

Preface

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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".

Computer

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:\Documents and Settings\All Users\Application Data\Prairie Technologies, Inc\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.

Multi-photon Imaging

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 Prairie Technologies 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; these are described in other parts of the Prairie View manual
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 of the Prairie View manual.

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 box next to **Single scan auto. save** if you want every Single Scan to be saved; this prevents loss of data but fills disk space quickly
 - d. Check the box next to **Live scan auto. save** 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 "Misc" tab. 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.

Load

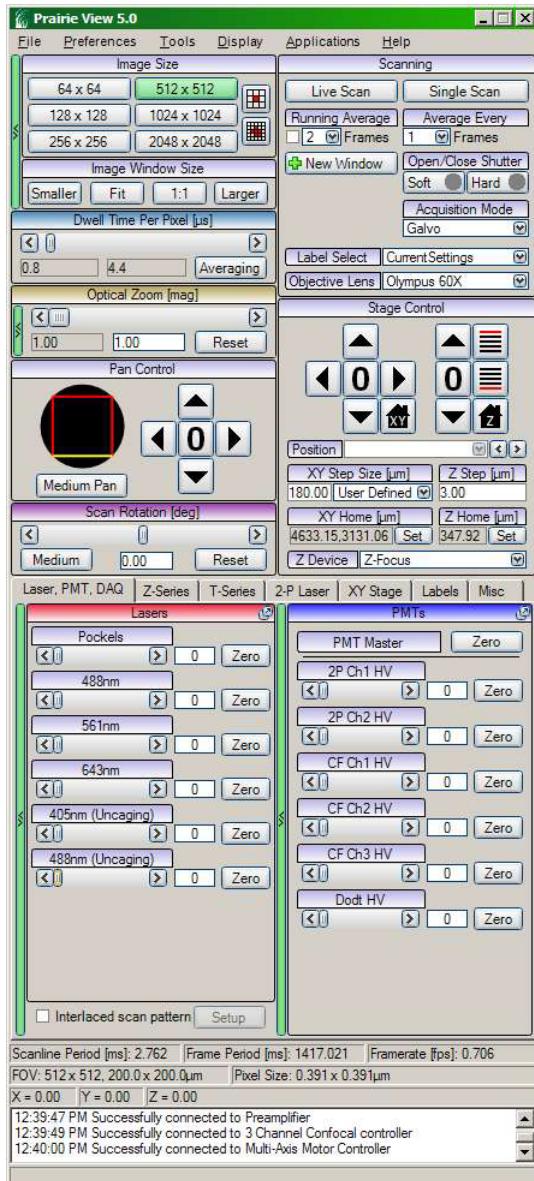
1. Go to File > Load Image(s)
2. Navigate to the directory and folder for the image you want
3. Open the XML file for the image

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.

Menus contain many options and features

Scan and stage controls are always visible



Tabs contain many controls for specific features

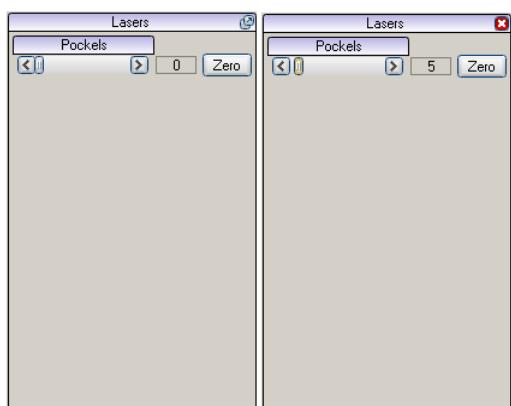
Current scan parameters and motor positions

Status messages about hardware and acquisitions

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.

Image Size

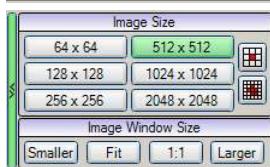
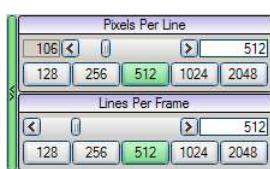


Image size is a definition of how collected data is acquired and displayed. In the case of a 512 x 512 acquisition, data is acquired at 512 bins in x by 512 bins in y, with each bin sampled according to the user-specified dwell time.

The information section at the bottom of the Main Control Window lists the image size in pixels and microns, based on the currently selected Image Size and Objective Lens.

Grid buttons to the right of the pre-defined options will increase or decrease the image size (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 size buttons while inside a Region Of Interest (ROI) will cause the software to exit the ROI and return to the full, square scan.



Clicking the green bar to the left of the Image Size 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, refer to the information section at the bottom of the Main Control Window for the pixel size. 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.

Image Window Size



There are four options for Image Window Size:

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

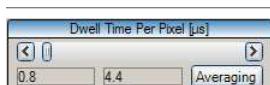
1:1 shows the image at its actual size as defined by the number of pixels in the current Image Size.

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

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.

Dwell Time Per Pixel



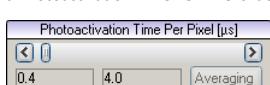
This slider allows the user to control the time that the imaging laser beam is scanning over each pixel location in the image. The minimum value allowed is displayed below and to the left of the slider. Changing pixels per line, optical zoom, and scan rotation may change the minimum allowed dwell time. 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. The button on the right side allows the user to choose whether the recorded intensity value is obtained by **Averaging** or **Summing** those 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. 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.

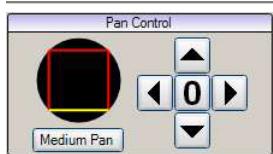
It is not possible to set the optical zoom to less than 1.

When scanning in Resonant mode, only the pre-set optical zoom buttons are available. Additionally, due to differences between traditional and resonant galvanometers, the lowest optical zoom available in Resonant mode may larger than 1x; in these cases, the 1x button will be replaced with the minimum value for that system, such as 1.1x or 1.2x.



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

Pan Control



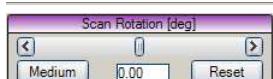
When working at an Optical Zoom of greater than one, Pan Control allows the user to change the position of the scanned area on the sample. On the left is a graphic that shows the relative position and orientation of the scanned area. A red and yellow box indicates the scanned area; the yellow side is 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 **0** 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 $+180^\circ$. 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 **Live scan auto. save** is checked in the **Misc tab**, a disk/save icon appears inside the **Live Scan** button and 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 (camera icon) 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.

Single Scan

When **Single Scan** is selected, 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 **Single scan auto. save** is checked in the [Misc tab](#), a disk/save icon appears inside the **Single Scan** button and every Single Scan is numbered and saved. If this option is not checked, the frame is stored only temporarily unless the **Snap** button (camera icon) 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.

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](#).

New Window

Selecting **New Window** will cause another image window to appear on the desktop. It is possible to have up to 8 image windows open at one time. The operator can, for example, display different combinations of channels in separate image windows.

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 multiphoton 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. The parameter is set in the Scan Settings dialog. 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. Contact Prairie Technologies personnel for assistance with this setting.

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.

When scanning in Resonant mode, only the pre-set optical zoom buttons are available. Additionally, due to differences between traditional and resonant galvanometers, the lowest optical zoom available in Resonant mode may larger than 1x; in these cases, the 1x button will be replaced with the minimum value for that system, such as 1.1x or 1.2x.

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, 0.1μs 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 will 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 $0.1\mu s$; lower values can help reduce photo-bleaching. Each $0.1\mu s$ 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.

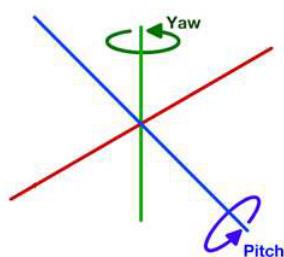
Label Selection

The **Label Select** drop-down menu allows the user to select previously defined and saved settings. These settings include dwell time, laser power, PMT voltages, etc. If any label other than "Current Settings" is selected in this menu, then changes to the scan settings will be automatically written to the selected label. Labels are discussed further in the Labels Tab section of this manual.

Objective Lens

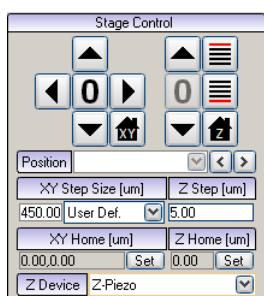
The **Objective Lens** drop-down menu allows the user to select a previously calibrated objective setting for an objective lens used on the system. Proper objective selection is important as it is used in determining pixel size. Failure to select or use a properly calibrated objective will result in invalid measurements. 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.

On systems configured with an Orbital Nosepiece to adjust the angle of the objective, an objective can be configured for use with the Orbital Nosepiece. This is done by checking the box in the "Orbital?" column in the Objective Calibration dialog. When an orbital-enabled objective is selected in the Objective Lens drop-down list, fields appear in the Scanning section for the user to enter the Yaw and Pitch angles of the Orbital Nosepiece. Entering these values allows the software Stage Control buttons to perform coarse movements accounting for all three axes of movement (x, y, z). Leave the software values set to 0 to retain independent control of the three axes, as one would have for controlling a micromanipulator.



Stage Control

Stage Control allows the user to move the stage in the x and y direction and the objective in the z direction. It also allows the user to recall saved locations, which can be modified on the XY-Stage Tab described later in this manual.



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 drop-down menu. Field of view measurements are based on the currently selected objective lens calibration.

Use the home feature to store an XY location to come back to at a later time. Selecting **Set** stores the current XY location as Home and pressing the button will return to that position.

0 Select **0** 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.

The **Position** drop-down menu allows the user to select from a list of previously saved stage positions. Storing and using these positions is described in the XY-Stage Tab section of this manual.

The z-motor 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.

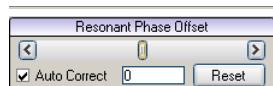
Use the home feature to store a Z location to come back to at a later time. Selecting **Set** stores the current Z location as home and pressing **1** will return to that position.

0 Select **0** to leave the motor 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.

If there is more than one z device available on the system, such as a high-precision piezo controller, the **Z Device** drop-down menu will be active to select which device will be moved by the software control buttons. More information about using multiple z devices can be found in the Z-Series Tab section of this manual.

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.

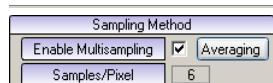
Resonant Phase Offset



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 un-check 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

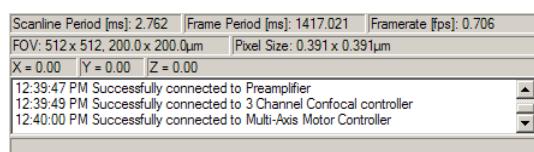


Sampling Method control is available only in Resonant acquisition mode. When the **Enable Multisampling** option is checked, the system collects as many samples as possible at each pixel. This number is indicated in the **Samples/Pixel** field and is based on the size and number of pixels in the image. 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.

Scan Information

At the bottom of the Prairie View Main Window, status bars to show basic information about the scan being performed.



The first line displays the Scanline Period, Frame Period, and Framerate.

- The scanline period is the number of milliseconds required to scan across one line in the frame. This includes acquisition time and retrace time for the galvanometers. To calculate the retrace time, use the following formula:
Retrace time = scanline period - (dwell time * number of pixels per line)
- The frame period is the number of milliseconds it takes to create one frame. This could also be arrived at by multiplying the scanline period by the number of lines per frame. When acquiring frames using a Label Group, the frame period will change as the individual labels are applied.
- The frame rate is the number of frames scanned per second.

The second line displays the Field of View (in pixels and microns) and Pixel Size. The field of view measurement and pixel size in microns are dependent upon the calibration of the objective lens selected.

The third line displays the X, Y, and Z motor positions.

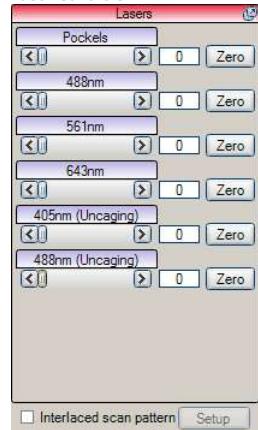
The large window is used to display informational messages at start up and update status during an acquisition.

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.

Laser, PMT, DAQ 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



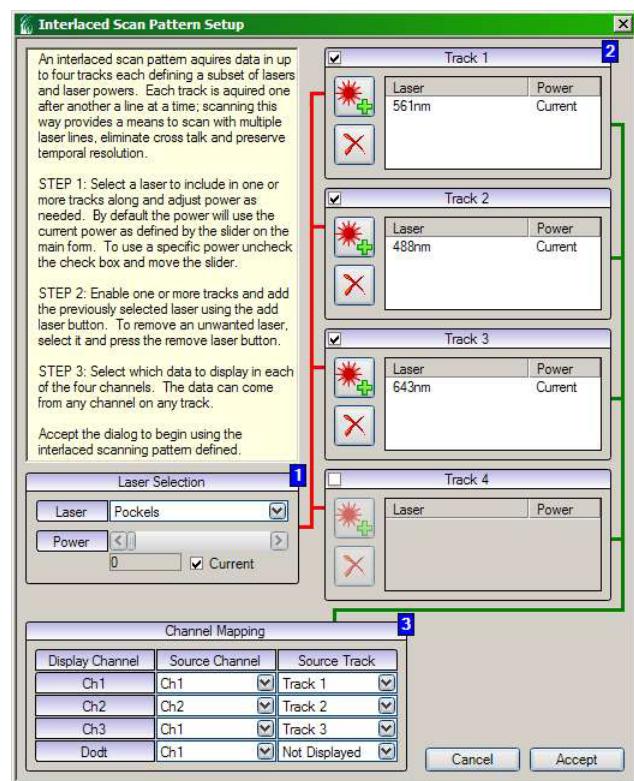
This section allows the user to control the laser power for all lasers configured in the system. Typically, lasers on the imaging light path are listed first, followed by lasers on the uncaging light path. For each laser, the user can drag the slider to change laser power, or enter the desired setting in the field to the right of the slider. Clicking the **Zero** button will return laser slider to 0. 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 labels 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.

