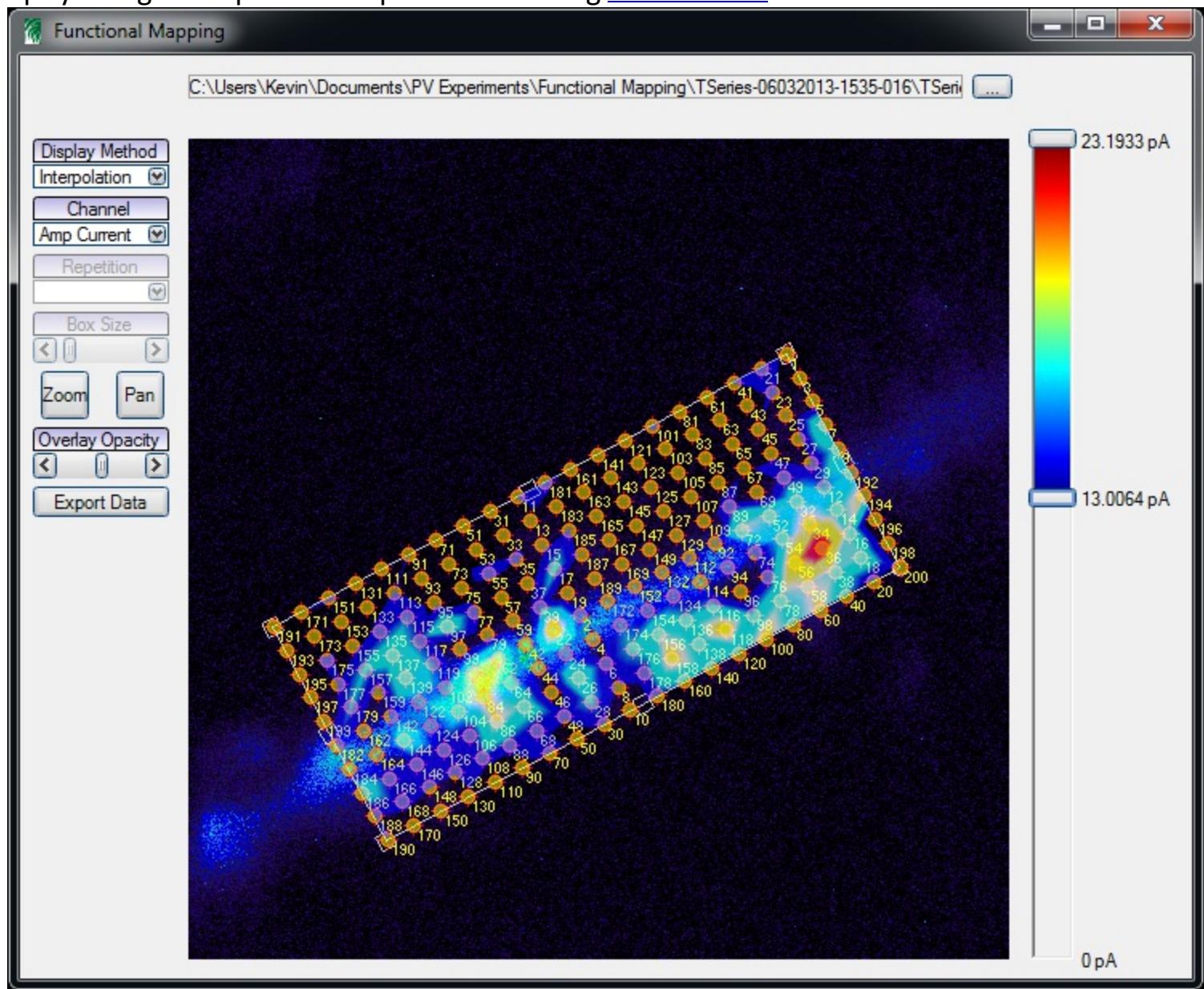
The **Run Test** button initiates recording of the signals from the patch amplifier. While a seal test is running, the button text will change to **Stop Test** and clicking it again will stop the recording; no data is saved.

Holding Voltage initiates application of the defined Holding signal to the amplifier.

Pulse initiates repeated application of the defined Pulse signal to the amplifier.

Functional Mapping

The Functional Mapping application is helpful for analyzing the data acquired from common ephysiological experiments performed using [Mark Points](#).



To generate a dataset that can be used in Functional Mapping, an acquisition must be run from Mark Points that includes activation at multiple points, with at least one of those points synchronized with a voltage recording. In this case, Functional Mapping will draw an overlay over the original reference image, with the overlay being pseudocolored to represent the strength of the reaction at that point.

Functional Mapping is launched from the Applications menu on the Main Form. When the application is first launched, a window like the one above will be opened, but without any image data. To open a dataset, click the button next to the filepath at the top of the window and navigate to the top level *.xml file that corresponds to an experiment that satisfies the Functional Mapping criteria.

At this point, Functional Mapping will load in the data from the chosen experiment, which will then cause the reference image to be shown and the controls on either side of the window to become enabled. Additionally, an overlay will be placed on the reference image representing the change in the synchronized voltage recording immediately following the laser pulse at that point. To generate the most appealing and informative image, the controls on the left side may need to be changed.

The **Display Method** drop down allows for a choice between four types of representations of the overlay. The default method, "Boxes", simply places a square over each point that was synchronized with a voltage recording. If this method is chosen, the **Box Size** slider becomes enabled, which allows changes to the size of the square placed over each point. The "Grid" method creates an overlay in which every pixel throughout the image is pseudocolored to represent the response of the nearest activation point. The "Interpolation" method (shown above) creates a smooth overlay where every pixel within the bounds of a group of points is calculated based on its neighboring points. Finally, the "IMD" method is similar to interpolation, but merges the reference image and overlay into one image. In the resultant image, the intensity is taken from the reference image, whereas the hue of each pixel represents the response.

The **Channel** drop down automatically populates with the names of each active voltage recording channel from the experiment. This control can then be used to change the overlay to represent changes in the recording from the indicated channel.

If the Mark Points experiment has multiple repetitions, the **Repetition** control will automatically populate and become active. At this point, the drop down menu can be used to cycle between different repetitions and see the overlay of responses at each repetition.

The **Zoom** and **Pan** controls allow for magnifying and moving the image shown in the window. When one of these buttons is clicked, the application enters the appropriate mode. In zoom mode, any point that is clicked in the image window will be magnified. Additionally, a box can be drawn on the image window, and the resulting area will be magnified to fit the window. Double-clicking or right-clicking will revert the zoom to its original value. When zoomed in, it may be desirable to shift the image around to see neighboring areas. In this case, the **Pan** button can be pressed to allow clicking and dragging of the image to see the rest of the image.

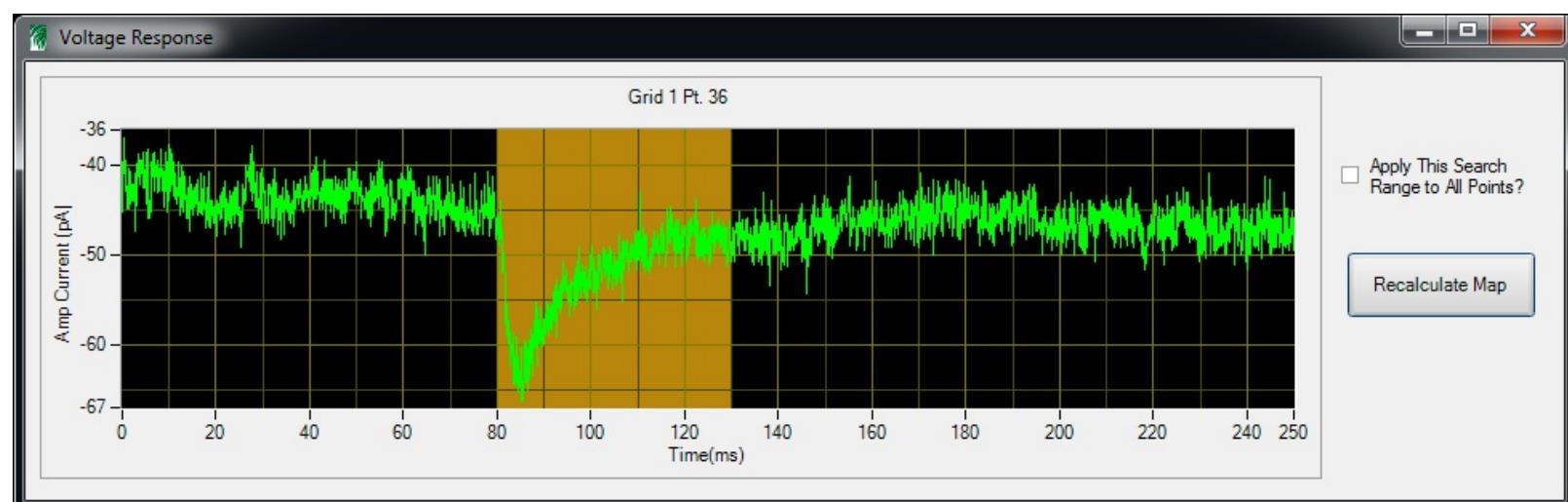
The **Overlay Opacity** slider allows for changing of the transparency of the overlay. If the slider is dragged all the way up (to the right), the underlying image will no longer be visible. On the other hand, if the slider is dragged all the way down (to the left), the overlay is no longer visible.

The **Export Data** button opens a save dialog that will save much of the calculated data. When a valid file name is entered into this window, three files are created. The first one is a copy of the original reference image. The second file is an image that contains the current overlay at its

current transparency. Finally, a comma separated value file is created that contains useful information about the reaction at each point. This file contains, for each point, a name, index, repetition number, x coordinate in pixels, y coordinate in pixels, and scaled response in each channel.

On the right side of the image is a colorbar that can be used to scale the lookup table used to color the overlay. By dragging the bottom limit upward, the lookup table is compressed upward, and vice versa. Additionally, any response that is below the lower limit of the colorbar will be set to completely transparent, and any response that is above the upper limit of the colorbar will be saturated at a deep red color. The limit controls of the colorbar also display a textbox that maps the indicated color to the scaled measurement made in that channel.

If any point in the image window is clicked while not in zoom or pan mode, a new window will be opened that shows the voltage recording that was synchronized with the nearest point to the clicked location.



In this window, the entire voltage recording can be seen, along with a title indicating which point was selected. Furthermore, an orange box indicates the window used to calculate the response (by default this window starts with the laser pulse and lasts for 50 ms). This box can be shifted left or right, or resized by clicking and dragging either endpoint. After resizing the window, the overlay can be recalculated by clicking the **Recalculate Map** button on the right side. Prior to recalculating the map, the checkbox above the button, **Apply This Search Range to All Points?**, can be checked to use the new window for every point in the overlay. If left unchecked, the other points will continue to use their previous windows. This process can be repeated as many times as necessary until the desired results are obtained.

Applications Menu Overview

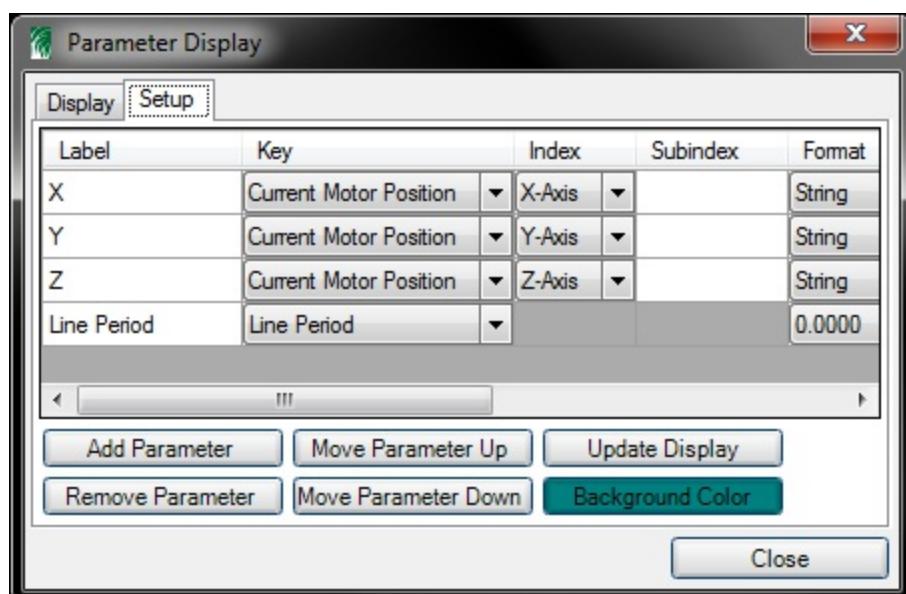
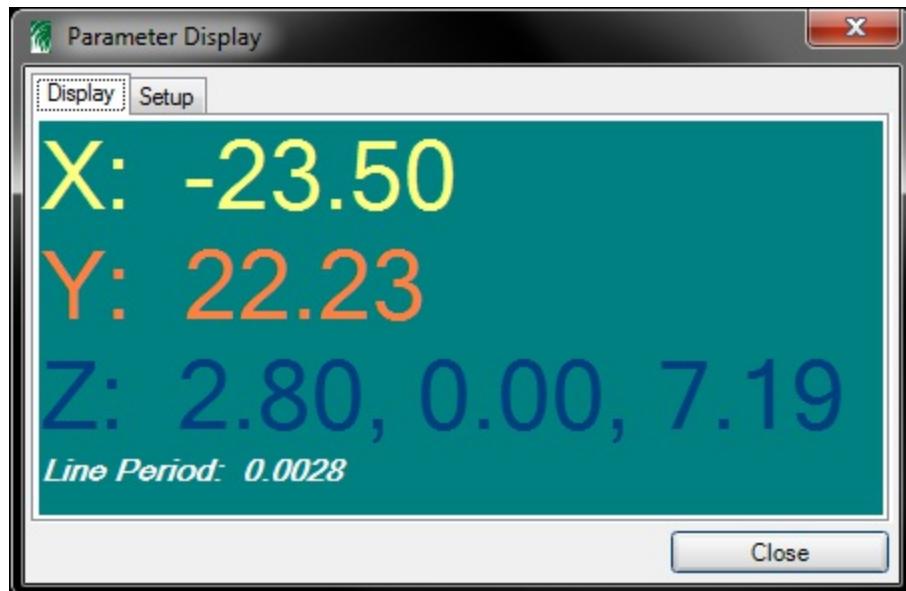
The Applications Menu contains a list of software modules that can be opened and run within Prairie View. Clicking an option will open the dialog of controls for that activity. Some activities are described in more details in their own sections of this manual.

- **Notes** opens an empty window for users to enter notes; these notes will be saved with each subsequent saved image, and will be displayed in the Metadata window during Playback
- **[Parameter Display](#)** opens a dialog which the user can configure to display any number of system parameters in different orders, fonts, colors and sizes
- **[Atlas Imaging](#)** allows for easy visualization of large sections of tissue in an automated process
- **[Image Block Ripping Utility](#)** converts raw data files into usable formats (TIFF for image data, CSV for Voltage Recording data, etc.) if the raw files were not converted automatically after acquisition

Additional programs can be added to the Applications menu by defining an Action to call that program's executable file. This can be a convenient way to launch other programs such as the Windows Calculator, ImageJ, etc., from within Prairie View. Additional information about [Actions](#) can be found elsewhere in this manual.

Parameter Display

The parameter display dialog can display any number of system parameters, in any order, using a custom font, color and size. The Display tab shows the current values of the selected parameters. The Setup tab consists mainly of a grid which the user can use to edit the list of parameters which buttons underneath can be used to determine the order and background color of the Display tab. For each parameter, the user can set up the **Label** (name for the parameter to display), **Key** (which parameter to display), **Index** and **Subindex** (used to narrow down a parameter with multiple values), **Format**, **Font**, and **Color** for each line of the Display tab. All parameter and color selections are automatically saved/recalled when Prairie View is exited/started.

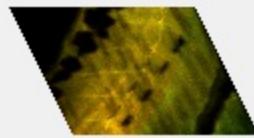


Atlas Imaging is a module that allows for easy visualization of large sections of tissue in an automated process. Furthermore, Atlas has tools that allow users to quickly and easily set up a 2D or 3D volume acquisition.

Atlas Imaging allows the user to see a thumbnail preview of their current location placed into a map representing the surrounding tissue. As the stage location is changed, the map is updated in real time to show the relative position of the current field of view. Additionally, any stage locations at which a future acquisition should take place can be saved and viewed using Atlas Imaging.

Open Atlas Imaging by selecting it from the Applications menu on the main form. At this time, Atlas Imaging will open two windows, the Atlas Image window and the Atlas Overview window.

Atlas Image Window



4053.33 um

Set Z Top

Set Z Bottom

Z Step Size (um):

The Atlas Image window functions as a normal Image window, showing the current field of view at the resolution selected on the Main Control window. In addition, to the right of the image is a representation of the tissue as a slice of a z stack. In this window, the stage position can be changed by clicking and dragging on the image. With a properly calibrated objective and stage, any point clicked on in the Atlas Image window will remain under the mouse cursor as it is dragged around. Additionally, the focal plane can be changed by clicking and dragging on the Z slice to the right of the image window. In fact, any change to the current Z position, including those from hardware, the buttons on the main form, and the mouse scroll wheel, will be reflected by a change of the position of the z slice in this window. Furthermore, the **Set Z Top**, **Set Z Bottom**, and **Z Step Size** controls can be used to define the range for a Z-Series (at one or multiple XY locations). Use the **Set Z Top** button to set the start of a Z-Series, the **Set Z Bottom** button to set the stop of the Z-Series, and the **Set Z Step Size** to define the distance between consecutive slices. When using Atlas Imaging, the Z series definition is stored within each location, so it is possible to acquire irregular volumes using this module.

Atlas Overview Window

Atlas Overview



Overview Size

2 Wide x 2 High

15 % Overlap

Set as Center

Add Location / Update Z

Generate Montage

Interpolate Z Ranges

Remove Locations

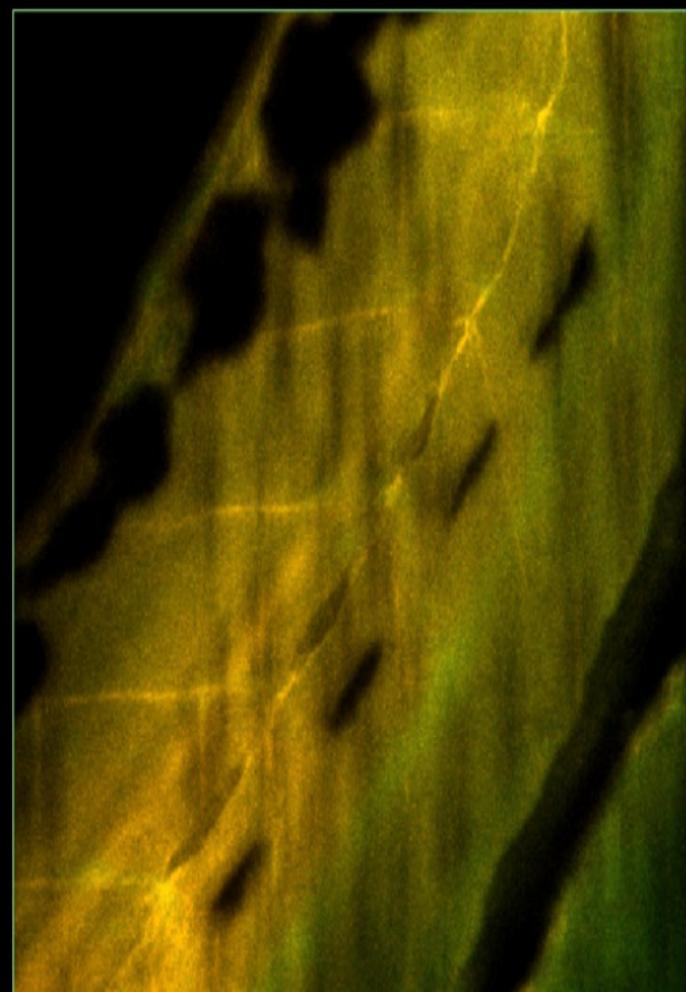
Scan Overview

Preview Locations

Take Snapshot

Clear All

Acquire Volume



Save Path



AtlasVolume-01142016-1

66

The Atlas Overview window contains a map of the current position in relation to previous positions. In addition, it shows the location of previously saved positions to reference the relative placement of the current field of view. Clicking anywhere in the overview image will move the stage such that the current field of view lies underneath the mouse cursor, which can then be dragged around to view the surrounding sample in real time. If the size of the overview is not large enough, simply move the current field of view out of the range of the current overview window, and it will automatically rescale to include the new position. If desirable, the size of the overview window can also be set using the text boxes in the upper left corner of the window, which will set its size to fit the corresponding number of fields of view (shown as 2x2). In addition, the overview window has several useful features that are run using the buttons on the left side of the window.

Set as Center will take the current stage position and place it in the center of the Atlas Overview window. This button will preserve any previous information in the overview window by shifting it appropriately along with the new center. This feature should be used when a location other than that at the time Atlas was opened is determined to be the center of interest for upcoming acquisitions.

Add Location/Update Z can be used to save the current location as an area at which future acquisition should take place. When this button is pressed, the current image will be permanently placed in the overview window and outlined with a blue rectangle, indicating that this position will be used for upcoming acquisitions. If this button is pressed when the current field is on top of an already existing field, the Z series associated with the existing location will be updated to the current Z series. Use this to set up an acquisition at any number of sparse locations, or to define the boundaries of a continuous volume.

Generate Montage will calculate and show the locations needed to continuously cover the area defined by the locations furthest away from the current center. Prior to pressing this button, the **% Overlap** should be entered in the text box in the upper left corner of the overview window. This overlap will then be used to lay out a grid that completely covers any previously defined locations. The grid will then be shown on the overview window for visualization. As part of the calculation of each stage location, linear interpolation of the known Z series is also performed. Therefore, irregularly shaped 3D volumes can be quickly and easily set up.

Interpolate Z Ranges is used to recalculate the Z series at any stage location outlined with a red square. For each of these locations, the Z series is interpolated from the user-defined Z series added/updated at all other stage locations (denoted by a blue square outlining the location). This feature is typically used after a montage is generated and then the Z series at one or more of the calculated locations is updated by the user. The interpolation will only take place if there are at least two locations with user-defined Z series.

Remove Locations provides an interactive way to remove a previously defined field from upcoming acquisitions. When this button is pressed, it will highlight green indicating that Atlas is now in removal mode. At this point, any field that is clicked in the overview window will be removed from the list of locations to be used for upcoming acquisitions. This change will be

represented by erasing the red rectangle around the previous location. This feature is particularly useful when a square grid was defined to cover an irregular shape. After the montage is generated, this feature can be used to remove fields that don't contain useful information, thus increasing the speed of acquisition. Click the button again to exit removal mode.

Scan Overview will fill the entire overview window with image data. When this button is pressed, the stage will drive to a location corresponding to a corner of the overview window and take an image. It will then move to a neighboring location and take another image. This process is repeated until the stage reaches the opposite corner of the overview window (using a "snake" pattern). This allows for a quick preview of the area of tissue surrounding the centered field of view. Next to the button is a small drop down menu that allows a choice of whether or not to save the image data. If the option to save is checked, a new folder will be created with the text "Overview" in the name where the images will be saved.

Preview Locations will become enabled when at least one stage location is defined. After that point, clicking this button will cause the X and Y motors to drive to all defined stage locations, without changing the Z position, and take an image. That image will be permanently added to the overview window. This method is intended for getting a "single slice" of a defined 3D montage and can be used to ensure that all fields are as desired prior to starting the whole volume acquisition. Once again, there is a smaller button next to this which contains a drop-down menu to choose whether or not to save the data acquired. If the option to save is checked, a new folder will be created with the text "Preview" in the name where the images will be saved.

Take Snapshot will capture an image of the current data in the overview window and open a dialog to save it in tiff format. This feature is useful to have a reference of what the surrounding area of the sample looks like before acquisition.