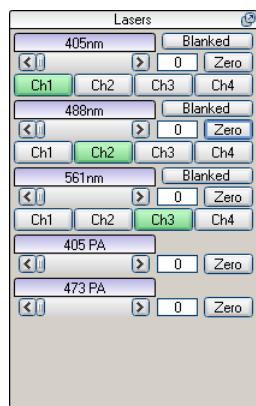
Laser Selection specifies one of the lasers currently configured on the system as well as a power setting. The laser and power here can be added directly to an active track. By default the **Current** power check box is checked which indicates that the laser slider power setting should be used even as 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.

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.

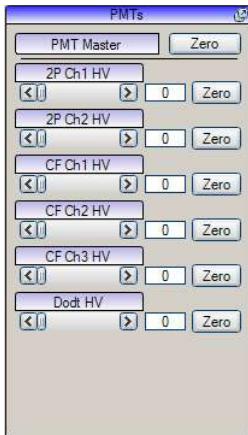
For a channel to be acquired, it must also be activated in an Image window. To do this, click one or more of the channel buttons on the left side of the Image window. In the example below, channel 1 is active in Image window 1, and is pseudocolored green. The pseudocolor is chosen by clicking the narrow 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 Labels (see further discussion in the Labels 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.

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** at the end of a PMT slider sets that PMT to zero.

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.

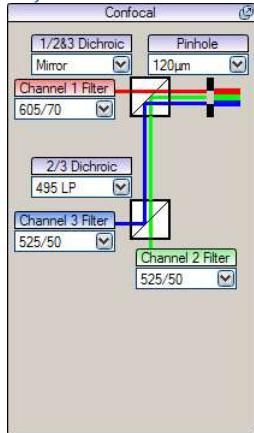
When used together with the PMT voltage and laser power settings, the controls in this section allow the user to maximize the range of data collection for the sample.



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 (Volts)
0.2	0 – 50
0.5	0 – 20
1	0 – 10
2	0 – 5
5	0 – 2
10	0 – 1.0
20	0 – 0.5
50	0 – 0.2

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 it 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.

Confocal Controls

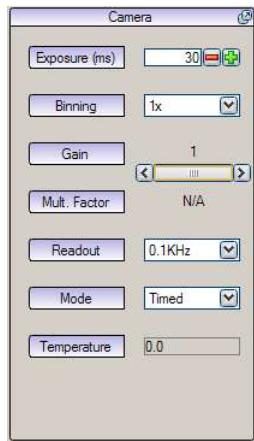
For systems configured with a 3-channel descanned confocal module, this section allows the user to select the dichroic mirrors, band pass filters, and confocal pinhole in the emission/detection light path. The controls are located behind the Laser control section of the Laser, PMT, DAQ tab; click the green bar on the right side of the Laser control section to reveal Confocal controls. Filter positions and names are configured by Prairie Technologies 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 120µm 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

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.

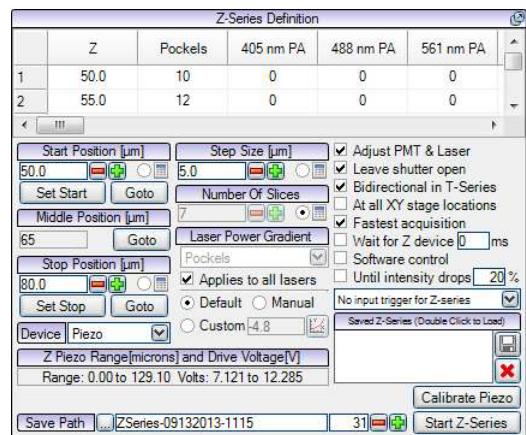
Many cameras supported by Prairie View have two types of gain control, which amplify signal after it is acquired by the camera. Options in the first **Gain** slider or menu determine the way signal is digitized – they describe the number of photoelectrons that make up one unit of digital signal. The 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.

Z-Series Tab

Use the Z-Series controls to define, save, and recall Z-Series definitions, and to acquire image stacks. The top 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 **Goto** buttons to move the motor to the start, middle, or stop position.

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 in the Z-Series tab, or by clicking the button 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 in the Z-Series tab, or by clicking the button 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 0.1μm
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 corresponding **Goto** button 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 1000 slices. If the user attempts to define a stack of more than 1000 slices, the Step Size and Number of Slices will be adjusted to cover the range between the Start and Stop location without exceeding 1000 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, Misc, 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 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 and PMT Power Gradient

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. When the **Adjust PMT & Laser** checkbox is checked, the laser power slider setting and PMT voltages are recorded along with the Z device position when the start and stop positions of a Z-Series are set. These settings are displayed in the table at the top of the Z-Series tab.

PMT voltages between the Start and Stop position are incremented in a linear fashion.

The Laser Power Gradient control affects the way imaging laser power is controlled throughout a Z-Series. When **Adjust PMT & Laser** is checked, the Laser power Gradient control is active. When more than one imaging laser is present, the user can choose the laser(s) to which gradients are applied via a pull down menu and checkbox. The settings are retained for each Z-Series definition and each saved Z-Series can have a different gradient setting. There is also a check-box to allow the settings to be applied to all imaging lasers.

The user can choose the equation applied to the laser power by selecting Default, Custom, or Manual control. When using a Manual gradient, the user can type laser power values into the table at the top of the Z-Series tab. The Default laser power gradient is a slight parabola defined by Prairie View. Custom allows the user to enter a gradient line for the acquisition. The user can enter a number in the field to the right of Custom or click the graph icon button.

If the graph is selected, a dialog will appear for viewing the Custom gradient versus the Default. There may be additional lines for other imaging lasers. The x-axis (horizontal) represents the Z position of the current Z-Series, and the y-axis (vertical) indicates the laser power setting as a function of the Z position. The Default curve shows the relationship of laser power to Z position if the user selects the Default laser power gradient option (radio button). The second curve shows the relationship of laser power to Z position as the **Adjust Gradient** control is adjusted. As the **Adjust Gradient** slider is adjusted, the graph will automatically update with the new relationship between the laser power and Z position. In addition, the operator may type in a **Gradient** value in the text box located below the **Adjust Gradient** slider. If more than one imaging laser is present, then the operator may click on the **Show All Laser** checkbox to display the graphs of all laser powers as a function of Z position for the current Z-Series definition. If the user clicks the **Cancel** button, the dialog will be dismissed and the gradient setting will revert to the value when the dialog was first opened. If the user hits the **Accept** button, then the new gradient setting will be applied to the selected laser line(s).

Note that keeping the **Adjust PMT & Laser** box checked will implement the laser and PMT power gradients defined when setting the Start and Stop positions, even when the user does not change those values between slices. Thus, having the box checked will over-ride laser and PMT powers changed in the Laser, PMT, DAQ tab, as well as laser powers defined for Photoactivation Masks (described later in this manual).

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 can not 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 Prairie 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.

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 Prairie Technologies 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 Prairie Technologies 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 **No Input Trigger for Z-Series** is selected, then the Z-Series will start immediately when the Start Z-Series button is pressed.

If **Start Z-Series with Input Trigger** is selected, 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 **Use Input Trigger for each Image** is selected, 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.

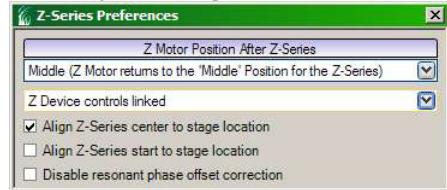
These controls only affect Z-Series executed as stand-alone experiments. They do not affect a Z-Series executed as a cycle in a T-Series.

Triggers sent to the Prairie 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 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.

Z-Series Preferences dialog



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** drop-down menu 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.

A second drop-down menu 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 of this manual.

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.

T-Series Tab

The T-Series tab gives users the ability to generate a series of images and data using different labels, 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 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.

The **Remove Selected** button deletes the currently selected cycle (row) of the T-Series table. **Clear All** removes all 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.

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. The T-Series Preferences dialog (Preferences > T-Series) contains an option to **Update display during max speed acquisitions**; un-checking the box for this option will allow the user to decrease the amount of computer resources used to update the display.
- **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, Label, 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 Label 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 Label settings are used. When performing multiple repetitions of a Z-Series in one cycle, to 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](#).

- **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 Label 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 Label settings are used. More information about [BOT](#) features is provided elsewhere in this manual.
- **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.
- **Label** is a modifier cycle that will apply to all subsequent lines in the T-Series. Select the previously-defined Label from the Resource Selection drop-down list; the settings of that Label will be applied. No scans are performed in this cycle; follow it with an Image Sequence or other cycle to acquire images. See more information discussed with [Labels 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 Labels (see further description of [ROIs](#) and [Labels](#) in other sections of this manual). If multiple ROIs and Labels 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 Label. Also, frame averaging defined in the main form Average Every ___ 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-written 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 > Output Trigger Type, select either "Start of Frame Trigger" or "End of Frame Trigger". In order for the **Triggers Before Starting** feature to be used, the Trigger Mode must be set to "Generate Start of Frame OR End of Frame Trigger OR Neither", and one of the two frame triggers must be selected. 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="▼"/> Current	<input type="checkbox"/> Current <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="button" value="▼"/> Current	<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="button" value="▼"/>	<input type="checkbox"/> No Trigger <input type="button" value="▼"/>	0	None	<input type="button" value="▼"/> None	<input type="checkbox"/> None <input type="button" value="▼"/>
V Rec	---	---	---	4	Current	<input type="button" value="▼"/> None	<input type="button" value="▼"/>	---	---	---	<input type="button" value="▼"/> None	<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, Misc, 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 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 Prairie 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 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:

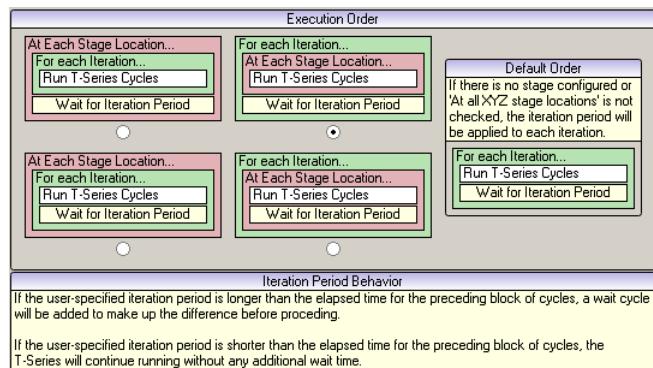
1. Click **Image Sequence**. A row with default settings will be created.
2. To select the number of images or acquire, click on the **# Reps** field, type "10", and press <Enter>.
3. Click on the **Period** field and type "30", the total time for the system to wait between start of one repetition and 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.

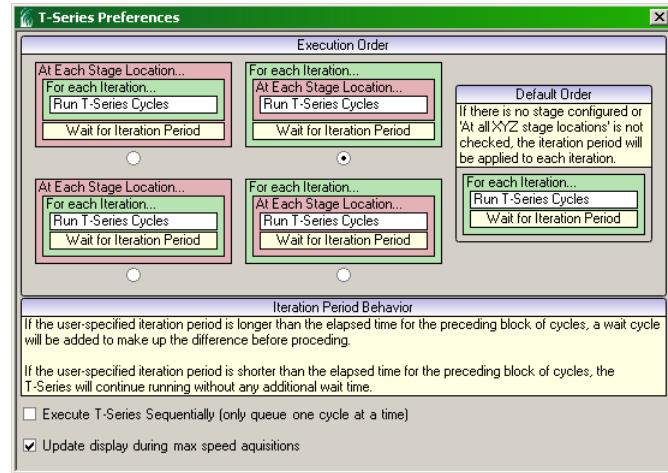


Consider as an example a T-Series which contains 5 cycles totaling 3 minutes, 2 iterations, the **Run at all XYZ stage locations** box is checked, 4 stage locations are saved, and the Period is set to 3600 seconds. The option selected in the screen shot above is "For each Iteration, At Each Stage Location, 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.

T-Series Preferences

The T-Series Preferences dialog (Preferences > T-Series) contains options that affect the display update and file saving during acquisitions.



The top portion of the T-Series Preferences window contains options for the T-Series Execution Order, described elsewhere in this manual.

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 **Update display during max speed acquisitions** option determines whether the Image window is updated during an imaging sequence with the Max Speed option is checked. It can be useful to disable the update on older computers to save resources for acquisition, but the option can safely be left enabled on most systems.

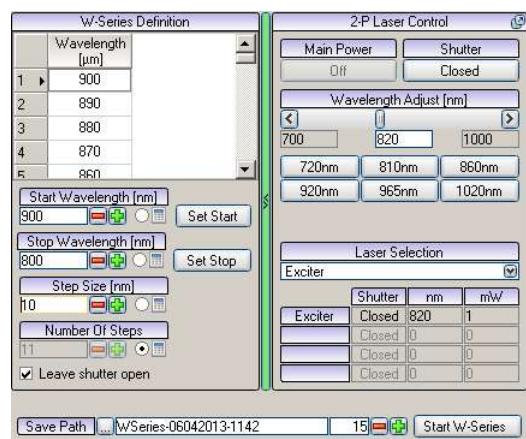
Additional selections in the Preferences menu pertain to T-Series acquisitions.

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.

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-fast 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, Misc, 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 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 Prairie Technologies personnel before making any changes in the Prairie Configuration Utility.

The **Main 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 On/Off** 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.

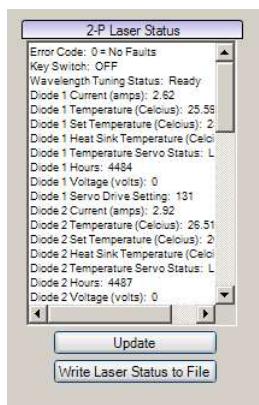
Change the laser wavelength by clicking one of the pre-set wavelength buttons or typing in the text box below the **Wavelength Adjust** slider. The text field to the left of the slider displays the minimum allowed wavelength for the laser, while the text field to the right of the slider displays the maximum allowed wavelength for the laser. These limits are properties of the laser and are defined in the Prairie Configuration Utility; contact Prairie Technologies 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.