Clear All will remove all currently saved stage locations, any image data in the overview window, and the currently defined Z series. This button should only be used to "start over" when using Atlas Imaging, as everything related to Atlas up to that point will be lost.

Acquire Volume is used to perform an acquisition of the defined 3D volume. When this button is pressed, the stage will drive to the first location and perform the Z series associated with that location, then to the second location and perform its Z series, and so on and so forth. If none of the locations were stored with a Z series (or if the Z series top and bottom were set to be equal prior to adding/updating each location), a single image will be taken at every stage location, resulting in a 2D surface. The resulting data will be saved in the current save path under the Atlas Volume directory.

Each Atlas acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the ... button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the ... button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the Preserve User-Modified File Names option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, Misc, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and – buttons. Note that decreasing the counter value can result in overwriting data.

Atlas Imaging and Playback Mode

When Atlas Imaging is opened in conjunction with Playback mode, the Atlas Overview is filled with all image data taken from the same Z position as the currently selected frame.

Additionally, the Atlas Image window is updated with the image data from the currently selected frame. Changing the frame of the Playback dataset will cause the Atlas Overview to search through the dataset, find all images taken at the same Z position, and fill their image data into the Overview window. If the Sequence of the Playback dataset is changed, the newly selected image's Z position will be compared to the previous image's Z position. If these positions are the same, the data in the Overview window will remain as is, and only the green square indicating the X-Y position of the currently selected image is updated. If the Z positions are not (approximately) the same, the dataset will be searched for all images with the same Z position as the currently selected image, and any images which have the same position will be filled in in the Overview.

When Playback is exited, the Atlas Overview will be returned to the exact state it was in prior to entering Playback mode.

Procedures

Below is a typical workflow for using Atlas Imaging to acquire a 3D volume:

1. Obtain an image in Prairie View's normal image window roughly near the center of where you want to acquire
2. Launch Atlas Imaging from the Applications menu in the Main Control window
3. Estimate how large of an area (in number of fields) will be needed to cover the desired acquisition and enter this number into one of the text boxes in the upper left corner of the overview window
4. Acquire a preview of the current overview using the **Scan Overview** button on the overview window
5. Navigate to one corner of the grid of fields that will be acquired
6. Set the top, bottom, and step size of the Z-Series to be associated with this location
7. Click **Add/Update Location** to store this as one of the locations to be acquired
8. Navigate to the opposite corner of the grid to be acquired and set the top, bottom, and step size of the Z series to be associated with this location
9. Click **Add/Update Location** to store this as another location that needs to be covered
10. (Optional) Navigate to any other locations for which the associated Z series will not be linearly interpolated from the corner points. At these locations, define the top, bottom, and step size of the Z series to be associated with this location and click **Add/Update Location**
11. Enter the desired **% Overlap** into the text box in the upper left corner (common values are around 15%)
12. Click on the **Generate Montage** button to calculate and show the grid of locations that will be used. Additionally, the Z series for each location will be interpolated from all previously defined locations
13. (Optional) Drive the Z motor to a position in the middle of your defined Z range. Click the **Preview Locations** button to acquire a single image at every X-Y location and ensure that all images are as desired. If necessary, change imaging parameters and repeat this step until all images are as desired.
14. (Optional) Navigate to an existing location and change the top, bottom, and step size of the current Z series. Click the **Add/Update Location** button to store the modified values and the **Interpolate Z Ranges** button to recalculate the Z series for all calculated locations (outlined in red)
15. (Optional) Click the **Remove Fields** button to enter field removal mode and click on any

locations that do not have any useful image data

16. Click the **Acquire Volume** button to acquire and save the 3D image data

Optional recommended post-processing steps:

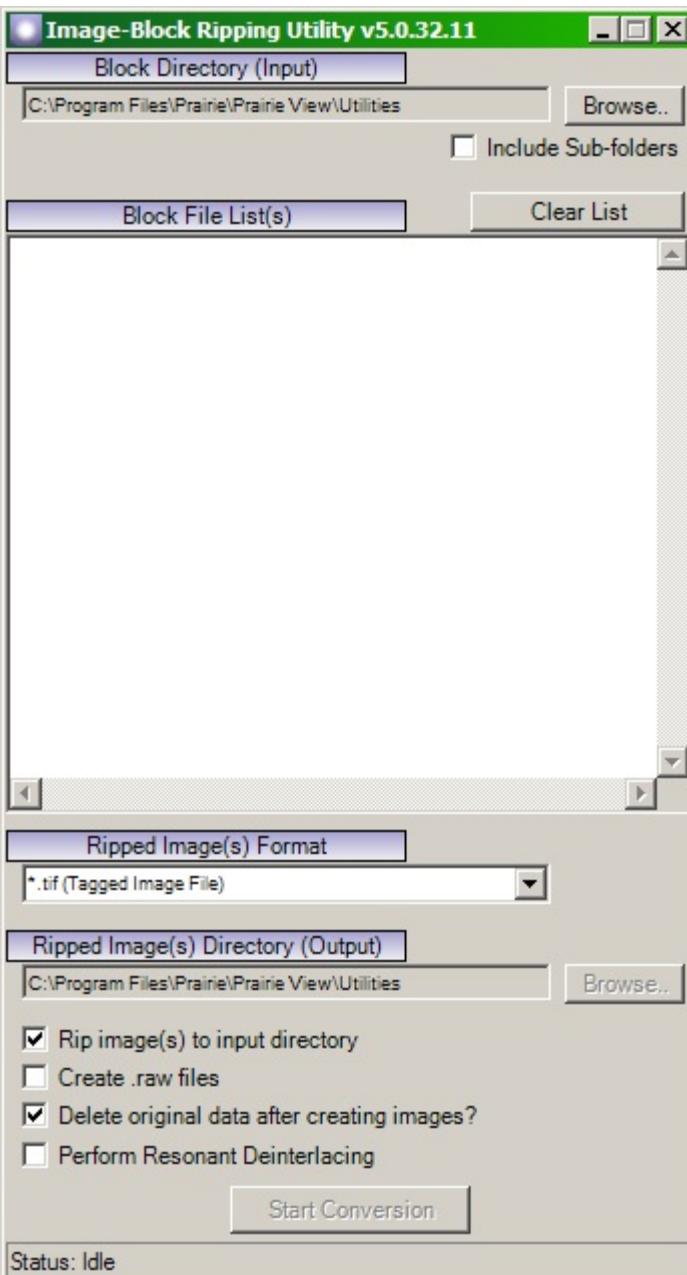
1. Open Fiji to stitch and render the data set
2. In Fiji's Plugins menu, choose Stitching -> Grid/Collection Stitching
3. Choose "Type: Positions from file" and "Order: Defined by image metadata" and click OK
4. For the "Multi-Series File", navigate to the directory in which your T-Series is saved and choose the first *.ome.tif file
5. It may be necessary to check one or both of the "Invert X Coordinates" and "Invert Y coordinates" options. This is unique to each system, and it will be obvious if this is not set correctly when the results are generated
6. Click OK to stitch the Z planes together and create a 3D volume dataset

Image-Block Ripping Utility

This is a program which converts raw data into usable files (TIFF for image data and CSV for other data). It can be run from within Prairie View, or executed independently when Prairie View is not running.

In many cases, raw data files are automatically converted after an acquisition is complete. This allows for immediate viewing and use of the data. During conversion, the user cannot run another acquisition. In most cases, this is not a problem, because the time required to convert the raw files is negligible. However, some experiments generate large amounts of data that can take a long time to convert. This can happen with long T-Series experiments with a Resonant Scanner, long Brightness Over Time acquisitions, or large Voltage Recording experiments. In these cases, it can be useful to delay conversion of the raw files until a later time, so the user can start another acquisition immediately.

The user can choose whether or not raw data is automatically written to TIFF and/or CSV files after an acquisition using the Preferences menu options to [Automatically Convert Raw Files](#). If images are not automatically converted, there is less processing overhead at the end of the acquisition before another image can be acquired. The images can be converted later using the Image-Block Ripping Utility.



The user selects the location of the original file and the destination for the images, which need not be the same location.

Use the **Browse** button at the top of the window to navigate to the folder containing the raw data to be converted.

Enable the **Include Sub-folders** option if selecting a parent directory that contains multiple acquisitions.

The Block File List(s) window lists the files waiting to be converted. Use the **Clear List** button to start over making selections of files to convert.

At this time, the only Ripped Image(s) Format available is TIF, so the user does not need to make a selection.

Use the **Browse** button for the Ripped Image(s) Directory (Output) to choose a destination folder for the converted files. Check the option to **Rip Image(s) to input directory** to put converted files back into the original directory; this will disable the controls to browse to a

user-defined input directory.

The option to **Create .raw files** will write a raw data file of pixel intensities for each image in the acquisition (breaking up the combined raw data file from the original acquisition). These file types may be used by some third-party analysis software packages.

Check the option to **Delete original data after creating images** to delete the original raw files after conversion. This saves disk space.

When converting images acquired with a Resonant Scanner, the option to **Perform Resonant Deinterlacing** applies a post-processing algorithm during conversion to clean up artifacts of the bi-directional scanning and variable dwell time. These processed images will be saved in a sub-directory, alongside the images converted without post-processing.

Click the **Start Conversion** button to run the Image-Block Ripping Utility on the selected acquisitions. An information bar at the bottom of the window informs the user whether the program is currently running or has finished all specified conversions.

Templates Menu

The Templates menu is used to manage a collection of basic experiments which can be loaded quickly without potentially overwriting or clearing out anything that takes a measurable amount of time to set up. For example the current t-series is not affected when loading a template since it takes some time to set it up, the same is true for mark point locations or scripts, but simple things like image resolution, laser power, and window positions are affected by Templates. Another way to think about Templates is as a [Parameter Set](#) with everything selected, plus a little more like acquisition mode.

The following options are available:

Load Template will apply a previously saved Template after selecting an [Environment file](#)

Save Template will save the current settings as a new [Environment file](#) which can be loaded later as either an Environment or a Template

Edit Quick Load List brings up a dialog where it is possible to add custom items to the Templates menu to quickly apply specific Templates; each additional item added to the menu can be given a custom name so that multiple users can use the same template, but call it whatever makes sense to them

What is loaded by a Template? Behind the scenes a Template file is simply an [Environment file](#), but different portions of the file are loaded depending on the context of where it is being loaded from. This ensures that everything is always saved, even if it wasn't thought to be important at the time. For example importing a previously exported t-series definition only loads the t-series portion of the Environment file, but it was an environment file which was originally exported. When an Environment file is loaded as a Template the following software settings are changed:

- **Everything that can be changed using a parameter set;** this list is system and acquisition mode specific and can be viewed by creating and/or selecting a new [Parameter Set](#) and looking at the list of available parameters
- **Acquisition Mode**
- **Custom Output Waveforms**
- **FLIM: Photon Counting Mode**
- **Image Windows** including how many were open, where they were, what channels were selected, channel color mappings, as well as overlay font, color and transparency level
- **Interlaced Scan Settings** including track count, laser powers for each track, and which track each channel is getting its data from

- **Window Locations** of all the forms and dialogs in Prairie View
- **XY and Z Step Sizes**

Templates are meant to be an unobtrusive way to switch to a different experiment, share common settings between users, or provide a starting point for a new user. So in general a Template will not change any of the following:

- **Anything that takes time to set up:** t-series definition, parameter sets, scripts, actions, uncaging point locations, etc.; if these were overwritten it could take a user a significant amount of time to recover what was lost
- **Anything that is difficult for a user to notice it has changed,** like obscure menu options not normally used; if these settings were changed it could leave the user wondering why the software is all of a sudden behaving differently
- **Anything that is a matter of personal preference:** control caption colors, scale bar location, automatically starting playback mode, when to convert raw files, etc.; Templates are meant to be shared so they will only change what is needed to perform an experiment and leave everything else the way the user prefers it

Keyboard Shortcuts: Opens a dialog to display the list of keyboard shortcuts available on the system

Luigs & Neumann Technical Note: Provides information specific to systems configured with a stage manufactured by Luigs & Neumann

User's Manual: Opens a compiled HTML file containing the user's manual for Prairie View software

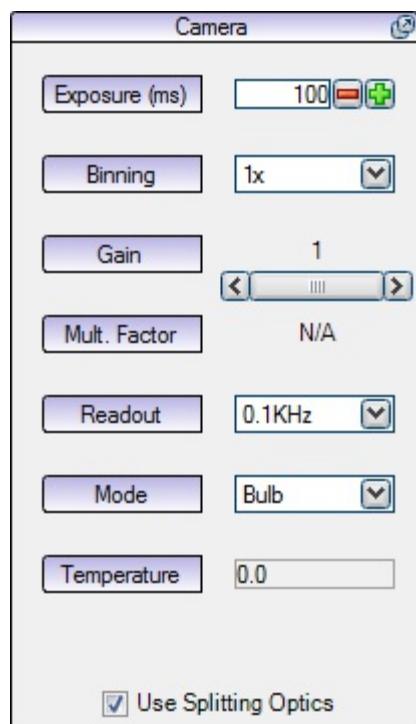
Remote Support: Launches a window enabling Bruker Fluorescence Microscopy personnel to remotely access the computer for service and support; ensure an internet connection is established and [contact](#) Bruker Fluorescence Microscopy support personnel to set up a session

About: Provides information about software version, system ID(s), device firmware versions, and the Prairie View license agreement

Splitting Optics Overview

Prairie View includes support for dual and quad image splitting optics in SFC and Camera modes. These image splitting optics enable simultaneous collection of two or four channels of data using a single CCD or CMOS image sensor.

When the Image Splitting Optics configuration is enabled, a check box will appear in the user interface. In SFC mode, the checkbox is labeled **Use Splitting Optics** and is located just below the **Emission Filter** selection in the upper right hand corner of the **General** tab. In Camera mode, the checkbox is labeled **Use Splitting Optics** and is located at the bottom of the camera settings section on the main form.



SFC Settings

General Triggers Scan Settings Terminal

Exposure (ms)
100

Detection Mode

- Confocal
- Spectral
- Widefield

Emission Filter
488/561/640

Binning
1x

Image Splitting

Use Splitting Optics

Aperture
30um pinhole

Optimal Aperture

Galvo Mode
Normal (Linear)

Camera Settings

Gain

EM Gain

Readout Mode

4e-/ADU

301

10 MHz (EM)

-70.3°C

Stopped

Stopped

24 Cycles/Frame

Dual Image Splitting Optics

The presence of dual image splitting optics enables simultaneous collection of two channels of data using a single CCD or CMOS image sensor.

When the check box is checked to use the splitting optics is checked, the software will separate a single camera image into two channels of data. Channels are acquired in pairs, where channels 1 and 2 are taken from one exposure, and channels 3 and 4 are taken from a separate exposure. Any software-controlled light sources (lasers, LEDs, etc.) enabled for either channel in the pair will be active during the exposure for that pair.

In the metadata information for an image data set, the relativeTime and absoluteTime will be the same for both channels of each pair acquired in a single exposure.

Quad Image Splitting Optics

The presence of quad image splitting optics enables simultaneous collection of four channels of data using a single CCD or CMOS image sensor.

When the check box is checked to use the splitting optics is checked, the software will separate a single camera image into four channels of data. Any software-controlled light sources (lasers, LEDs, etc.) enabled for any channel will be active during the exposure.

In the metadata information for an image data set, the relativeTime and absoluteTime will be the same for all channels, as they are acquired in a single exposure.

Dual Camera Splitting Optics

The presence of dual camera splitting optics enables simultaneous collection of two channels of data using two CCD or CMOS image sensors (two different cameras of the same model).

When the check box is checked to use the splitting optics is checked, the software will collect pairs of channels simultaneously. Channels 1 and 3 are acquired on one camera, and channels 2 and 4 are acquired on the other camera. Any software-controlled light sources (lasers, LEDs, etc.) enabled for either channel in the pair will be active during the exposure for that pair.

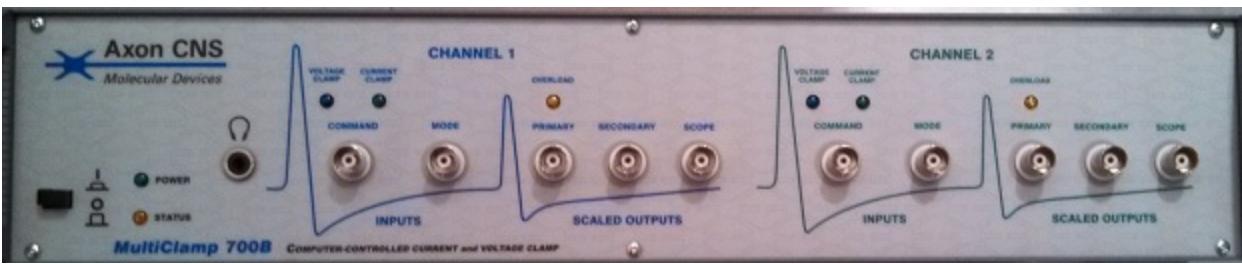
In the metadata information for an image data set, the relativeTime and absoluteTime will be the same for both channels of each pair acquired in a single exposure.

Patch Amplifiers Overview

Prairie View can be used to send and receive signals from a variety of patch amplifier devices. Integrated support is provided for reading the scale factors of the MultiClamp 700B and the Axopatch 200B; other amplifiers can be used by manually entering the scale factors.

Cabling and configuration instructions for the [MultiClamp 700B](#) and [Axopatch 200B](#) are provided in this manual. These instructions are guidelines for an initial setup. Actual connections and usage may vary based on system configuration.

MultiClamp 700B



Cabling

USB connections

MultiClamp 700B Back Panel, USB type B → Computer Back Panel, USB type A

BNC → BNC connections

MultiClamp 700B Front Panel	GPIO Front Panel
Channel 1 Primary	PCI-6052E AI 0
Channel 1 Secondary	PCI-6052E AI 1
Channel 1 Command	PCI-6713 AO 0
Channel 2 Primary	PCI-6052E AI 2
Channel 2 Secondary	PCI-6052E AI 3
Channel 2 Command	PCI-6713 AO 1
Channel 1 and/or 2 Mode	PCI-6713 AO2 (Optional)

Other input and output channels can be used; adjust the channel numbers in the instructions below to match actual system configuration.

Software

MultiClamp Commander 700B

- Start MultiClamp Commander software before running Prairie View
- Make sure the MultiClamp Commander software finds the attached device
- Use the Options dialog (from the button that looks like a wrench) Gains tab to configure settings
 - Voltage Clamp

- Select desired Feedback Resistor
- Set External Command Sensitivity to 20mV/V
- Current Clamp
 - Select desired Feedback Resistor
 - Set External Command Sensitivity to 400pA/V
- Choose the signals to be sent on the Primary and Secondary outputs by right-clicking on those outputs in the main MultiClamp Commander window
 - In the examples below, the Primary output is assumed to be used for Current and the Secondary output is assumed to be used for Potential

Prairie View Configuration Utility

Near the bottom of the Misc tab, check the box to enable Patch Clamp Device and select MultiClamp 700B from the drop-down list.

Prairie View Software

Use the [Seal Test](#) feature when patching the cell. To incorporate electrical recording and analog output signals during an experiment, use the [Voltage Recording](#) and [Voltage Output](#) modules.

- [Seal Test](#)
 - Select input and output channels to match cabling
 - When using Channel 1
 - Device – MultiClamp 700B Ch1
 - Current – 6052 Ch 0
 - Potential – 6052 Ch 1
 - Command – 6713 Ch 0
 - When using Channel 2
 - Device – MultiClamp 700B Ch2
 - Current – 6052 Ch 2
 - Potential – 6052 Ch 3
 - Command – 6713 Ch 1
 - Gains are read automatically from MultiClamp Commander software
 - Pulse can be defined by the user
 - **Acquire** begins reading and displaying signals
 - **Pulse** begins sending the pulse defined by the user
 - **Holding Voltage** begins sending the holding potential defined by the user
 - **Stop** ends the acquisition and pulsing
 - [Voltage Recording](#)
 - The Name of each input can be set to reflect the connections to the amplifier. Based on the cabling listed above, that would be:
 - Input 0 = Ch1 Primary
 - Input 1 = Ch1 Secondary

- Input 2 = Ch2 Primary
- Input 3 = Ch2 Secondary
- Range for each input can be set to $\pm 10V$, or narrower to get more resolution if signals are of smaller amplitudes
- Units can be read automatically from MultiClamp Commander software settings
 - Input 0 – check the box to Read from Multiclamp 700B Ch 1 and choose Primary Output from the drop-down menu
 - Input 1 – check the box to Read from Multiclamp 700B Ch 1 and choose Secondary Output from the drop-down menu
 - Input 2 – check the box to Read from Multiclamp 700B Ch 2 and choose Primary Output from the drop-down menu
 - Input 3 – check the box to Read from Multiclamp 700B Ch 2 and choose Secondary Output from the drop-down menu
- Define recording time and sampling rate needed for the desired experiment/protocol
- Voltage Output
 - Scaling must be defined manually by the user on channel AO 0 and AO 1
 - Set up two separate experiments for Voltage Clamp and Current Clamp protocols
 - Voltage Clamp
 - Click **Config Line 0** and **Config Line 1** to define the outputs
 - Click the  button to set units and scaling
 - Set 1 V actual output = 20mV
 - Define pulses in the Patch Amplifier waveform type
 - Optional: If the Mode selection in MultiClamp is set to EXT, Configure Line 2 to be a fixed voltage of 5V, maintained indefinitely, to put the amplifier into Voltage Clamp mode
 - From the Voltage Output window
 - Set Update Rate
 - Save the entire output definition
 - Current Clamp
 - Click **Config Line 0** and **Config Line 1** to define the outputs
 - Click the  button to set units and scaling
 - Set 1 V actual output = 400pA
 - Define pulses in the Patch Amplifier waveform type
 - Optional: If the Mode selection in MultiClamp is set to EXT, Configure Line 2 to be a fixed voltage of 0V, maintained indefinitely, to put the amplifier into Voltage Clamp mode
 - From the Voltage Output window
 - Set Update Rate
 - Save the entire output definition
 - Load the settings for the desired experiment (Voltage Clamp or Current Clamp)

- Define any other outputs needed for the protocol

Axopatch 200B



Cabling

All are BNC-to-BNC connections

Axopatch 200B Back Panel	GPIO Front Panel
Scaled Output	PCI-6052E AI 0
10Vm Output	PCI-6052E AI 1
Gain (Telegraph Outputs)	PCI-6052E AI 2
Ext Command Input Front Switched	PCI-6713 AO 0

Other input and output channels can be used; adjust the channel numbers in the instructions below to match actual system configuration.

Software

Prairie View Configuration Utility

Near the bottom of the Misc tab, check the box to enable Patch Clamp Device and select Axopatch 200B from the drop-down list.

Prairie View Software

Use the [Seal Test](#) feature when patching the cell. To incorporate electrical recording and analog output signals during an experiment, use the [Voltage Recording](#) and [Voltage Output](#) modules.