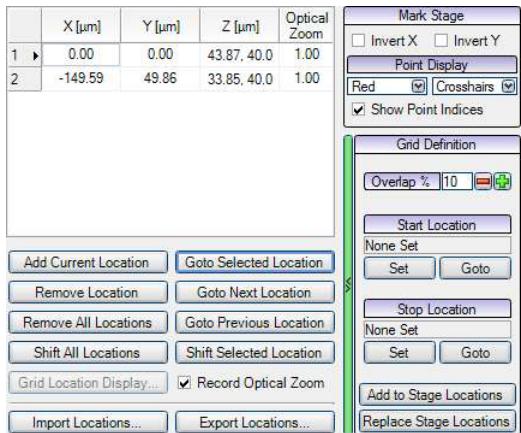
This section displays a text readout of the commands and information received from the laser itself. It is useful for diagnosing errors and checking the status. The **Update** button will query the laser for the most recent information.

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

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.



Saved positions appear in the list in the upper portion of 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 Location 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.

Remove Location erases the currently selected location from the table.

Remove All Locations clears the entire table.

The **Goto Selected/Next/Previous Location** buttons move the stage to the selected, next, or previous location in the saved locations list, respectively.

Shift Selected Location 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 Locations 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.

The **Record 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.

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 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.

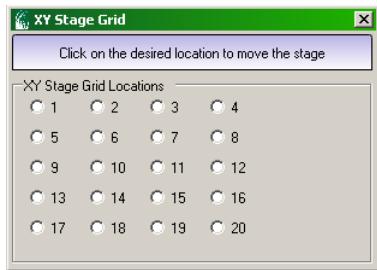


Grid Definition allows the user to define an array of stage positions by setting diagonal corners of the array and defining the overlap of neighboring images. These positions can be added to an existing list of stage locations or used to replace the current list of locations. The list of locations can then be used to acquire an array of overlapping images to be stitched together later into larger mosaic images; this is accomplished using a variety of other software programs.

1. Set the position of one corner by driving to that location and clicking the Start Position Set button. The current location will be displayed.
2. Set the position of the diagonally opposite corner by driving to that location and clicking the Stop Position Set button. The current location will be displayed.
3. Define the amount of overlap of adjacent images by typing in the Overlap text box or using the + and – buttons. The required amount of overlap may vary based on the software package used for stitching the images after acquisition.
4. 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 grid of locations will be used to collect a 3-dimensional montage, it is suggested that the user set the start and stop positions of the grid using the same Z position. A Z-Series can be defined based on the thickest part of the sample, and the Z-Series can be executed at all saved stage locations from the Z-Series or T-Series tab. When setting stage locations, keep in mind the Z-Series preference to align the saved stage locations to the start or the center of the Z-Series definition. For example, if stage locations are defined in the center focal plane of the sample, set the Z-Series preference to align the saved locations to the center of the Z-Series definition. Z-Series preferences are described elsewhere in this manual.

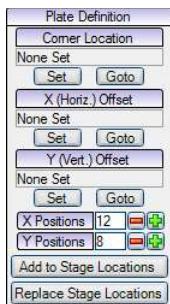
After a grid has been defined, the **Grid Location Display...** button becomes active. When clicked, this button opens a dialog to allow the user to navigate to positions in the defined grid.



Note that the locations and movements calculated by the Grid Definition depend on the currently selected objective and the accuracy of its calibration. If the currently selected objective lens is not calibrated, the **Add to Stage Locations** and **Replace Stage Locations** buttons will be disabled. Also, regardless of the state of the **Record Optical Zoom** checkbox, the Optical Zoom will be recorded when the **Add to Stage Locations** or **Replace Stage Locations** button in the Grid Definition controls is pressed. This is necessary because changing the optical zoom after defining the grid locations would invalidate the grid definition with regards to image overlap.

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.



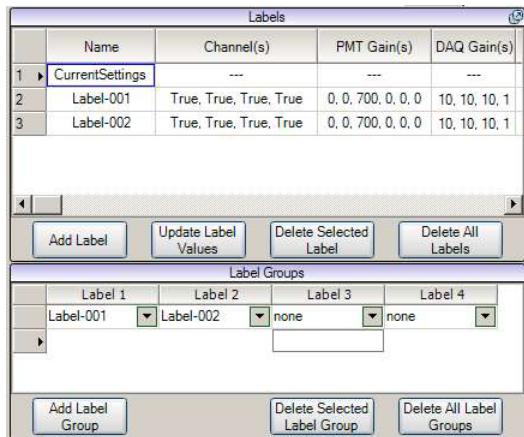
Set the position of one corner well by driving to that location and clicking the Corner Location Set button. The current location will be displayed.

- 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.
- Move back to the Corner Location by clicking the Corner Location **Goto** button.
- 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.
- 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.
- 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.

Labels Tab

The Labels tab gives the user the flexibility to define and save a set of operational parameters for imaging control including laser power, PMT settings, channels, and pixel dwell time; available parameters vary based on system configuration and acquisition mode. Each Label is essentially a snapshot of the acquisition parameters, which the user can recall at a later time. It is also possible to set up Label Groups for more complicated or multi-laser applications.



The values for each label created are displayed in the Labels chart. Each label can have a customized **Name** for easy identification. The status of each Channel in a label is shown as True for on or False for off. Additional columns display PMT or camera settings, emission filters, laser slider settings, etc.

Buttons below the Labels chart allow the user to add, update, and delete labels.

Creating a Label

1. Adjust all channel, PMT/camera, laser, and other settings to those intended to be saved in the label definition
2. Press **Add Label**; a row named "Label-001" with the current settings will be created
3. To change the name of the label, double-click in the "Label-001" box and type the desired name

Using a Label

To apply the settings of a label, select it from the **Label Select** drop-down menu in the Scanning section of the Prairie View main control window. Any subsequent changes to scan settings will be automatically applied to the label selected in this menu. The preferred method for loading a label without saving subsequent changes is to select the desired label from the **Label Select** drop-down menu and then immediately select "CurrentSettings" from the same menu.

To use a label during a T-Series, use the **Label** button in the T-Series tab to add a Label cycle to the T-Series. Select the desired label from the drop-down menu in the Resource Selection column. The chosen label will be used for all subsequent cycles, until a new Label cycle is defined.

If the user wishes to change only one or a small number of parameters during a T-Series, a Script may be a more efficient way to make the change. More information about [Scripts](#) is available elsewhere in this manual.

Updating or Changing a Label

Any changes to settings will be automatically applied to the label displayed in the **Label Select** drop-down menu in the Scanning section of the Prairie View main control window. Thus, one way to make changes to an existing label is to select it from that menu and then make the desired changes to settings.

To change the settings of a current label without selecting it from the drop-down menu, do the following:

1. Adjust the setting in the appropriate place (for example, an increase in laser power would be done in the Laser,PMT,DAQ tab)
2. Click on the label to be updated in the Labels chart, so that the small black arrow appears to the left of the name of that label
3. Click the **Update Label Values** button

Label Groups

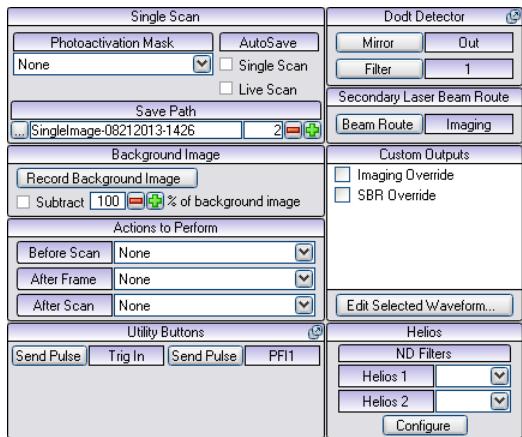
Label Groups allow the user to sequentially apply two or more labels during an acquisition. They are defined in the Label Groups table of the Labels tab.

To create a label group, click the **Add Label Group** button at the bottom of the Labels tab. Drop-down menus allow the user to assign existing labels to be labels 1 through 4 within the group. Additional buttons below this table allow the user to add and delete label groups.

Once created, a label group will appear in the **Label Select** drop-down menu of the main control window and will also be available when adding a Label cycle to a T-Series. When using a label group, each acquisition (Single Scan, Z-Series, T-Series repetition, etc.) will be performed sequentially with all the labels in the group. For example, in a Z-Series with a label group, the first slice will be acquired with all labels in the group, followed by the second slice for all labels in the group, etc., until the last slice is completed. A label group is not used in the following acquisitions: **Live Scan**, a T-Series in which the **Max Speed** option is enabled, a Z-Series in which the **Adjust PMT & Laser** option is enabled.

Misc Tab

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. This screen may contain fewer modules depending on the configuration of the system.



Single Scan

The **Photoactivation Mask** drop-down menu 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.

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, Misc, etc.) can cause data to be overwritten 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 in the Misc tab. When the Auto Save option for **Single Scan** is checked, a disk icon appears in the **Single Scan** button. 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, a disk icon appears in the **Live Scan** button and 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 tool button  on the Image window.

Background Image

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.

Dott Detector

The **Dott Detector** section controls the Dott gradient contrast detector, if one is configured. When the Dott mirror is **In**, the lamp house will automatically turn off to protect the Dott 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 Dott 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.

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 is part of a Label definition, allowing the operator to create labels 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 label, change the routing of the second laser, then use Mark Points, and so on).

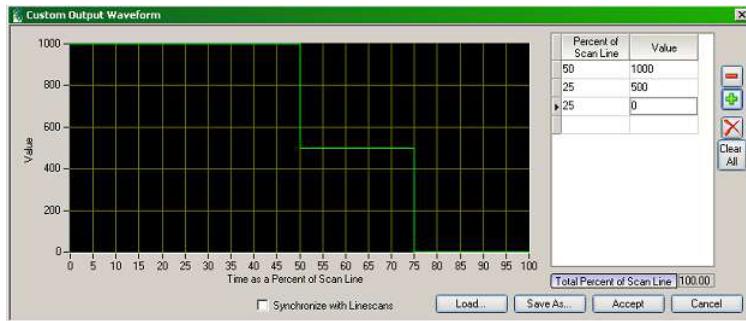
Custom Outputs

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.

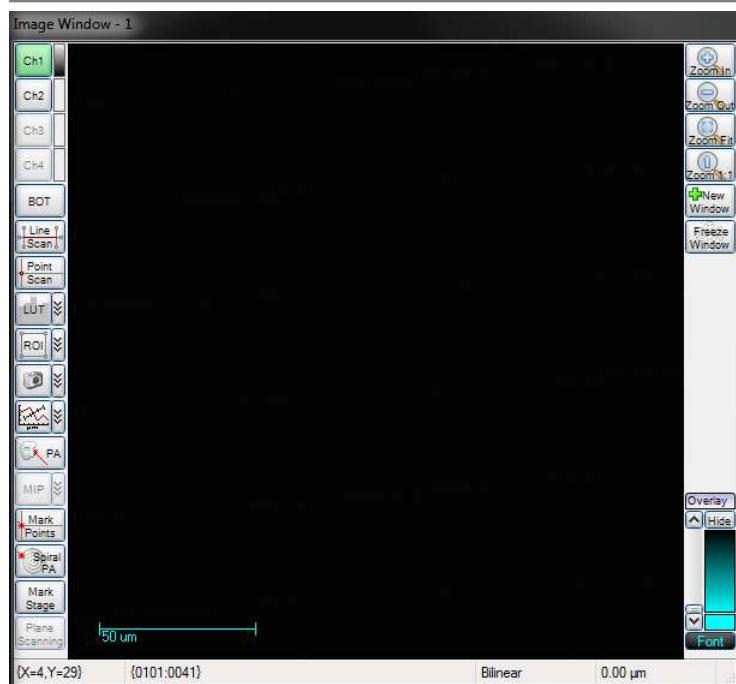
It is also possible to configure a Custom Output to produce a waveform based on a drive signal from a BOT (Brightness Over Time) region, to turn intensity data into an analog signal. This is option is set up in the Prairie Configuration Utility.

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.

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 side 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
- **LS (Line Scan):** Used for definition and acquisition of a Line Scan (2-dimensional)
- **PS (Point Scan):** Used for definition and acquisition of one or more Point Scans (1-dimensional)
- **LUT (Look Up Table):** Used to adjust the display intensity scale for an image
- **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
- **PA (PhotoActivation):** Used to define masks to limit laser exposure to certain areas
- **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 Points:** Opens a dialog to set up a [Mark Points](#) experiment
- **Spiral PA (Spiral PhotoActivation):** Opens a dialog to set up a Spiral PhotoActivation experiment
- **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

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** 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 descriptions of the image being shown. From left to right, these are:

- The pixel position of the cursor within the frame
- The intensity value (grey level) of the pixel in each of the four acquisition channels
- A description of the interpolation method used
- The length of the last line drawn with the Line Profile tool

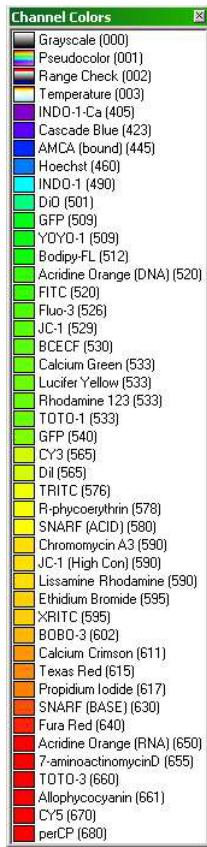
Input Channel Buttons

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,PM,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.