- [Seal Test](#)
 - Select input and output channels to match cabling
 - Current – 6052 Ch 0
 - Potential – 6052 Ch 1

- Output – 6713 Ch 0
 - Telegraph – 6052 Ch 2
 - Set Mode knob on 200B to V-Clamp
 - Gains are read automatically from Telegraph connection
 - Pulse can be defined by the user
 - **Acquire** begins reading and displaying signals
 - **Pulse** begins sending the pulse defined by the user
 - **Holding Voltage** begins sending the holding potential defined by the user
 - **Stop** ends the acquisition and pulsing
- [Voltage Recording](#)
- The Name of each input can be set to reflect the connections to the amplifier. Based on the cabling listed above, that would be:
 - Input 0 = Scaled Output
 - Input 1 = 10Vm Output
 - Range for each input can be set to $\pm 10V$, or narrower to get more resolution if signals are of smaller amplitudes
 - Unit scale factor for the Scaled Output connection can be read automatically from the Telegraph connection. Otherwise, set the units using the conversion fields in the Units pop-up dialog. Unit scaling for the 10Vm output connection must be entered by the user.
 - Input 0 (Scaled Output from amplifier)
 - Check the box to Read from Axopatch 200B
 - During Voltage Clamp experiments, choose “Current 6052 Ch 2”
 - During Current Clamp experiments, choose “Voltage 6052 Ch 2”
 - The 6052 channel for units corresponds to the channel connected to the Telegraph Gain signal from the amplifier
 - Alternatively, scaling can be set by the user
 - Input 1 (10Vm Output from amplifier)
 - Set scaling to 0.1 Volt = 1 Volt
 - Define recording time and sampling rate needed for the desired experiment/protocol
- [Voltage Output](#)
- Scaling must be defined manually by the user on channel AO 0
 - Set up two separate experiments for Voltage Clamp and Current Clamp protocols
 - Voltage Clamp
 - Click **Config Line 0** to define the output
 - Click the  button to set units and scaling
 - Set 1 V actual output = 20mV
 - Define pulses in the Patch Amplifier waveform type
 - From the Voltage Output window
 - Set Update Rate

- Save the entire output definition
- Current Clamp
 - Click **Config Line 0** to define the output
 - Click the  button to set units and scaling
 - Set 1 V actual output = 2nA if using WC mode x1, or
Set 1 V actual output = 20nA if using WC mode x0.1
 - Define pulses in the Patch Amplifier waveform type
 - From the Voltage Output window
 - Set Update Rate
 - Save the entire output definition
- Load the settings for the desired experiment (Voltage Clamp or Current Clamp)
- Define any other outputs needed for the protocol

The metadata associated with a Prairie View acquisition is saved as an XML file. The format of this file has changed over time, as Prairie View itself has evolved. The information below summarizes these changes. If you need additional information about the XML file for use in third-party applications, please contact Bruker Fluorescence Microscopy support personnel.

Note that Bruker Fluorescence Microscopy has developed a plugin for ImageJ/Fiji to open Prairie View acquisition files and associated metadata. This plugin is updated along with Prairie View software. The plugin, along with instructions for installation, can be found on the system computer in the following directory: C:\Program Files\Prairie\Prairie View\Support Files\ImageJ

Version 4.x and Earlier

This is the format most Prairie View users are accustomed to seeing, and most third-party applications are able to handle. The largest issue with customers trying to parse this format is trying to read the XML file like a text file, looking for certain strings or assuming a fixed order, when it should be read as an XML file using whatever XML parser is available in the programming language being used.

Another lesser-known issue with this format is that while the first line in the file says it is XML version 1.0, the file actually contains some characters that aren't valid for version 1.0 and instead require version 1.1. Some XML parsing libraries (for example, MATLAB's) are picky about this distinction and refuse to read the XML files unless the version at the top of the file is changed from 1.0 to 1.1. However, Microsoft/.NET doesn't support version 1.1, so if the file is changed, Prairie View can no longer read the file. Removal of those characters is beyond the scope of Bruker's current technical development projects.

Version 5.0

In addition to the traditional XML file format, Prairie View version 5.0 offers the option to 'Use Smaller XML File Format'; the user can toggle this option under the 'Preferences' menu in the Prairie View software.

The smaller XML file format eliminates the duplicate state key/value pairs by introducing a hierarchical structure where only what has changed is written out. This saves tens to hundreds of megabytes for larger data sets. However, most third-party applications designed to read the larger XML files will not read the smaller format because they don't know where to find data that can exist in any one of three places. The differences are illustrated in the following example.

In earlier versions each data set XML file looked something like the following:

```
<?xml version="1.0" encoding="utf-8"?>
<PVScan version="4.3.2.24" date="3/27/2013 5:01:57 PM" ... >
...
<Sequence ... >
  <Frame ... >
    <PVStateShard>
      ...All state key/value pairs are listed here, for every frame, even if they don't change...
    </PVStateShard>
  </Frame>
  ...There could be more frames here...
</Sequence>
...There could be more sequences here...
</PVScan>
```

XML files using the smaller format look something like the following:

```
<?xml version="1.0" encoding="utf-8"?>
<PVScan version="5.0.64.100" date="4/15/2014 9:01:26 AM" ... >
...
<PVStateShard>
  ...All state key/value pairs are listed here,
  this can be thought of as the grandparent state...
</PVStateShard>
...
<Sequence ... >
  ...
  <PVStateShard>
    ...Only state key/value pairs which differ from the grandparent state are listed here,
    this can be thought of as the parent state...
  </PVStateShard>
  ...
  <Frame ... >
    <PVStateShard>
      ...Only state key/value pairs which differ from the parent state are listed here,
      this can be thought of as the child state...
    </PVStateShard>
  </Frame>
  ...There could be more frames here...
</Sequence>
...There could be more sequences here...
</PVScan>
```

It is important to note that any XML parser capable of handling the smaller XML format can still

read the old format. The old, larger format is like a special case of the smaller XML file where the child state contains every state key/value pair and it is never necessary to look at the parent or grandparent state. It is still a good practice to check if those parent and grandparent nodes exist prior to trying to access them.

Version 5.1

There were no significant changes in the XML structure between version 5.0 and 5.1. The option for the user to select either the original or the smaller XML format exists in 5.1 as it did in 5.0.

In Prairie View 5.1, OME TIFF support was updated and extended to allow Fiji/ImageJ to import our datasets using just the TIFF files. Almost every TIFF file written in version 5.1 is an OME TIFF, as noted by the *.ome.tif file extension. In version 5.0, and earlier versions supporting OME TIFF, adding OME TIFF metadata to the TIFFs was done in post processing and did not change the file extension; that OME TIFF implementation was rudimentary and designed with a single case in mind. The changes in 5.1 fully implement the OME TIFF format.

A data set can be imported into Fiji/ImageJ using the OME TIFF metadata (instead of the Prairie View data set XML file) by selecting the first *.ome.tif file in the folder (instead of the XML file that would normally be used by the Bio-Formats import tool). The first *.ome.tif file is a little larger than the rest and contains information about all the other related TIF files.

Version 5.2

This version takes the smaller XML format introduced in version 5.1 and makes it the default format, with no option to go back to writing out the larger data set XML files. In addition, the format of the state key/value pairs has changed slightly to incorporate indices and sub-indices where applicable.

For example here are what some state key/value pairs looked like prior to version 5.2:

```
<Key key="linesPerFrame" permissions="Read, Write, Save" value="186" />
<Key key="pmtGain_0" permissions="Write, Save" value="605" />
<Key key="pmtGain_1" permissions="Write, Save" value="604" />
<Key key="pmtGain_2" permissions="Write, Save" value="0" />
<Key key="positionCurrent_XAxis" permissions="Write, Save" value="0.95" />
<Key key="positionCurrent_YAxis" permissions="Write, Save" value="-4.45" />
<Key key="positionCurrent_ZAxis" permissions="Write, Save" value="-9,62.45" />
```

Here is what those same state key/value pairs look like in version 5.2:

```
<PVStateValue key="linesPerFrame" value="186" />
```

```

<PVStateValue key="pmtGain">
  <IndexedValue index="0" value="605" description="Ch1 High Voltage" />
  <IndexedValue index="1" value="604" description="Ch2 High Voltage" />
  <IndexedValue index="2" value="0" description="Ch3 High Voltage" />
</PVStateValue>
<PVStateValue key="positionCurrent">
  <SubindexedValues index="XAxis">
    <SubindexedValue subindex="0" value="0.95" />
  </SubindexedValues>
  <SubindexedValues index="YAxis">
    <SubindexedValue subindex="0" value="-4.45" />
  </SubindexedValues>
  <SubindexedValues index="ZAxis">
    <SubindexedValue subindex="0" value="-9" description="Focus" />
    <SubindexedValue subindex="1" value="62.45" description="Piezo" />
  </SubindexedValues>
</PVStateValue>

```

Notice that in addition to the slight formatting changes, description fields have been enhanced to include some human-recognizable identifiers for key/value pairs which would otherwise only have a programmatically generated index associated with them. Since this new description field has just been introduced, not all key/value pairs have been implemented; more will be implemented over time.

It is also worth noting that keys, indices, and sub-indices in version 5.2 are written in alphabetical/numerical order, whereas the order in older versions could vary based on a number of factors.

Also new to version 5.2 is XML metadata related to Spectral Mode in Prairie View. If a sequence was collected in Spectral Mode, the sequence tag would have the SpectralMode attribute set to "True".

```
<Sequence SpectralMode="True" ...
```

The rest of the metadata format is consistent with a non-spectral dataset except that instead of having a maximum of four image channels, there can be 16 channels with each channel corresponding to subset of the entire emission spectrum. Each channel is denoted by the <File> tag and has attributes for channel name, channel number, timestamp and filename. Metadata for a spectral frame with 16 spectral channels is formatted as follows:

```

<Frame index="1" parameterSet="CurrentSettings" absoluteTime="0.374000000000024"
relativeTime="0">

<File absoluteTime="0.374" relativeTime="0" filename="TSeries-06052014-1318-
003_Cycle00001_Ch1_000001.ome.tif" wavelengthMax="531" wavelengthMin="526"
channelName="SpectralChannel_01" channel="1"/>

```

...

```
<File absoluteTime="0.374" relativeTime="0" filename="TSeries-06052014-1318-  
003_Cycle00001_Ch16_000001.ome.tif" wavelengthMax="828" wavelengthMin="808"  
channelName="SpectralChannel_16" channel="16"/>  
  
<ExtraParameters lastGoodFrame="0"/>  
  
<PVStateShard/>  
  
</Frame>
```

Multi-User Systems

By creating separate user profiles in Windows, Prairie View can keep track of usage and settings for multiple users. All settings which would normally be retained between sessions are also user specific, with the exception of scan settings specific to the hardware present and optical alignment which are shared by all users. Some settings, such as stage or focus device position, are polled from the hardware at startup and are never restored from the previous session.

Logging Activity

The file 'Prairie View.log' keeps track of several functions and actions taken while a user is in Prairie View. Lines are added to the log when certain events occur: starting and stopping Prairie View, execution of an acquisition, and editing of the Scan Settings dialog. The date, time, user name and, in the case of an acquisition, file location, are shown for each line of action recorded in the log.

Limiting Access to Configuration and Scan Settings

It is possible from the Configuration Utility to add password protection to the configuration files, which in turn is used by Prairie View to password protect the scan settings dialogs. This allows a system administrator to prevent unauthorized or unintentional modifications to these settings which could negatively impact other users. Contact a Bruker Fluorescence Microscopy representative for help implementing this feature.

Third-Party Integration

There are a number of ways to have Prairie View use, or have Prairie View used by, a third party application.

Actions

Actions, which are described in more detail in the [Actions](#) section, provide a way for Prairie View to run, and optionally pass along some data to, a third-party application and have that third-party application do something with the data and in some cases pass some data back to Prairie View. For example an action could be set up to run after each line scan which would pass the metadata xml file to a third-party application which would do some post-processing which can be reviewed before starting another acquisition.

Scripts

Scripts, which are described in more details in the [Scripts](#) section, are a way to automate (or program) otherwise complicated experiments or tasks within Prairie View, but they can also be used by a third-party application to control Prairie View externally. The conduit for this control can be as simple as instructions provided at the command line, or as complicated as communicating directly with Prairie View over a TCP/IP connection which can be done from anywhere on the network/internet. More information on specific scripting commands and how script commands can be called can be found in the [Script Commands](#) section.

PrairieLink

PrairieLink is an API (Application Program Interface) which can be used by software developers to communicate with Prairie View using a set of predefined methods/functions, which are described in more detail in the [PrairieLink](#) section. Some of the methods/functions provided by PrairieLink can be used to execute script commands without the additional hassle of dealing with TCP/IP communication since that is already written into PrairieLink.

PrairieLink is an API (Application Programming Interface) which allows direct two-way communication with Prairie View without understanding the underlying communication protocols. Put simply, it uses script commands to communicate with Prairie View via TCP/IP. For more information please reference the script command documentation accessible from the script editor under the Tools menu in Prairie View, or use [this link](#).