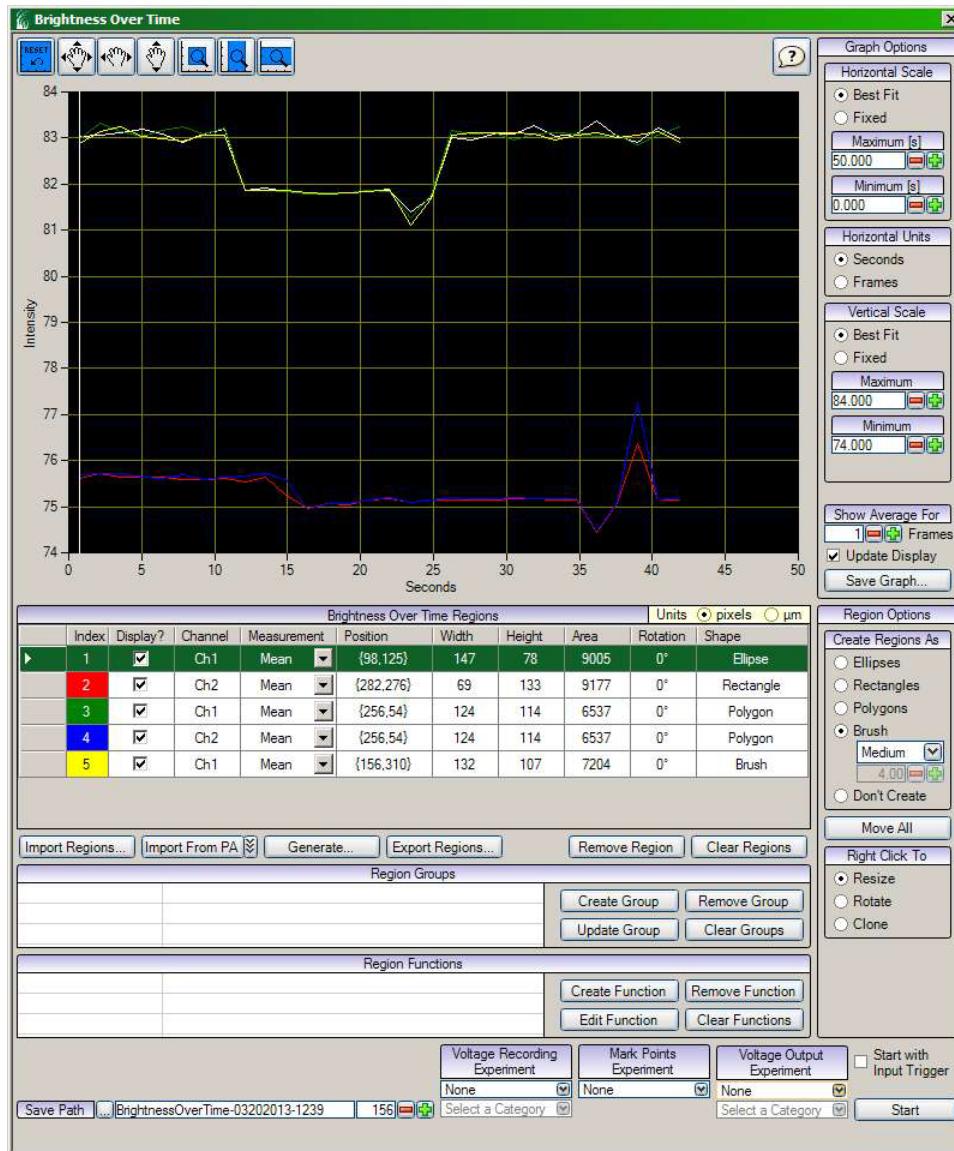
Channel Menu

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.



Graph Options

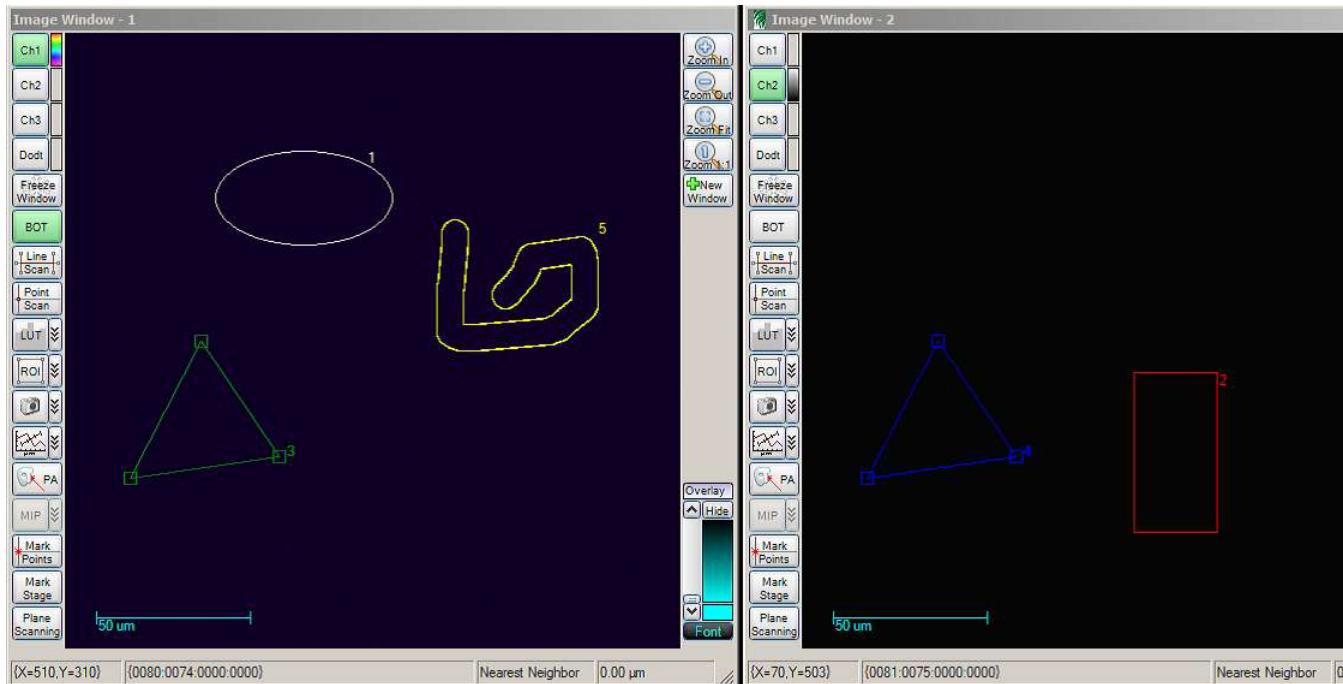
Along the right hand side of the graph window are controls for the graph display. There are separate controls for both the horizontal (x) and vertical (y) axes. Both axes can either be scaled as **Best Fit** (an automatic scale) or **Fixed**. **Best Fit** will adjust the visible values as necessary according to the data set, 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 overwritten 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 data set 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 set. **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 data set 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.

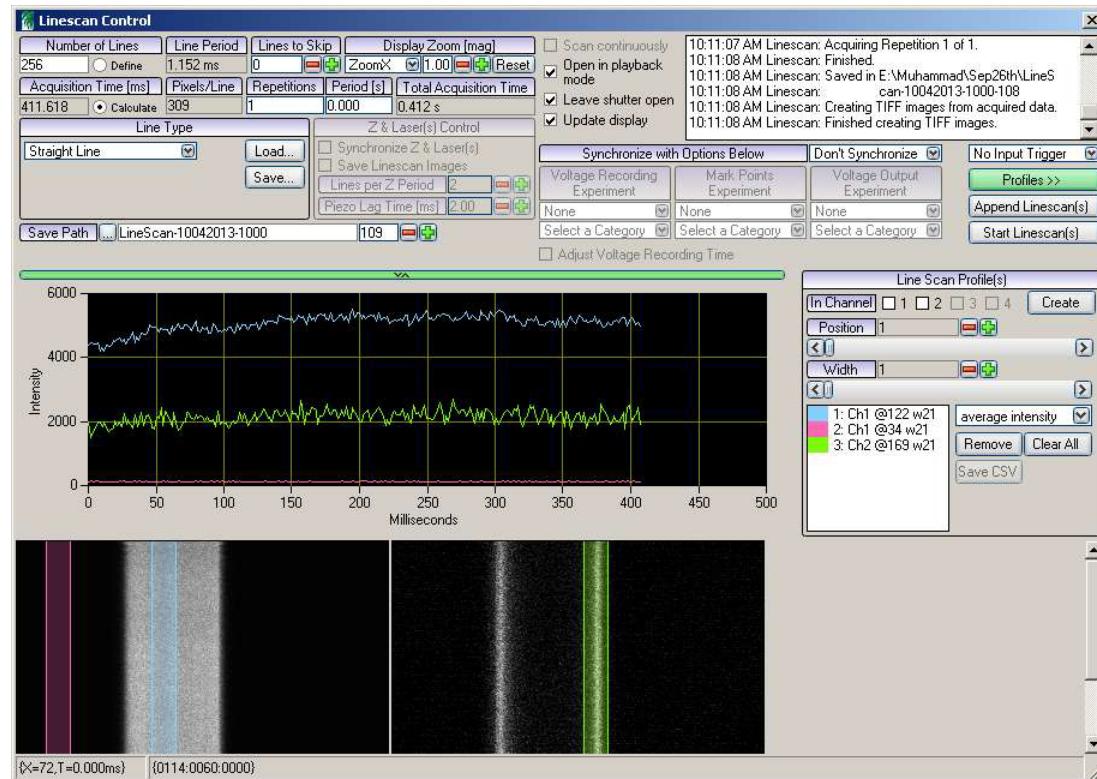
Files Created by the BOT Experiment

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.

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.

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 Spirial 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. 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.

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 overwritten 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 Prairie 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.

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.

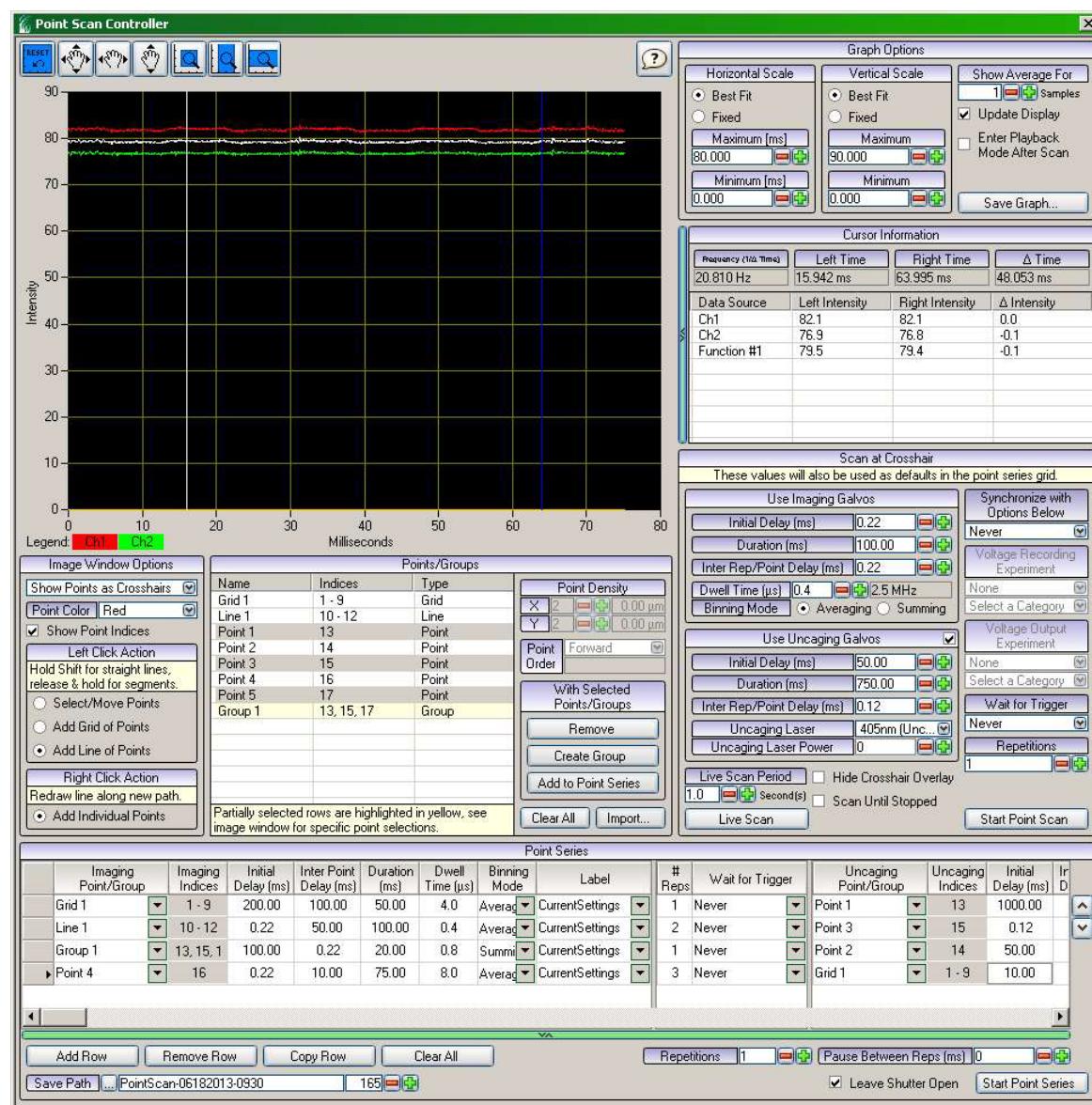
Click the left mouse button on the displayed data to display a vertical yellow cursor. A yellow box will appear on the Image window showing the position of this pixel on the reference image. These yellow markers can be used to correlate locations along the defined line scan with intensity information 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.



Simple Point Scan - Scan at Crosshairs

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:

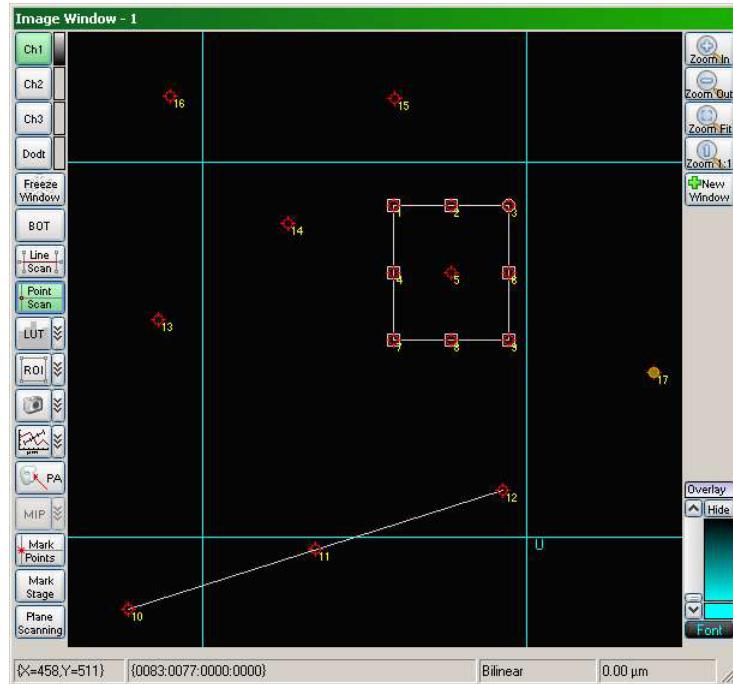
1. Ensure that the **Hide Crosshair Overlay** checkbox is not checked.

2. Position the Imaging crosshair (denoted with an I) 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 Rep/Point Delay** to the amount of time that should be elapsed between repetitions of the experiment.
7. In the same panel, set the **Dwell Time** to the time desired between recording samples, in us.
8. In the same panel, set the **Binning Mode** to either averaging or summing, depending on which behavior is desired. This controls whether the samples acquired from one pixel are averaged or summed throughout the dwell time.
9. 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.
10. 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.
11. In the same panel, set the **Duration** to the length of time for which the uncaging laser is powered, in ms.
12. In the same panel, set the **Inter Rep/Point Delay** to the amount of time that should be elapsed between repetitions of the experiment.
13. In the same panel, set the **Uncaging Laser** to the laser that will be used for uncaging.
14. In the same panel, 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 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.
16. 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.
17. Choose the number of times the experiment should run by changing the value in the **Repetitions** control.
18. Choose the **Save Path** using the control at the bottom left of the point scan window.
19. 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



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, 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, **Select/Move Points**, allows for points to be defined individually by right-clicking in the image at the desired position. 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, **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 header entitled, “Point Density,” which allows for a more densely defined grid. By changing the density in the X, Y, or both, directions, the resolution of the grid can be changed to include more or less 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 when the experiment is started to when intensity recording should begin.
3. Set the **Inter Point Delay** to the amount of time that should be elapsed between recordings at each point, if the row corresponds to a group of points.
4. Set the **Duration** to the length of time for which the intensity is to be recorded at each point, in ms.
5. Set the **Dwell Time** to the time desired between recording samples, in us.
6. Set the **Binning Mode** to either averaging or summing, depending on which behavior is desired. This control toggles between averaging and summing the samples acquired at one point throughout the dwell time.
7. Choose the **Label** which corresponds to the imaging settings desired for recording intensity at the point/group.
8. Set the **# Reps** to the number of times that the row should run before moving onto the next row.
9. If desired, set the **Wait for Trigger** option accordingly to wait for a stimulus prior to starting the row.
10. 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.
11. Set the desired **Initial Delay** in ms. This is the time to wait from when the experiment is started to when uncaging should begin.
12. In the same panel, set the **Inter Point Delay** to the amount of time that should be elapsed between uncaging of points, if a group of points is selected for uncaging.
13. Set the **Duration** to the length of time for which the uncaging laser is powered, in ms.
14. Set the **Uncaging Laser** to the laser that will be used for uncaging.
15. Set the **Uncaging Laser Power** to the desired value representing the power of the laser used for uncaging.
16. 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.
17. Repeat the above steps for every row in the table.
18. 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.
19. 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.
20. Choose the **Save Path** using the control at the bottom left of the point scan window.
21. 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 of the graph panel in the point scan window. Note that if a group is selected in the point scan series table, intensity from one point will be appended to the intensity recorded from the previous point, and not plotted separately. This is done to minimize the amount of time spent processing data, allowing for the highest sampling rate possible.

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 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 **? 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, **? Intensity**, shows the difference between the **Left Intensity** and the **Right Intensity**.

Data Evaluation

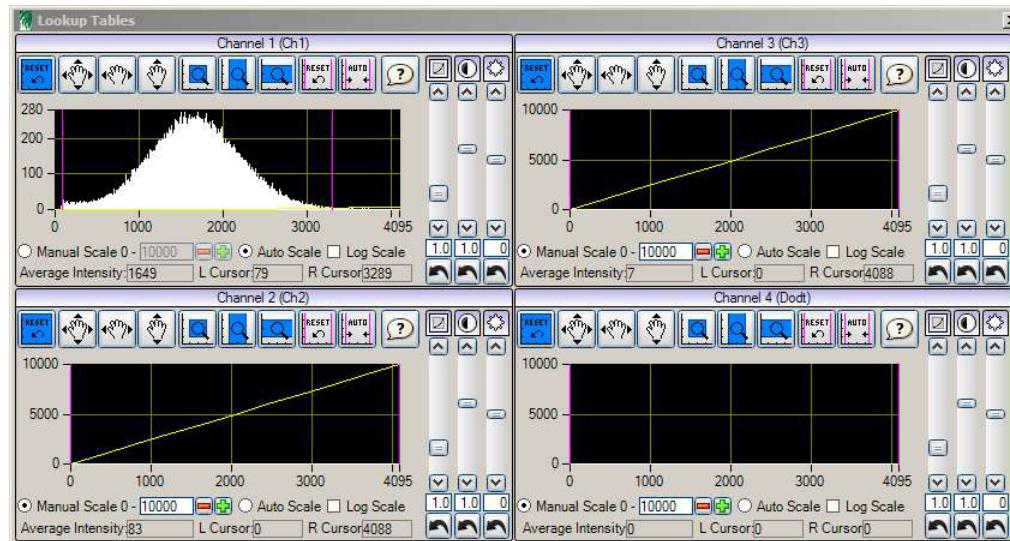
When the green button next to the "Cursor Information" panel is pressed, the "User Defined Functions" panel is displayed. Here, the user may enter custom mathematical functions to perform on the data. By clicking **Create Function**, a new window opens which allows for entry of a new function. Additionally, 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.

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  button moves the LUT cutoffs back their minimum and maximum values.

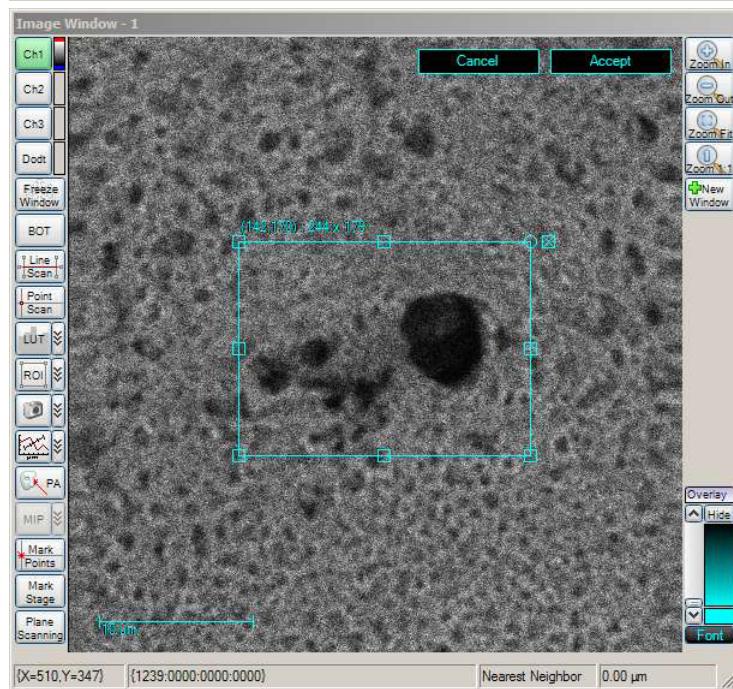
The  button adjusts the LUT to maximize the contrast of the current image in the display. This is not a continuous adjustment, but rather an instantaneous one using the current frame's channel data.

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.

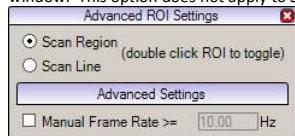
For point scanning systems, the pixel size used within the ROI will be the same as that of the image on which the ROI was drawn. To change the pixel size within the ROI, use the Increase/Decrease Pixel Size buttons in the Image Size section of the Main Control Window:



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 is no longer used. In the past, it was used as a way to improve the accuracy of inter-image timing when scanning an ROI in a T-Series at a period other than the fastest possible frame rate. This is no longer necessary, as inter-image timing accuracy in the T-Series has been optimized.
- **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.

Snap Tool



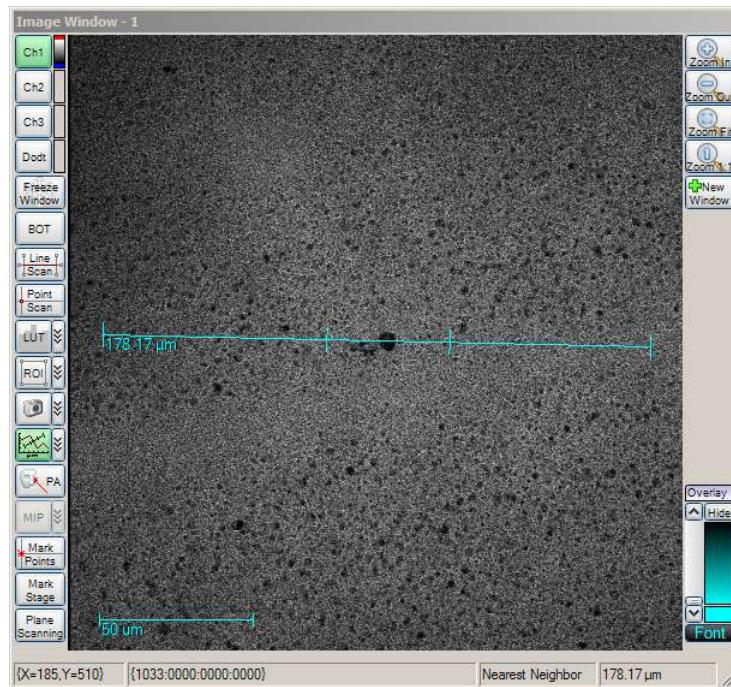
The snap button is used to save the current image displayed in an Image Window. The image will be saved to the filename and path specified in the Prairie View Misc tab, and the

incremental image counter on this tab will be increased by one to prevent over-writing data.

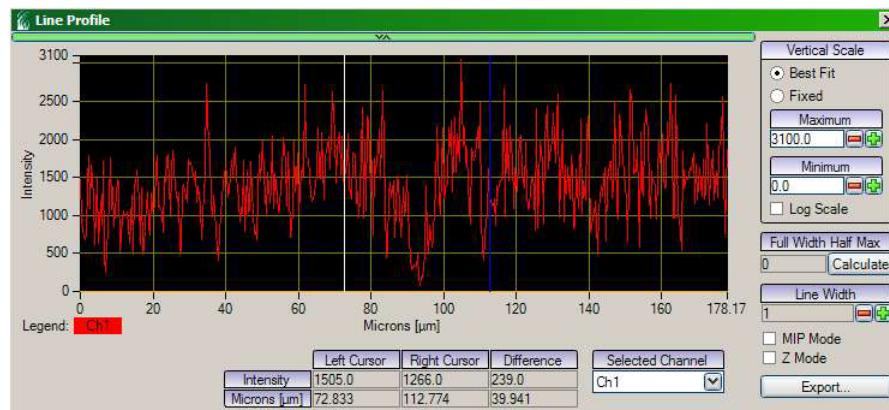
The displayed image will be saved as if it were a single image acquisition (a metadata file). If any overlays are present on the image, the exact displayed view (including display zoom) and overlays will be saved as an 8-bit TIFF.

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**.

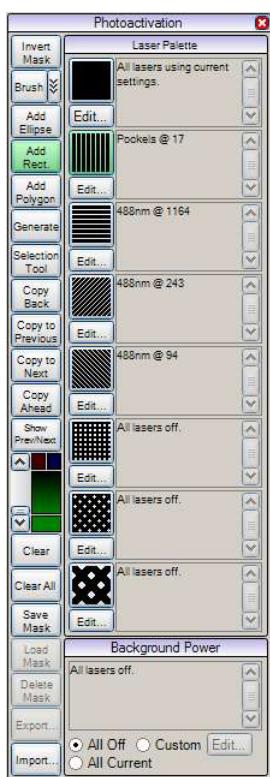
By clicking the green bar above the line profile graph, the user can access buttons for zooming and panning within the graph display.

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.

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.

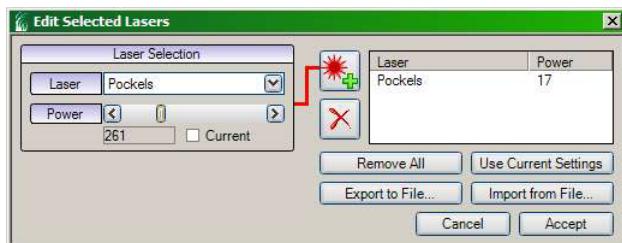
In the Preferences menu, the option to Save Images Generated With Photoactivation determines whether TIFF files are made when a PA mask is being scanned. In most cases, these files do not contain useful images of the sample. However, enabling the option provides the user with a quick reference image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

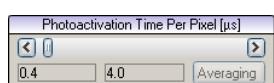
Using a Photoactivation Mask – SFC and Camera Mode Acquisitions

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

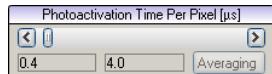
Cycle Type	# Reps	Period [s]	Max Speed	Duration [s]	Resource Selection	Photoactivation	BOT	External Trigger
Async P	1	-1	---	0.12959	---	Current	---	---

"Current" mask will be photoactivated one time.

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, Labels, 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.