Using PrairieLink

Using PrairieLink will differ based on what language you are using, for example VB .NET or MATLAB. To use PrairieLink in a .NET language first add PrairieLink.dll as a reference then instantiate a new object of type PrairieLink.Application, for example:

Dim pl As New PrairieLink.Application

To use PrairieLink in MATLAB the equivalent line would be:

pl = actxserver('PrairieLink.Application');

Once a PrairieLink object has been instantiated you must connect to Prairie View using the Connect function:

pl.Connect()

Optionally include the IP address parameter to connect to Prairie View running on another computer:

pl.Connect("127.0.0.1")

If a remote access password is set up on the [Edit Scripts](#) dialog accessible under the 'Tools' -> 'Scripts' menu that password will need to be passed as the second optional parameter:

pl.Connect("127.0.0.1", "password")

The Connect function will return a Boolean value: true if the connection succeeded or false if the connection failed.

Once connected any of the methods described below can be used, without an active connection to Prairie View they not work.

For example, the following will return the current dwell time in microseconds:

pl.GetState("dwellTime")

When finished, use the `Disconnect` method to let Prairie View know so it can clean up afterwards; failure to disconnect properly could result in undesirable behavior.

pl.`Disconnect()`

PrairieLink Methods

Connect(*optional* IPAddress as String) as Boolean

This function connects to the currently running instance of Prairie View at the address provided and returns true if the connection was successful or false if the connection failed. If the optional IP address is not provided PrairieLink will connect to the currently running instance of Prairie View on the same machine. Note that some methods only work on the same machine, those exceptions will be mentioned in the descriptions of any affected methods.

Connected() as Boolean

This function returns true if a connection has been made or false if no connection is present.

Disconnect()

This method disconnects from Prairie View. Prairie View needs to do a little bit of cleanup when a communication channel isn't being used anymore, so failure to disconnect, particularly after numerous connections are made in the same session, could result in undesirable behavior.

SendScriptCommands(commands as String) as Boolean.

This function sends a string of script commands to Prairie View which will then be run. If the script commands run successfully the function will return true, otherwise it will return false. For a complete listing of available script commands please reference the script command documentation accessible from the script editor under the Tools menu in Prairie View, or use [this link](#). Some script commands can return values; each time a script command returns a value an event will be raised for which a handler can be registered to do something with the value. There are also specific functions to call a single script command which returns a value without using events. See the [event handling](#) section below for more details.

PixelsPerLine() as Integer

This function returns the number of image pixels in the width/X dimension as an integer.

LinesPerFrame() as Integer

This function returns the number of image pixels in the height/Y dimension as an

integer.

GetState(key as String, *optional* index as String, *optional* subindex as String) as String

This function returns the value for the specified state key as a string. Some state keys are indexed or subindexed which requires the optional parameters to be used. Use the PVStateShard section of the environment file as a reference.

GetMotorPosition(axis as String ('X', 'Y' or 'Z'), *optional* deviceIndex as Integer) as Double

This function returns the position of the specified axis as a double precision floating point value. On some systems there are multiple devices for an axis so the optional device index parameter can be used to differentiate between them. The device index parameter is zero index, so the first device is zero.

GetImage(channel as Integer) as Integer()

This function returns a two dimensional array containing the image data for the specified channel. In cases where there are multiple samples for a pixel the values are summed, not averaged. Note that this function will only work on the same computer that Prairie View is running on, it will return an array of zero's if run on a different computer.

GetImage_2(channel as Integer, pixelsPerLine as Integer, linesPerFrame as Integer) as Integer()

This function is a slightly more efficient version of the GetImage function to be used in cases where the image dimensions are known. This eliminates the need to poll Prairie View for the image dimensions, saving a little time. Note that this function will only work on the same computer that Prairie View is running on, it will return an array of zero's if run on a different computer.

DroppedData() as Boolean

This function returns true if the current acquisition has dropped data or false if all the data has been saved successfully.

ReadRawDataStream(*out* samplesRead as Integer) as Short()

In order to use this function raw data streaming needs to be enabled by calling the

script command ‘–srd true’, otherwise this function will never return any samples. This function will return an array of samples as they are read off of the acquisition card. This function will guarantee that the chunks of data returned will form contiguous whole frames. If frames of data are not read off fast enough, only the most recent frames will be kept and the stream will omit the frame(s) in between (unless the buffer frames parameter passed to the –srd command is non-zero which will return all data and stop streaming if the data is not read out fast enough to keep up). Making sense of the data stream requires knowledge of the acquisition being run, like how many pixels are in a line, how many lines are in a frame, how many samples are acquired for each pixel, and how many channels of data are being acquired. All of these things can be polled for using script commands. For example a galvo mode acquisition for two channels at a 4us dwell time will have two 16-bit values for each sample (one for each channel) and 10 samples for each pixel (4/.4). The SamplesPerPixel function can help figure this out for other acquisition modes. Multiple channels for a camera based acquisition will not be interleaved. Using this function will definitely require some trial and error as every application will be different.

SamplesPerPixel() as Integer

This function returns how many samples are acquired for each pixel in the image. This is mainly useful when used in conjunction with the ReadRawDataStream function to figure out how to parse the raw data stream.

PrairieLink Event Handling

There is currently only one event raise by PrairieLink which is used to handle script command responses asynchronously. There are specific methods to call a single script command and return the response synchronously in the [methods](#) section above. The following code will register for script command response events in VB .NET:

```
AddHandler pl.ScriptCommandResponse, AddressOf  
ScriptCommandResponseHandler
```

Where the handler subroutine looks something like:

```
Private Sub ScriptCommandResponseHandler(ByVal response As String)  
...  
End Sub
```

To register for the same event in MATLAB the code would look like this:

```
pl.registerevent({'ScriptCommandResponse' 'ScriptCommandResponseHandler'});
```

Where the handler subroutine would be in an M file with the same name, in the current working directory, and look something like:

```
function HandleScriptCommandRequest(source, eventId, response, args, type)  
...  
end
```

The response parameter in both cases contains Prairie View's response as a string.

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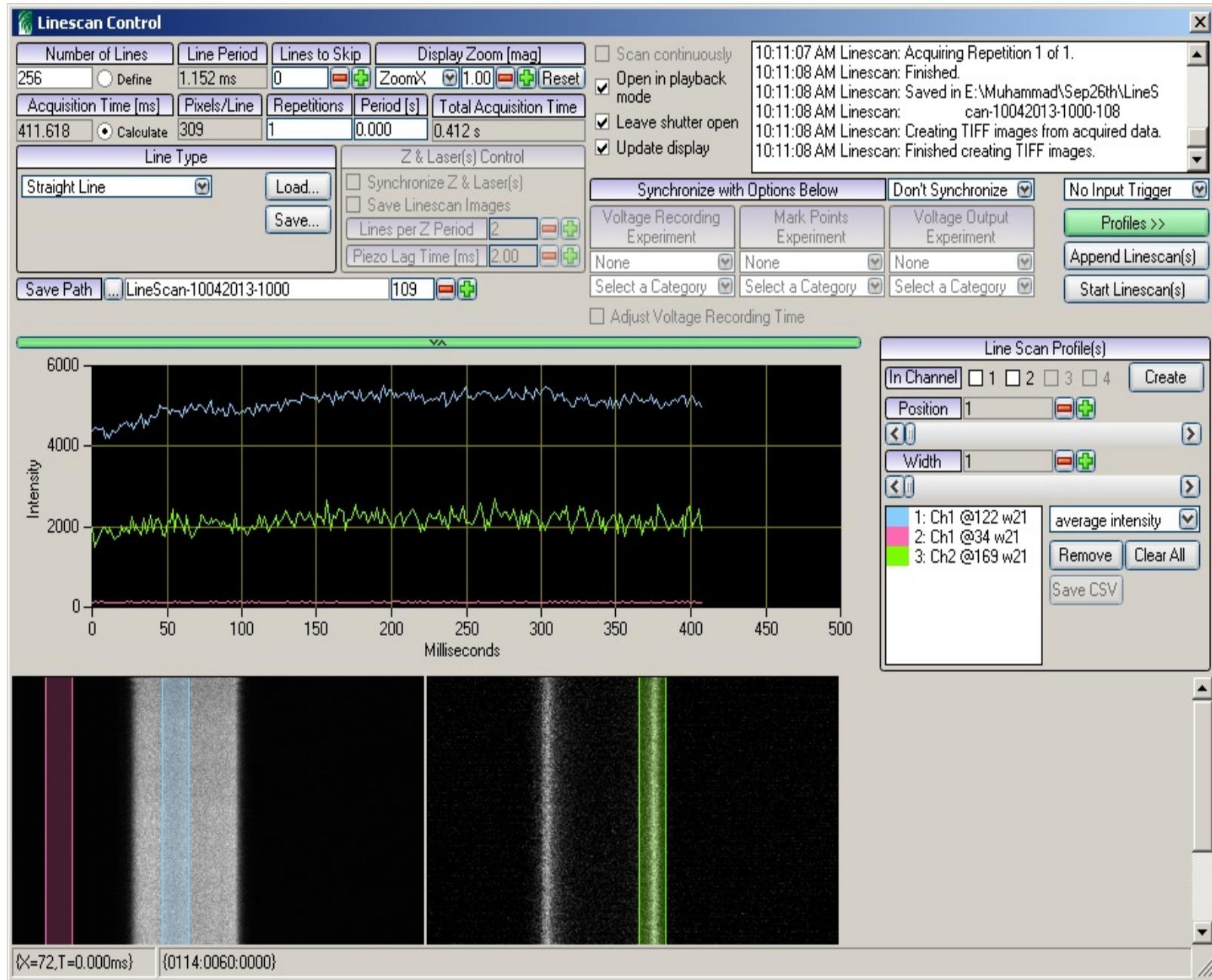
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Line Scan

The Linescan Control dialog opens when the user clicks the **Line Scan** button on an Image window. This window is used to define a one-dimensional acquisition along a user-defined line.



The Linescan Control window is divided into three main sections. The top section contains controls for the user to define the acquisition. The middle section of the window allows the user to define profiles to display intensity data in a graphical format; reveal this section by clicking the **Profiles** button in the upper control section. The bottom section of the window displays the acquired data as an image. These sections are described in detail below.

Defining a Line Scan

Parameters of the Definition

The user can choose to define the **Number of Lines** or the **Acquisition Time** for the scan; the other value is calculated automatically by the software. Use the **Define** and **Calculate** radio buttons to select which parameter text box to activate.

Line Period is based on the line defined by the user and the current scan settings defined in the main control window. Hovering over this field will display a tooltip showing which portion of the line period is spent scanning vs. retracing.

The **Pixels/Line** field displays the number of pixels scanned in the current definition.

During a line scan, the system will repeatedly scan the defined line. If the user wants to collect intensity data less frequently, a number of **Lines to Skip** can be defined. This number must be an integer. Scanning continues during these skipped lines, but laser power is set to minimum levels. For example, if the user sets the **Number of Lines** to 200 and the **Lines to Skip** to 1, the system will scan the line 400 times but only apply laser power and collect data every other line. The **Line Period** field will update to show the time from the start of one acquired line to the start of the next acquired line (taking into account the time for skipped lines).

The user can define a number of **Repetitions** for the experiment, and define a **Period** for those repetitions. For example, 3 repetitions with a period of 10 seconds would perform the defined number of lines 3 times, with 10 seconds between the start of the first repetition and the start of the second repetition. **Leave shutter open** determines whether the hard shutter will be closed between repetitions (if not checked), or left open until the entire acquisition is complete (if checked). The **Total Acquisition Time** field will update to display the amount of time acquiring data and waiting between acquisitions for the defined period.

For some line types (described below), the user can check the box to enable the **Scan continuously** option. The line will be scanned in both directions; essentially, the defined pattern will be mirrored back on itself. For example, if the user draws a Freehand line 500 pixels long and checks **Scan continuously**, the scanned line will be 1000 pixels long, where the scan will go from start to end to start as one line. If the user selects a Sprial line, the scan will go from the outside to the center and then back from the center to the outside. The Line Period and Acquisition Time fields will be updated to reflect the new scan parameters. Note that Line Period does not double when checking **Scan continuously**, as there is no retrace time in a continuous scan. This option is automatically checked for Circle and Lissajous modes, and is not available for Line mode.