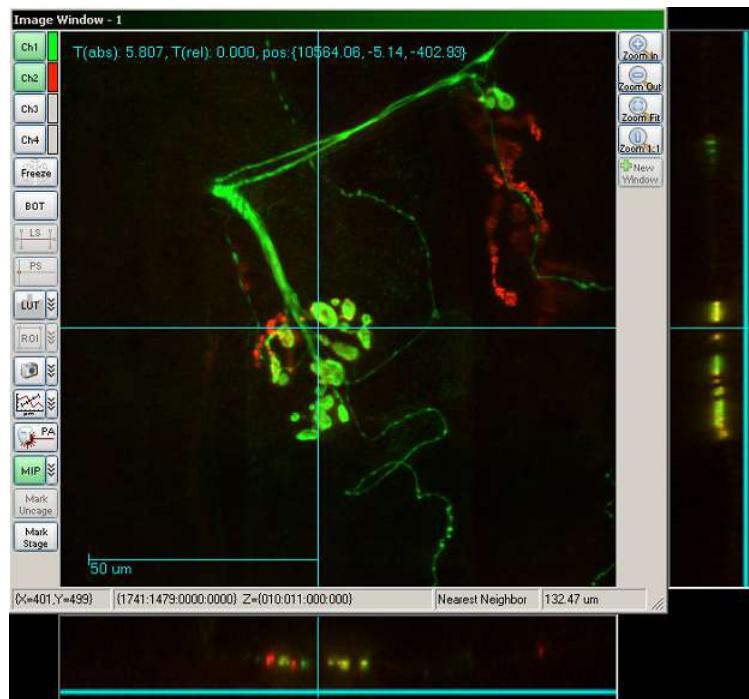
In some cases, the laser power applied during a photoactivation mask can result in the PMT signal becoming saturated. This is especially a problem with GaAsP detectors, which are more readily damaged than multi-alkali PMTs when exposed to bright light. Therefore, the user may opt to lower the PMT power 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.

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 dataset 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. To use MIP, a Z-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 re-sized 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.

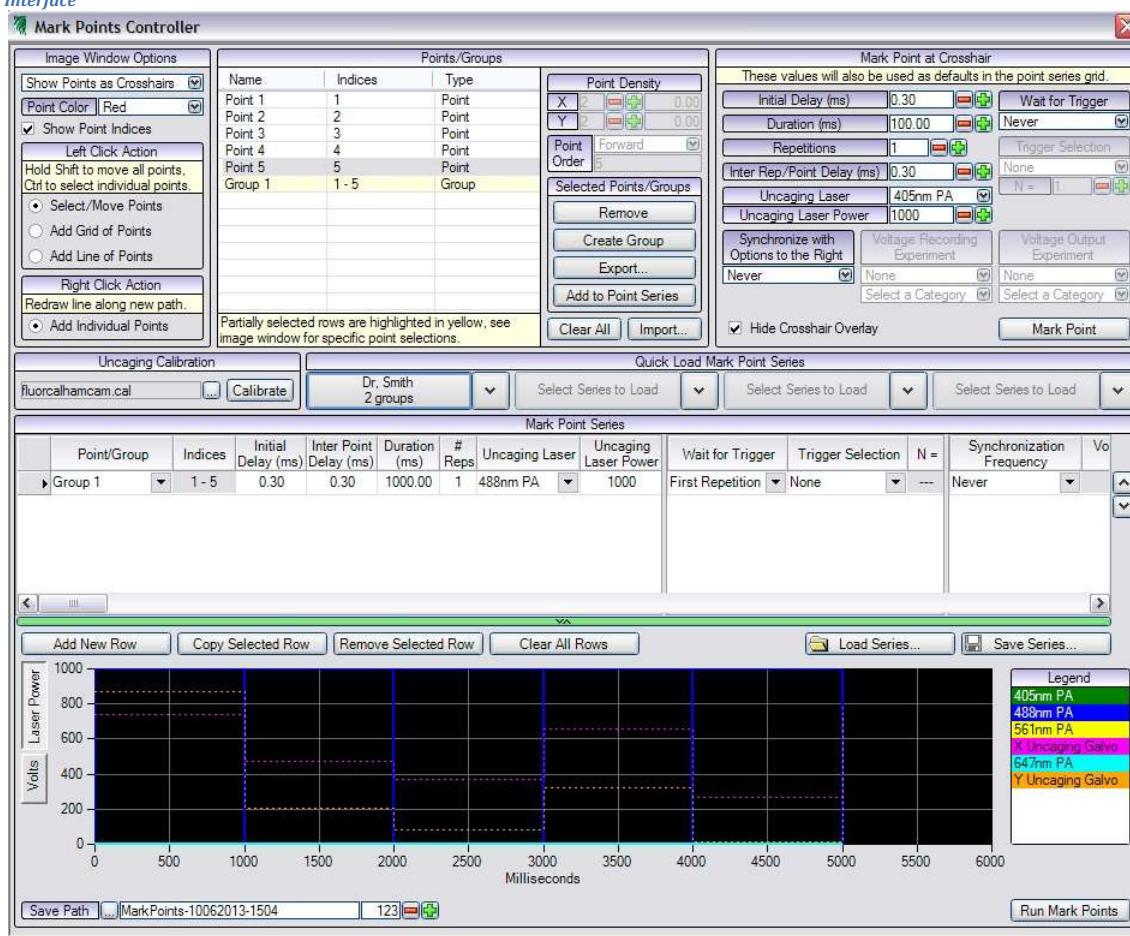
The user can choose to display an **Average Projection** to show the dataset at the average for each pixel instead of a **Maximum Projection**. **Cancel** allows the user to exit the MIP menu without making a selection.

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 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 Voltage Output signals that are used to control electrophysiology data as well as any other devices that can be controlled by analog voltage outputs and TTLs, and Voltage Recording of analog signals from electrophysiology amplifiers or an device generating an appropriate analog signal.

Mark Points Interface

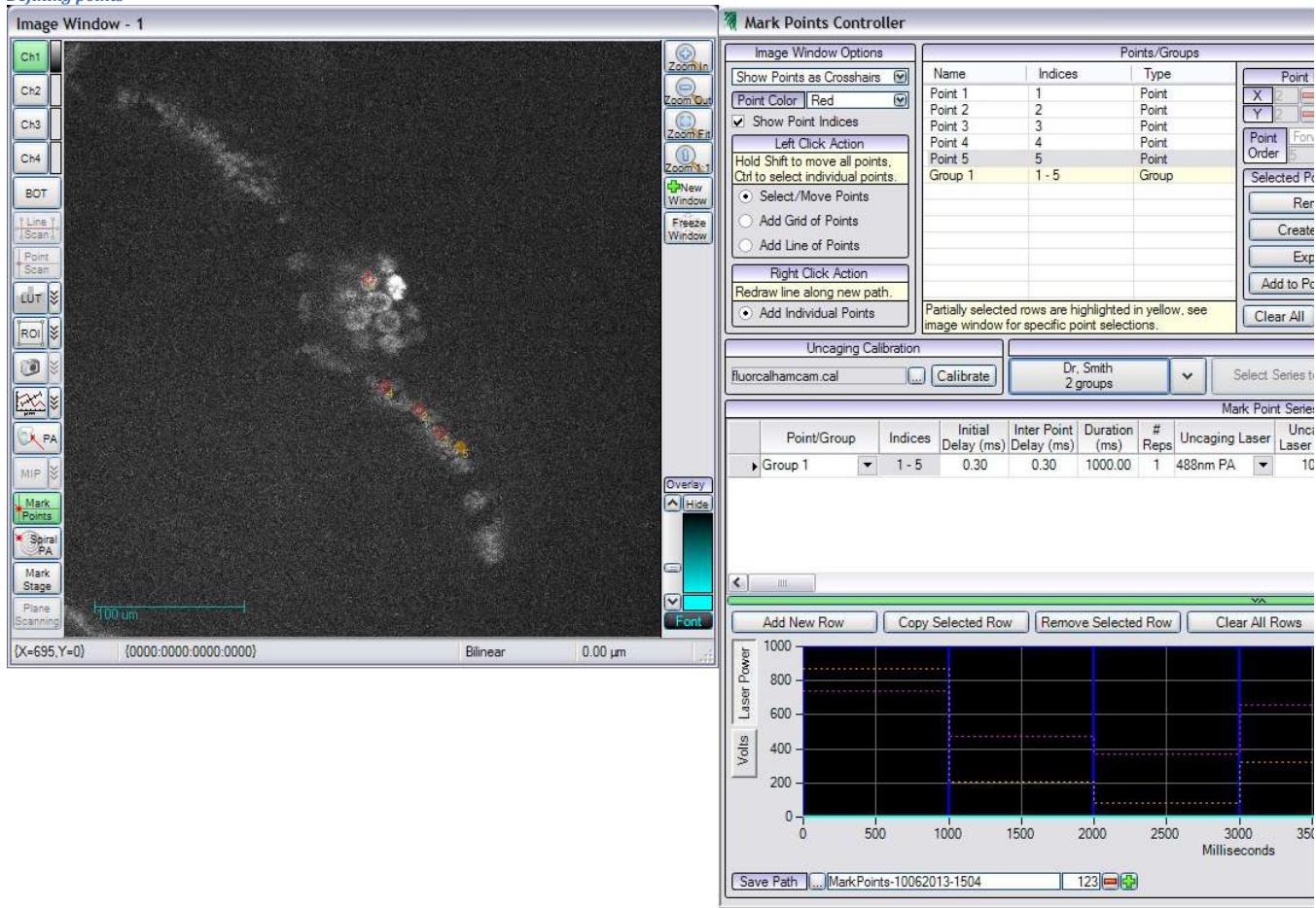
Left click on the **Mark Points** on the Image window or select Mark Points from the Application menu to launch the Mark Points interface.

The Mark Points interface is divided into following sections:

- Image window options: Sets display of graphic overlays as well as control of the mouse pointer when defining and editing point setup
- Point Groups: Lists points and groups defined on the image window. Allows user create groups of points as well as delete points, lines and groups. Point density of lines and grids can be defined. Custom point order can be defined. Sets of points, groups, grids and lines can be saved and loaded.
- Mark Points at Crosshair: Allows configuration of parameters for protocols using the interactive cross hair. These parameters are the same as those used in a Mark Point Series; see below for descriptions. The settings in this section also define the default values for parameters when points, groups, lines and grids are added to the Point Series table.

- Point Series Table: Defines the protocol to be run. Points, lines of points, groups of points and grids are added to the table and selected laser, laser intensity, pulse duration, intervals, triggering and synchronization are defined.

Defining points



After acquiring a reference image, the user selects Mark Points from the Image window or Application menu to launch the Mark Points interface.

Individual points (which can later be grouped), lines of points or grids of points can be defined. Select the type of points to be defined in the Left Click Action of the Image Windows Options.

To select individual points, right click in the desired locations on the image in the image window. Note: the image window can be zoomed when creating points or sets of points. To move a point, left click in the point and drag to a new location. Points can be moved after a Mark Points experiment is run. This feature is useful in evaluating location based on response.

To add a line of points, select **Add Line of Points**. To add a free hand line, point the mouse cursor to the beginning location, hold down the left mouse button, draw the desired line, and release the mouse button. The point density can then be set using the X point density controls to the top right of Points/Groups. To draw a straight line, hold down the shift key while drawing the line. To move a line, point at a point on the line, and while holding down the left mouse button move the line. To rotate the line, place the mouse cursor over an endpoint of the line, and while holding down the left mouse button rotate the line.

To add a grid of points select **Add Grid of Points**. Place the mouse cursor over the desired location of the upper left hand corner of the grid, hold down the left mouse button, then drag down to the desired lower right hand corner of the grid and release the mouse button. To move or resize the grid, place the mouse cursor on a point on the interior of the grid, hold down the left mouse button, and move the grid. To resize, place the mouse cursor on a point on the edge of the grid and drag. Placing the mouse cursor on the upper right corner allows the grid to be rotated. X and Y density are set with the controls to the upper right of Points/Groups.

There are two ways to create groups of existing points. One way is to set Left Click Action to **Select/Move Points** and while holding the left mouse button drag the cursor over the area containing the desired groups. The other way to create a group is to select the points in the Points/Group list by using control+click or shift+click. Then click the **Create Group** button on the right side of Points/Groups.

Defining protocols

Protocols are defined in the Point Series table. To add points, groups, lines or grids to the Point Series table, click on the desired entries in the Points/Group list and click the **Add to Point Series** button. The selected items will now appear in the Point Series table.

Note: If the parameters for selected laser, laser intensity, pulse width, etc. will be the same for all entries in the Point Series table, it is convenient to set those values in the Mark Point at Crosshair section as they will then be the defaults when entering items to the Point Series table.

	Point/Group	Indices	Initial Delay (ms)	Inter Point Delay (ms)	Duration (ms)	# Reps	Uncaging Laser	Uncaging Laser Power	Wait for Trigger	Trigger Selection	N =	Synchronization Frequency	Volts
Group 1	1 - 3	1.00	1.00	10.00	1	405nm (Uncal)	1000	Never	---	---	---	Never	
Group 2	4 - 6	1.00	1.00	10.00	1	405nm (Uncal)	1000	Never	---	---	---	Never	
Group 3	7 - 9	1.00	1.00	10.00	1	405nm (Uncal)	1000	Never	---	---	---	Never	

The Mark Points variables to be set for any experiment are:

- Initial Delay is the time until the first point in a group, line or grid is illuminated with the selected laser. Note: The shortest possible initial delay is the move time set for the galvanometers in the scan settings defined for the system; this parameter is set by Bruker Fluorescence Microscopy personnel during installation of the system.
- Inter Point Delay is the time between points in the group, line or grid. In the case of individual points this variable is used only if there are repetitions of that point.
- Duration is the length of time the laser is on at each point.
- # Reps is the number of times the group, line or point is repeated.
- Uncaging Laser is the laser to be used for that line in the Point series table. Note that different lasers can be selected for different lines. The lasers available for selection depend on system configuration.
- Uncaging Laser Power is the power of the laser when turned on as defined by the scaling set in the system configuration. This power setting uses the same scale as the laser power slider in the main control window.

At the bottom of the Point Series table is a graph showing the timing of the protocol. This graph can be hidden or displayed by clicking the green bar at the bottom of the Point Series table.

Running Mark Points

Mark Points protocols can be run in a number of ways, typically synchronized with imaging, in conjunction with electrophysiology recording, or both.