Line Type

The **Line Type** drop-down menu allows the user to choose the type of line to be scanned.

- **Straight Line** defines a horizontal line across the image. The two vertical bars define the portion of the line that is acquired. Reposition the line vertically on the image by dragging either of the outer boxes on the Image window. Change the lateral range of the

acquisition by dragging the two inner vertical bars. This is the only type available when using the Resonant Scanner or AOD to scan the sample. Use the Scan Rotation controls in Galvo mode to change the orientation of the sample relative to the horizontal line on the Image window.

- **Circle** defines a continuous circular scan pattern. In the Image window, left click and drag in the square markers to change the size and position of the circle, right click and drag to translate circle. The first pixel of the circle is indicated by an arrow head marker, which also indicates the direction of the scan.
- **Freehand** allows the user to define a custom line. Click and drag the left mouse button in the Image window to draw the line; hold shift to make straight segments. A green box marks the start of the line, and a red box marks the end. Click the left mouse button in either of these boxes and drag to extend the line. Click the right mouse button in either of these boxes to move the line.
 - The speed and accuracy of a Freehand line is limited by the response of the galvanometers scanning the line. Tight corners will be followed more closely at slower dwell times and higher pixel densities. To prevent over-driving the galvanometers, a Minimum Freehand Dwell Time parameter is defined in the Scan Settings. This parameter is defined at a 512x512 image size and scaled accordingly for other image sizes. The Line Period and other fields account for this minimum during setup. Additionally, if the user defines a Freehand line with a dwell time below the minimum time, a message will appear in the information box on the right side of the window to indicate that the dwell time was temporarily increased. The parameter is set during installation; contact Bruker Fluorescence Microscopy support personnel for assistance or to request changes to the setting on the system.
- **Action(s)** allows the user to import Freehand line definitions from external software programs, such as MatLab. See more information about [Actions](#) elsewhere in this manual, and contact Bruker Fluorescence Microscopy support personnel for more assistance.
- **Spiral** defines a spiral pattern over the image. Two additional controls allow the user to define the spiral. The **Shape** slider adjusts the shape of the pattern, with 1.0 being a circular spiral and smaller values making the scan more of a square shape. The **Spirals** slider adjusts the number of spirals, affecting the length of the line and the density of the scan. The Minimum Freehand Dwell Time parameter (described above) also applies to Spiral line scans.
- **Lissajous** is another continuous pattern. The **Freq** slider adjusts the density of the pattern.
- **Segmented** is a pattern of individual, unconnected straight line segments. The **Auto Order Segments** check box switches between scanning the segments in a user defined order, or an order optimized for scan speed. The **Show Separators** check box turns on and

off the overlays in the linescan window image. Individual segments can be deleted, or their scan order can be changed using the context menu available when right-clicking one of the ends of a segment. Line scan profiles are automatically created for each channel and every segment when enabling line scan profiles.

Click the **Save...** button to save the defined line, which can be recalled again later by clicking the **Load...** button.

Collecting the Line Scan

Click **Start Linescan(s)** to start the acquisition. The message box in the upper right corner of the window will update to show the status of the acquisition.

Each Linescan acquisition is saved in its own folder. The location of the folder is determined by the **Save Path**, which can be viewed or changed by clicking the ... button next to the **Save Path** box. The name of the folder is the name displayed in the text box next to the ... button, followed by the number displayed in the iteration counter text box.

The default folder name includes the type of acquisition and the date and time code for the last time the software was loaded. The user can type a custom name in this field for the current session. The folder name will revert back to the default type-date-time-counter format each time Prairie View is started. To retain the custom folder between sessions, check the **Preserve User-Modified File Names** option in the Preferences menu. Note that using the same folder name in multiple tabs (Z-Series, T-Series, Misc, etc.) can cause data to be over-written if counter values are the same on multiple tabs.

The counter is automatically increased by 1 after each acquisition. The user can change the counter value by typing in the box or using the + and - buttons. Note that decreasing the counter value can result in overwriting data.

A reference image is saved along with the line scan acquisition. The reference image is a TIFF image of the contents of the Image windows, with any overlays (including the defined line) currently displayed on the image. The channels active in each Image window will be listed in the name of the corresponding reference images.

Each defined Repetition will be saved as a separate file in the folder for the current line scan data set.

The **Append Linescan(s)** button starts the defined acquisition but does not start a new dataset. Instead, the acquisition is treated as one or more additional repetitions in the previous acquisition, reflected in the acquisition count messages in the message box in the upper right corner of the window.

When the **Update display** box is checked, newly acquired intensity data will be displayed in the bottom section of the Linescan Control window.

When the **Open in playback mode** box is checked, newly acquired intensity data will be

displayed in the Image window(s) at the end of the acquisition. Some features of Linescan Profiles (described below) are available only in playback mode.

Triggered Line Scans

A Line Scan can be started on its own as described above, or set to respond to input triggers from other equipment. Triggering options are selected via a drop-down menu in the Linescan Control window.

If **No Input Trigger** is selected, then the acquisition will start immediately when the Start Linescan(s) or Append Linescan(s) button is pressed.

If **Trigger First Rep.** is selected, then after Start or Append Linescan(s) is pressed, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first repetition. All subsequent repetitions are acquired automatically.

If **Trigger Each Rep.** is selected, then after Start or Append Linescan(s) is pressed, the Laser and PMT settings will be set, the hard shutter will open, and the software will wait for the arrival of an external input trigger before acquiring the first repetition. This process is then repeated for each subsequent repetition in the defined acquisition.

Triggers sent to the system should be 5V TTL-style signals on a BNC cable. If the system is configured with a GPIO box, input triggers are received by the Trig 1 In through Trig 8 In connections in the middle row on the front on that box.

Synchronizing Functions With Linescan

It is possible to synchronize Voltage Recording, Mark Points, and Voltage Output experiments with a line scan acquisition. The [Voltage Recording](#), [Mark Points](#), and [Voltage Output](#) experiments are configured in their own control windows; more information is provided elsewhere in this manual. The synchronization with the line scan is defined in the Synchronize with Options Below section of the Linescan Control window.

The drop-down menu next to Synchronize with Options Below allows the user to choose when the synchronized experiments run:

- **Don't Synchronize** means that no Voltage Recording, Mark Points, or Voltage Output experiments will be called from the Linescan Control window
- **Once at Start** means that the synchronized experiment(s) will be started along with the first repetition of the line scan
- **Each Repetition** means that the synchronized experiment(s) will be started at the start of every repetition of the defined line scan acquisition

After a synchronization option is chosen, the experiment selection options become active. Use the drop-down menus to select the Current or previously saved experiment for Voltage Recording, Mark Points, and/or Voltage Output.

Checking the **Adjust Voltage Recording Time** changes the acquisition time of the selected Voltage Recording experiment to match the time required for the line scan and synchronized Voltage Output and/or Mark Points experiments.

The **Total Acquisition Time** field will be updated to reflect the duration of the line scan including the synchronized experiment(s).

It is recommended that a user make a global decision on where to configure Voltage Recording and Voltage Output experiments for a given line scan acquisition. It is possible to configure a Mark Points experiment that calls a Voltage Recording or Voltage Output experiment from inside the Mark Points configuration. If using this option, it is not advised to also call Voltage Recording or Voltage Output from the Linescan Control window.

Synchronized Voltage Recording and Voltage Output experiments will start as defined in the Linescan Control window and not wait for any trigger defined in the Voltage Recording or Voltage Output windows. Mark Points experiments will override a trigger on the very first point of the experiment (first point of first repetition of first line), if defined, but will respect all other triggers defined in the Mark Points Series.

More information about Mark Points, Voltage Recording, and Voltage Output can be found in other sections of this manual.

Z & Laser(s) Control

The controls in this section allow the user to drive a Z-piezo device during a line scan acquisition. The goal is to collect intensity data from a volume in the sample at higher speeds than are possible when performing a Z-Series with traditional raster imaging. Alternatively, high-speed volume imaging can be carried out using the Z-Series feature with a Z-piezo and Resonant Scanner or AOD.

This feature requires specific hardware and software configurations which must be implemented by Bruker Fluorescence Microscopy personnel. Additionally, analysis and reconstruction of position and intensity data must be carried out using third-party software tools. Contact Bruker Fluorescence Microscopy support personnel for more information about this feature.

In this mode, the galvanometers are driven along a user-defined path defined as a Freehand, Spiral, or Lissajous line, while the Z-piezo device is driving in a sinusoidal pattern by analog signal control. Laser power can also be adjusted as a function of depth.

The intended data acquisition method for this type of scan is to record position and intensity signals on analog inputs in the Voltage Recording window, for reconstruction and analysis later

in other software packages. Positional information is obtained by recording feedback signals from the X and Y galvanometers and the Z-piezo device. Intensity data is acquired by routing the Preamplifier output signals to Voltage Recording inputs (rather than to the typical Preamplifier inputs used for other imaging).

A Custom Output must be defined for the Z-piezo drive signal, and an additional Custom Output must be defined for each laser to be synchronized with the acquisition.

Define a Voltage Recording experiment to collect analog signals routed from the X and Y galvanometer feedback, Z-piezo feedback, and Preamplifier signal(s) for the channel(s) from which intensity data will be acquired. Choose a sampling frequency (Samples/Second) appropriate for the data resolution needed in the analysis.

Define the Z range and laser power gradient by defining a Z-Series in the Z-Series tab.

In the Z & Laser(s) control section of the Linescan Control window, configure the synchronization of Z and XY control with the following parameters:

Check the **Synchronize Z & Laser(s)** box. Lines to Skip parameter of the line scan definition will be automatically set to zero.

Save Linescan Images can be checked if the user wishes to save the image segments generated in Prairie View software during the 3D acquisition. Intended operation will leave this option un-checked, as Prairie View does not reconstruct the 3-dimensional data acquired, and both the intensity and positional information must be recorded through Voltage Recording and reconstructed using other software packages. Thus, saving the images takes up disk space with files which will not be used.

Lines per Z Period is the number of XY line scan traces that should be executed for each Z period. One Z period is movement of the Z-piezo device from its start to stop position and back to its start position.

Piezo Lag Time allows the user to define the time difference between the application of the drive voltage to the piezo and the piezo achieving the intended location. The parameter is associated with and saved as part of the calibration for the currently selected Objective Lens.

Synchronize the Voltage Recording experiment to run with the line scan acquisition, and check the box to **Adjust Voltage Recording Time**.

Start the acquisition by clicking **Start Linescan(s)** or **Append Linescan(s)**.

Profiles

Line Scan Profiles allow the user to define regions of the line from which to plot intensity data in a graph during acquisition. Intensity data from these plots is saved as a CSV file in the directory containing the line scan data. Click the **Profiles** button in the Linescan Control window to reveal the profile definition section of the window. For **Segmented** line scans

profiles corresponding to all channels and every line segment are automatically created and acquired when the profiles interface is visible. For other line types the user has to define the profiles of interest manually.

To define a profile, check the box for one or more channels and click the **Create** button. One definition will be created for each channel whose check box was activated. Then drag the **Position** and **Width** sliders to adjust the definition of the profile. The profile definition(s) will be displayed in the list box below the definition controls. The name of the profile reflects the channel, position, and width information for the profile, and a colored rectangle indicates the color of the trace and the overlay used to display the profile. The profile definitions are displayed along the line on the Image window and overlaid on the data displayed at the bottom of the Linescan Control window.

Within the list of profiles, the currently selected definition will be displayed with a green background behind the text. Any changes to the Position and Width sliders will be applied to the currently selected definition.

A drop-down menu allows the user to define whether the intensity information recorded is the average or sum of the pixels encompassed by the profile definition. Buttons in the interface allow the user to **Remove** the selected profile or **Clear All** profile definitions.

During acquisition, intensity information for each profile is plotted in the graph within the Linescan Control window. Clicking the green bar above the graph reveals controls for zooming and panning within the graph display.