Stand-Alone Mark Points Experiment

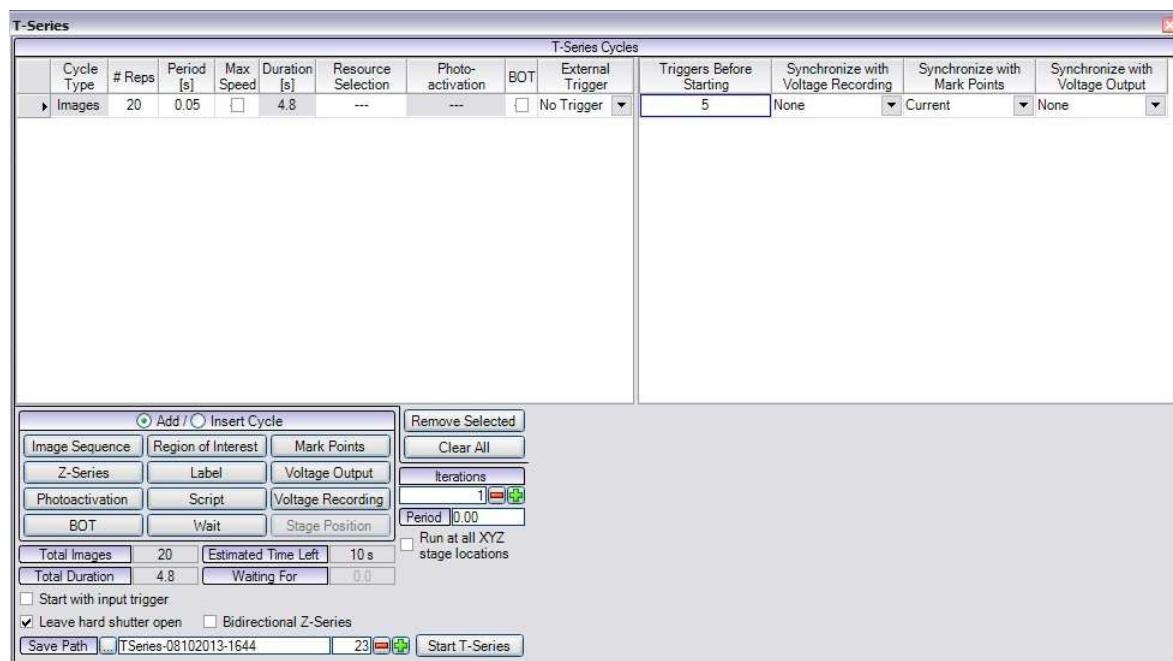
Mark Points can be run alone, which is typically done to verify that protocols are operating properly by using a marker slide or a slide with caged fluorescein. Simply click the **Run Mark Points** button at the bottom right corner of the Mark Points interface.

If more than one line is present in the Mark Points table, and no triggers are set, the entire table will run to completion.

To run a single point as a stand-alone experiment, use the Mark Point at Crosshair controls.

Triggering Mark Points with Imaging or Line Scans

Mark Points is often run with imaging, either full frame or ROI images or line scans. Simultaneous execution of Mark Points with imaging requires that the system have separate light paths (galvanometers and lasers) for the two operations. On systems with only one set of galvanometers, imaging and Mark Points must take place sequentially, rather than simultaneously.



When running Mark Points as part of a T-Series of images, the user can select on the T-Series line on which frame the Mark Points protocol should run. Scrolling to the right in the T-Series Cycles table will show a field for Synchronize with Mark Points. Selecting this field will open a drop down with the options to use the current Mark Points settings or a saved Mark Points protocol. This selection is only available on systems configured with the hardware needed for simultaneous Mark Points with imaging. To the left of the Mark Points synchronization field is the Triggers Before Starting column, in which the user enters the frame on which they want the Mark Points protocols to run. Frame triggers are discussed [here](#).

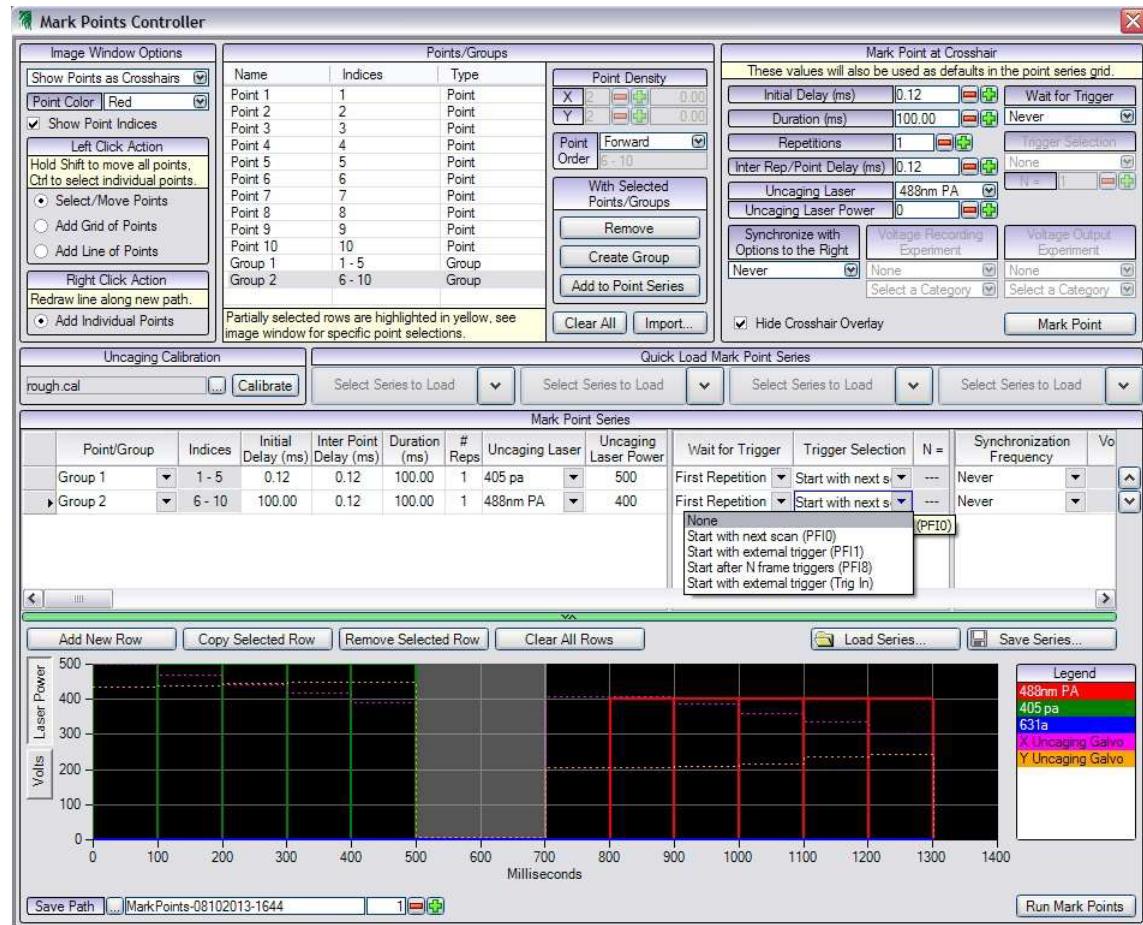
A Mark Points experiment can also be added to a T-Series as a separate cycle, by clicking the **Mark Points** button to add that type of cycle.

More information about T-Series setup is available [here](#).

In the Linescan Control window, Mark points is synchronized by selecting a Synchronize With option and then selecting Mark Points, with options to run the current configuration or a saved protocol. Note that with Line Scan the Synchronize with option gives two choices, Once at Start or Each Repetition. Once at Start will run the Mark Points protocol once when the **Start Linescan(s)** button is clicked, even if Line Scan is configured to run multiple repetitions. If Each Repetition is selected, Mark Points will run once for each repetition.

More information about line scan acquisition is available [here](#).

Triggering and Synchronization in Mark Points

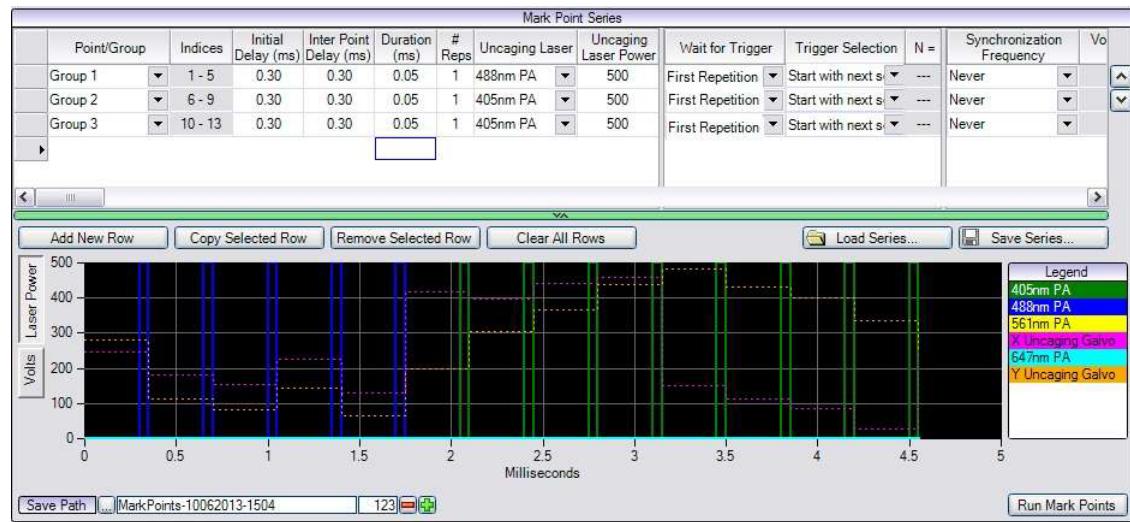


Mark Points has internal trigger variable that can be set to run Mark Points when an external trigger is received, or for more complex experiments involving multiple internal image triggers.

Scrolling to the right in the Point Series table will show the triggering section. Mark points can be triggered in two ways in conjunction with internal image triggers as well as being triggered by external triggers such as those that can be generated by an electrophysiology recording system.

Image triggers are PFI 0 (start of next scan) and PFI 8 (a specific frame). Frame triggers are discussed in more detail [here](#). These triggers are not available if the system is configured to use the same galvanometers for both imaging and Mark Points functions. If PFI 0 is selected for a line, that line will start when the next scan is started, a scan being defined as a T Series line, Live Scan, Single Scan, or a line scan. PFI 0 would be used when individual Point Series lines are to be matched up with individual T Series lines or line scan repetitions.

As an example, a user wants to do an experiment where they want to evaluate the effect of three different sets of points on eliciting a rise in calcium as measured by a calcium indicator. They define 3 groups of points, put each group on a line in the Mark Points Point Series table, and define the laser and timing variables. They set Wait for Trigger in Mark Points for each line to First Repetition and select the PFI 0 trigger.



In T series they set up three T Series lines, in this each with the same number of frames and frame interval. They DO NOT select triggering or synchronization in the T Series line, as the triggering is being set in Mark Points.

To start the experiment, they first click on **Run Mark Points** in the Mark Points window. Mark Points is now waiting to be triggered. They then click **Start T Series**, and the T Series starts to run. The start of each T Series line triggers Mark Points to run the next line.

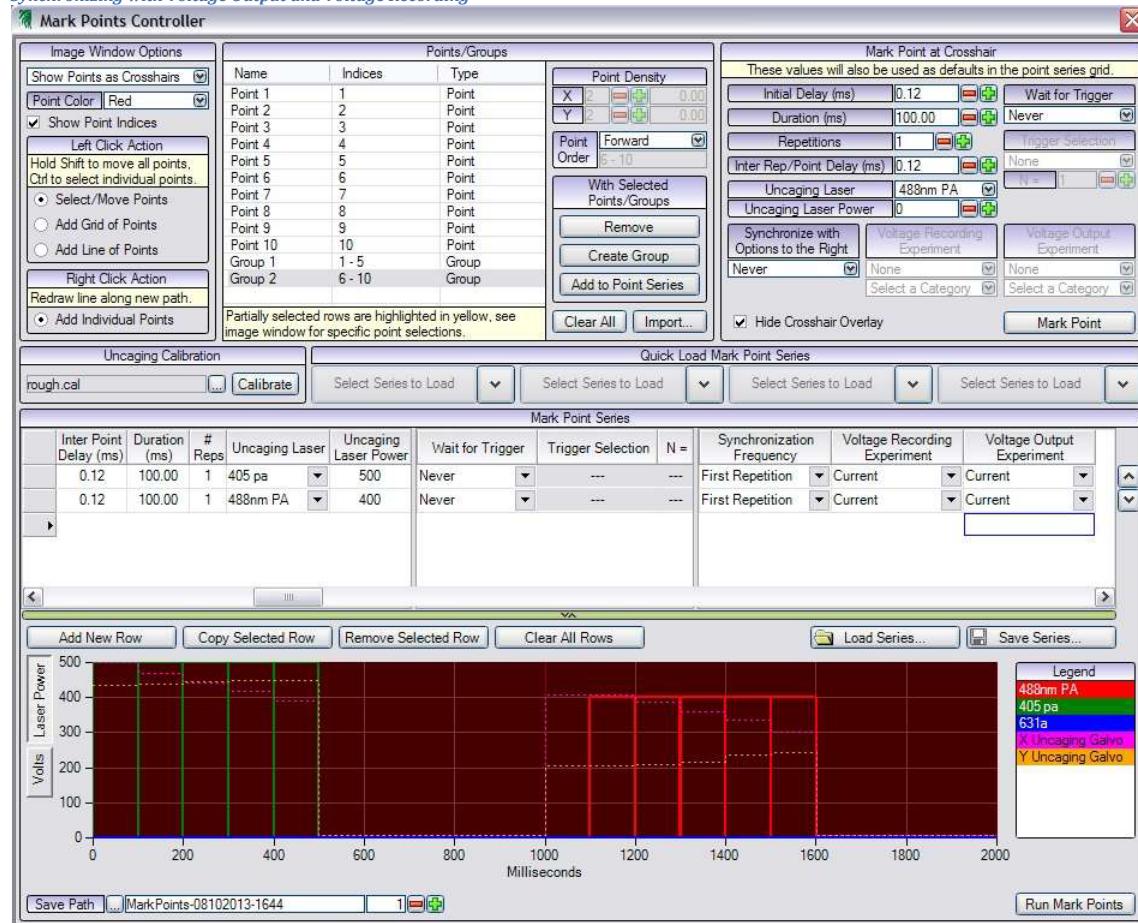
In another variant of the above experiment, the user wants to have a single T Series, and have three different groups of points run after a certain number of frames. They again set up 3 groups in Mark Points in the Points Series table. In this case they select as a trigger PFI 8, and select the number of frames to wait until the line in Points Series table runs. In this case they are going to run a T Series of 100 frames. They want to wait 25 frames before triggering the first line of the Point Series, and every 25 frames thereafter. They put 25 in as the number of frames for each line in the Point Series table. They click **Run Mark Points** so that it is waiting to be triggered, and then start the T Series.