Profiles can be moved by clicking and dragging the position of the colored markers in the Image window or in the data display at the bottom of the Linescan Control window. The rectangle marking the profile definition will move to the new location defined by the user. Dashed lines in the same color will mark the original position of the profile, corresponding to the trace still visible in the Profiles section of the window.

If the user is viewing the line scan data in Playback mode, moving a profile definition will cause the intensity graph to be recalculated. The user can save the new intensity plot data by clicking the **Save CSV** button. If the user is not viewing the data in Playback mode, the intensity graph will not be recalculated, but the new profile position will still be used in subsequent acquisitions.

Profile data can also be displayed in the Voltage Recording window. Defined profiles will appear as buttons next to each Voltage Recording plot. This allows users to overlay intensity data with electrical recordings that were synchronized with the collection of the line scan. Note that the Previous, Average, and History display options will be applied to line scan profiles acquired with a Voltage Recording, but not to stand-alone line scan acquisitions.

Data Display

Intensity data for each channel is displayed as an image in the bottom section of the Linescan

Control window. The horizontal axis is the position along the acquired line. The vertical axis is the line number, which can be interpreted as time. Each channel active during the acquisition is displayed. A scroll bar on the right side of the window allows the user to view more of the data without expanding the window.

The user can get information about a particular pixel in the displayed data by hovering the mouse over that pixel. Information boxes at the bottom of the Line Scan window display the position of that pixel along the line (X) and the start time (T) for that trace of the line, as well as the intensity of that pixel in each channel.

Click the left mouse button on the displayed data to display a vertical yellow cursor. A yellow box will appear on the Image window showing the position of this pixel on the reference image. These yellow markers can be used to correlate locations along the defined line scan with intensity information from the acquisition. The user can reposition the yellow markers by clicking elsewhere in the data display or dragging the yellow box in the Image window.

The position and width of any defined profiles are displayed on the image(s) as colored rectangles corresponding to the color used in that profile's definition and trace. To move a profile to a new location for the next acquisition, click and drag the rectangle in the data display at the bottom of the Linescan Control window. The rectangle marking the profile definition will move to the new location defined by the user. Dashed lines in the same color will mark the original position of the profile, corresponding to the trace still visible in the Profiles section of the window.

Long acquisitions are broken into multiple files during acquisition and reconstructed at the end of the scan. In these cases, only the last portion of the acquisition will be displayed in the Linescan Control window. To see the entire data set in the Image window, open the file in Playback Mode. This will happen automatically after any acquisition where the **Open in playback mode** option is checked; otherwise, load the data set from the File menu.

Playback Mode

After a multi-frame acquisition completes or a set of images is loaded from the file menu, Prairie View enters Playback mode indicated by the playback controls visible below the first image window.



An option in the [Preferences menu](#) allows the user to decide whether Playback will automatically open after an acquisition. In order for the images to display, the raw acquisition data must have been converted to image files. This can be done automatically after acquisition via an option in the [Preferences menu](#), or manually via the [Image-Block Ripping Utility](#) in the Tools menu. If the images files have not been created when Playback opens, a message box will give the user the option to convert the files at that time.

When in Playback mode, many controls associated with scanning become disabled until the playback mode is disabled by clicking **Exit**.

Playback Controls

Depending on the image sequence selected, one or both of two scroll bars may be available: one vertical for Z stack image data and one horizontal for time lapse image data.

Each image sequence represents a cycle of a T-Series, a simple Z-Series, or a single image. It is possible to navigate sequences by selecting one from the drop-down menu to the right of the horizontal scroll bar, or by using the arrow buttons next to the drop-down menu.

If the number of frames is the same for all sequences in the open data set, then both scrolls bars become active. The vertical scroll bar controls the selected frame while the horizontal scroll bar controls the selected sequence.

Arrows at the ends of the scroll bar allow the user to navigate in single-frame increments.

Next to each scroll bar is a set of five navigation buttons. The double-arrow buttons jump to the first or last frame/sequence. Single-arrow buttons play through the images in the indicated direction. The square button stops any playback currently in progress.

The **Loop** checkbox will continue playback in the reverse direction when the last/first image is reached. The **Wrap** checkbox will jump from the first to the last image or vice versa rather than reversing direction when the last/first frame is reached.

When playing through a set of images, the speed at which the images change is determined by the **Delay** text box (in milliseconds). Enter a new number in this box to change the speed of

playback.

When in Playback mode, information about the absolute and relative time of each frame's acquisition is displayed in the title bar of the Image window.

A text box in the lower right portion of the Playback window displays the file path for the acquisition. The user can open the directory containing the acquisition by clicking the  button on the right side of the window.

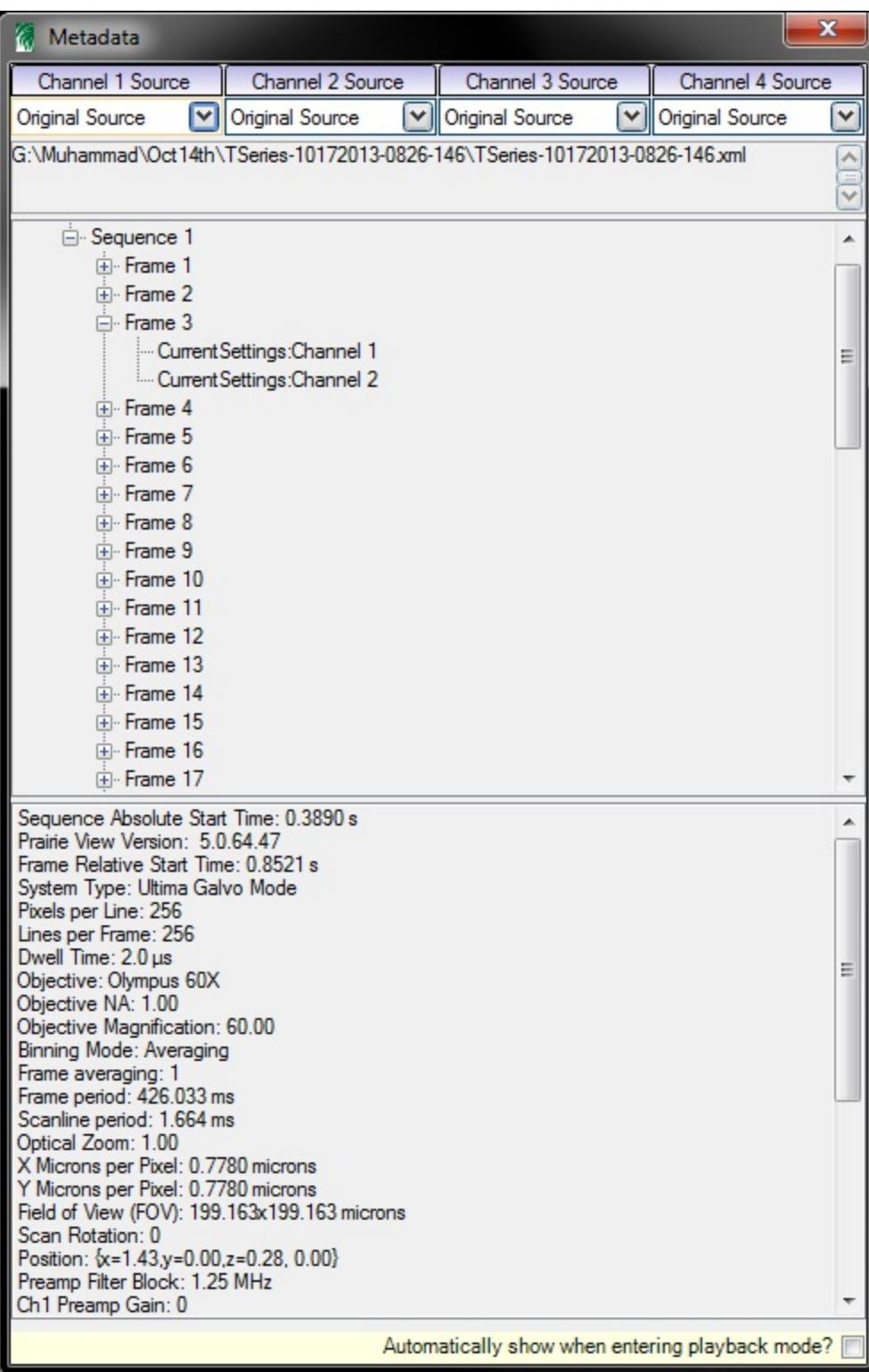
The user may choose a previously defined After Scan Complete/Playback action from the **Action** drop-down menu and execute it by clicking the **Perform** button. If the **Perform Action** box is checked, the action displayed in the drop-down menu will be executed every time the image in the Image window changes; that is, when changing slices in a Z-Series playback or changing time points or sequences in a T-Series playback. Actions are described [here](#).

The text box in the lower right corner of the playback controls will display an error message if the current image sequence is corrupt due to missing samples at the time of the acquisition. A red warning icon  will also appear in the lower right corner of the window. Due to hardware constraints while acquiring multiple channels with heavy processing, it is not always possible to keep up with the data throughput. Saving any heavy processing until after the data is acquired is a good practice in such cases.

While in Playback mode for a Z-Series or T-Series image sequence, the user can display projections of the images. This is discussed in the [Maximum Intensity Projection](#) section of this manual.

Metadata Window

The Metadata window displays a tree view of information about system settings such as laser power, motor positions, and scan timing for each frame. The Metadata window can be brought up at any time in playback mode by clicking **Info**. A check box in the lower right corner of the Metadata window allows the user to specify that the Metadata window open automatically each time Playback is open.



Channel Mapping in Playback Mode

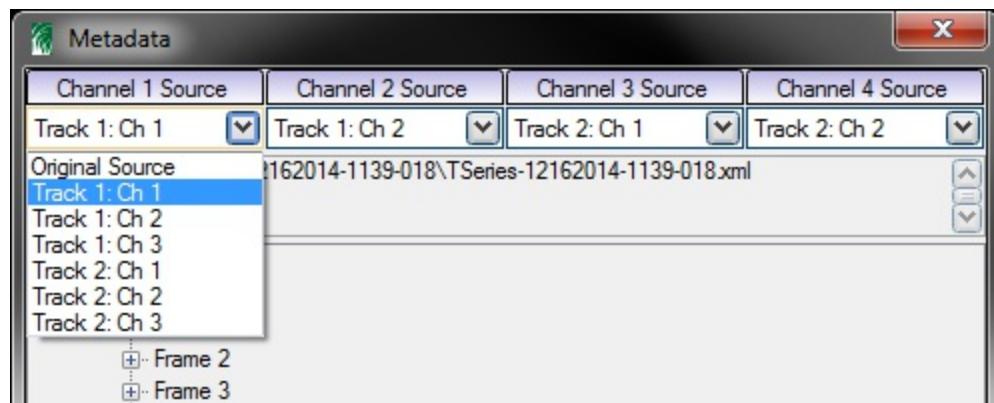
The Metadata window provides controls for mapping data associated with a certain acquisition channel, and an optional Parameter Set track, to a specific display channel. The playback mapping is important when looking at an image set that was acquired using multi-track Parameter Sets; the channels active when acquiring images within a specific track can be mapped to different display channels during playback.

The default choice for channel mapping is "Original Source", which is the most intuitive choice

and maps each channel of data to the channel in which it was acquired. However, in some cases it can be useful to change this mapping when viewing the data.

Consider an example where a T-Series has been acquired using a Parameter Set with two tracks (see further discussion in the [Parameter Sets Tab](#) section of this manual). Three physical channels of data were acquired for each of the two tracks, for a total of six logical channels of data to display. The user may wish to map these four of these logical channels (Track 1: Ch 1, Track 1: Ch 2, Track 2: Ch 1, and Track 2: Ch 2) to four different display channels to see them during playback.

While in Playback Mode for this T-Series, click the **Info** button on the right side of the Playback window. This will open the Metadata window. Across the top of this window are four drop-down menus which correspond to display channels 1 through 4. In this example, the data acquired on channel 1 with track 1, called “Track 1: Ch 1”, is mapped to appear in display channel 1 for playback.



Sometimes for a given sequence of frames there is no data for certain track/channel combinations so the image for that channel will be black.