Using Mark Points triggering with line scans is usually done with repetitions of line scans where a different line in the Point Series table is to be run with each repetition. For example, three groups of points, each to be run with a repetition of line scan. PFI 0 is used for the trigger for each line, and in line scan synchronization with Mark Point is not selected. Line scan would be set to run 3 repetitions. Mark Points would be started, then line scan started. At the beginning of each line scan a line from the Mark Points Point series would run.

Triggering by an external device can also be done from within Mark Points. Again, Wait for Trigger is selected, and the trigger source is selected. The trigger source can be PFI 1, which is the PFI 1 connector on the front of the GPIO box, or Trigger In, which is one of the 8 Trigger In connections on the front of the GPIO box. Again, **Run Mark Points** is clicked to put Mark Points in a state where it is waiting to run.

If there are multiple lines in the Mark Points Point Series, but only the first has a trigger set, the entire Points Series will run when the trigger is received. It is possible to set up protocols where there are multiple lines with triggers and lines without triggers. For example, a user wants to create 9 groups and run them 3 at a time with a line scan. They would create the 9 groups and enter them in the Point Series such that lines 1-3 were the groups for the first line scan, 4-6 were for the second line scan and 7-9 were for the 3rd line scan. On lines 1, 4 and 7 they would set the trigger to PFI 0. They would set line scan to 3 repetitions, click **Start Mark Points** and then start line scan. The first three lines would run with the first line scan, line 4 would wait for the next start of scan trigger from line scan, etc.

Synchronizing with Voltage Output and Voltage Recording



Lines in the Point Series can be set up to synchronize with defined Voltage Output and Voltage Recording. This type of synchronization can provide full control and recording of data for experiments where optical stimulation is being used in conjunction with electrophysiology recording. Voltage outputs can also be used as triggers for external devices, and Voltage Recording can record any type of -10 to +10 V signal.

In order to use Voltage Output or Voltage Recording, Voltage Output and/or Voltage Recording must be configured, or have saved experiments present (see Voltage Output, Voltage Recording).

To use Voltage Output or Voltage Recording, select First Repetition, Each Repetition or Each Point from the Synchronization dropdown. Then select Current or Choose Experiment from the Voltage Output and/or Voltage Recording experiment.

When using Voltage Output through Mark Points synchronization, the waveforms being generated are displayed in the graph below the Point Series. Voltage Recording time is displayed in the graph as a red overlay.

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

Spiral Activation

This feature is new in Prairie View version 5.0. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

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.

Plane Scanning

This section of the Help file is under construction. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

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 data sequence selected, one or both of two scroll bars may be available: one vertical for Z stack data and one horizontal for time lapse data.

Each sequence or dataset represents a cycle of a T-Series, a simple Z-Series, or a single image. It is possible to navigate datasets 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 scroll 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.

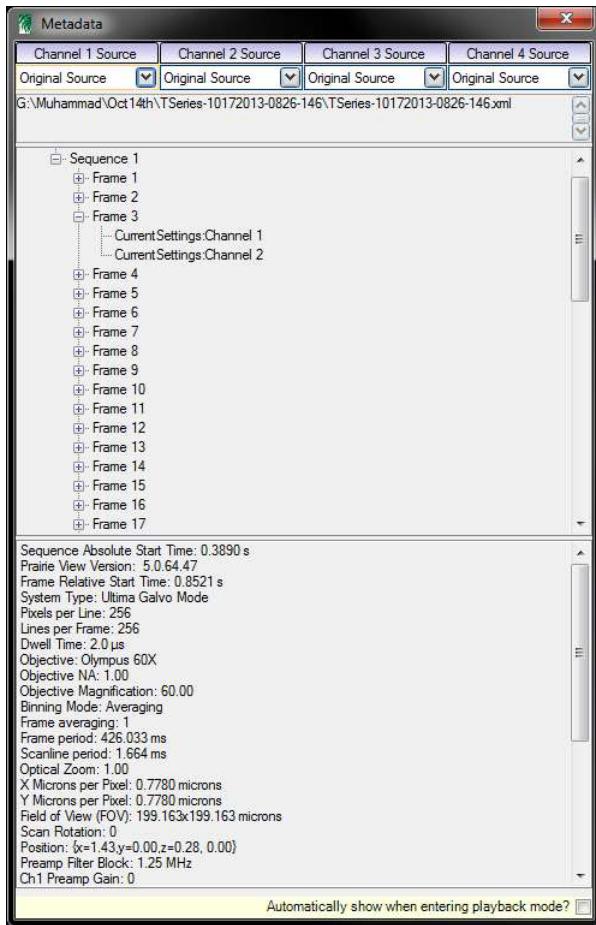
The user may choose a previously defined After Scan Complete/Playback action from the **Action** drop-down menu and execute it by clicking the **Perform** button. If the **Perform Action** box is checked, the action displayed in the drop-down menu will be executed every time the image in the Image window changes; that is, when changing slices in a Z-Series playback or changing time points or datasets 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 data set 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 dataset, 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.



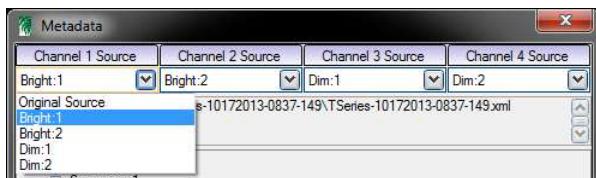
Channel Mapping in Playback Mode

The Metadata window provides controls for mapping data associated with a certain label and acquisition channel to a specific display channel. The playback mapping is important when looking at an image set that was acquired using Labels; the channels active when acquiring images within a Label 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 two different Labels (see further discussion in the [Labels Tab](#) section of this manual). The Labels used in this experiment were named "Bright" and "Dim", and each Label contained two active channels, 1 and 2. The user may wish to map these four acquisition channels (Bright channel 1, Bright channel 2, Dim, channel 1, and Dim channel 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 dropdown menus which correspond to display channels 1 through 4. In this example, the data acquired on channel 1 with Label Bright, called "Bright:1", is mapped to appear in display channel 1 for playback.



Sometimes for a given sequence of frames there is no data for certain label:channel combinations so the image for that channel will remain unchanged.

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.

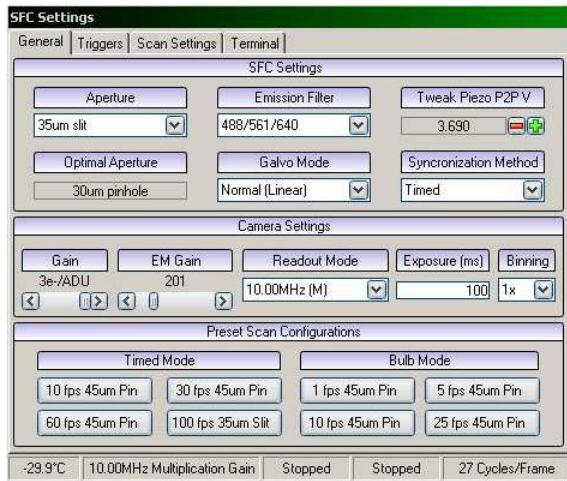
At the bottom of the window, an information bar provides status information about the system. From left to right, the information boxes report

- Temperature of the camera's internal sensor, if supported by the camera
- Readout Mode of the camera, as defined in the Camera Settings of the SFC General tab
- 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 scan settings for the SFC. The user can choose from 7 options in the **Aperture** drop-down menu. 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).

The **Emission Filter** menu 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".

The **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 **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.

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 **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 chosen readout mode is also displayed in the bottom of the window.

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** 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.

Preset Scan Configurations offer a quick pick for some common combinations of scan settings.

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.

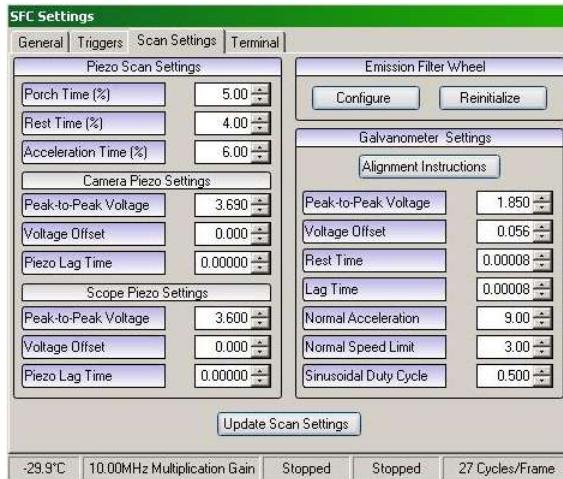
The user may also designate one Aux line to receive triggers from other equipment. This line is chosen in the SFC/Camera/Filters tab of the Prairie Configuration Utility and then becomes unavailable for use in the Triggers tab. For systems configured with a GPIO box, it is often better to use the numbered 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 Instructions** 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.



Terminal Tab

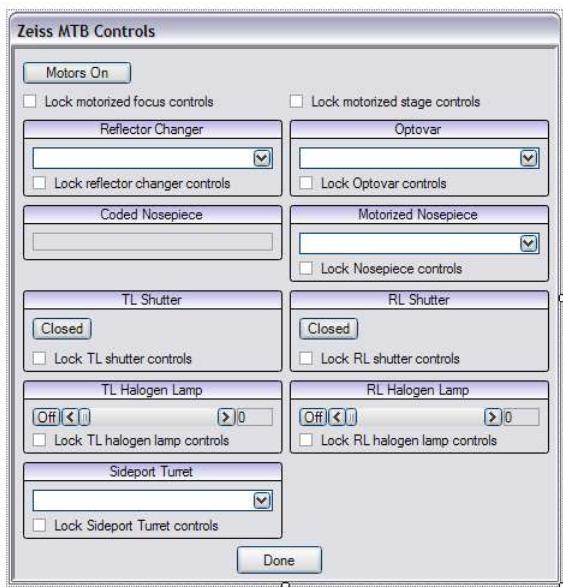
The Terminal tab can be used to send line commands to the SFC control box directly. This can be useful for technicians and support personnel during installation or when troubleshooting problems with hardware and software. Please contact Bruker Fluorescence Microscopy support personnel with questions 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

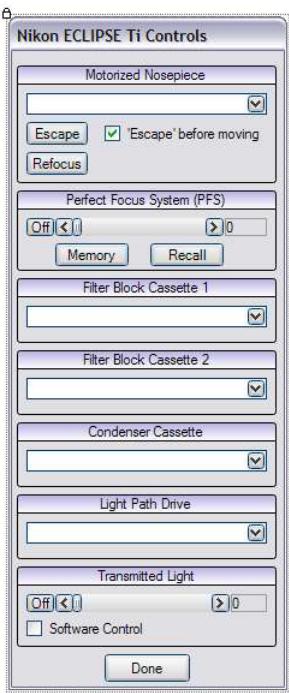
The **TL Halogen Lamp** slider allows the user to set the intensity of the transmitted light halogen lamp. The **On/Off** button toggles its light output on and off.

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 Ti Controls



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

'Escape' before moving automatically performs an Escape when the user selects a different nosepiece position, and refocuses after the movement is complete

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, the **Perfect Focus System** 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.

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.

The **Done** button closes the window. The user may display the window again by selecting Tools > Nikon Ti Controls in the Prairie View main control window.

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.

File Menu

Load Image(s): Allows the user to navigate to and load a saved acquisition

Load Recent Image(s): Displays a list of recent acquisitions for loading, without the need to navigate to the directory in which the acquisition was saved

Load Configuration: Loads a system configuration file, recalling control settings of the laser(s), PMTs or camera, etc.

Save Configuration: Saves a system configuration file using the current settings of the laser(s), PMTs or camera, etc.

Load Uncaging Calibration: Allows the user to navigate to and load a calibration file for the uncaging galvos, for use with [Mark Points](#) and [Spiral Activation](#)

Load Recent Uncaging Calibration: Displays a list of recent uncaging galvo calibrations for loading, without the need to navigate to the directory in which the calibration file was saved

Load T-Series Settings: Loads previously saved [T-series](#) settings

Save T-Series Settings: Saves the current [T-series](#) settings to a file for recalling at a later time

Load XY Stage Locations: Loads a previously saved list of XY stage locations; this option is the same as clicking the **Import Locations** button on the [XY-Stage tab](#).

Save XY Stage Locations: Saves the current list of XY stage locations this option is the same as clicking the **Export Locations** button on the [XY-Stage tab](#).

Exit: Stops Prairie View and closes the program

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.

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.

Output Trigger Type: Allows the user to determine which frame triggers are generated by the system during acquisition.

Save/Recall LUT settings: Retains the LookUp Table settings when Prairie View is exited, to be recalled the next time Prairie View is started

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, which is described in the [T-Series Tab](#) section of this manual.

Z-Series: Opens the Z-Series Preferences dialog, which is described in the [Z-Series Tab](#) section of this manual.

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.

Use Smaller XML File Format: The XML file contains all the meta-data describing an acquisition. The smaller version of this file contains all scan information while minimizing duplication; this format allows very large data sets to be re-loaded into Prairie View software. The legacy version of the XML file is much larger and contains many duplications, leading to file sizes that are difficult to load for viewing later. The option to use the older, larger file format exists to preserve compatibility with certain third-party analysis programs.

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 Affect Data Integrity!): 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 diverts some resources from data acquisition to processing, so there is a possibility that incoming data could be lost. Watch for warning messages during the acquisition or in Playback Mode that alert the user to lost data.

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 PrairieView 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).

Photo 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.

Output Trigger Type

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 Prairie 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 Prairie 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 > Output Trigger Type > Trigger Mode > Generate Start of Frame OR End of Frame Trigger OR Neither (the middle option in the sub-menu)
- Preferences > Output Trigger Type > 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 > Output Trigger Type > Trigger Mode > Generate Start of Frame OR End of Frame Trigger OR Neither (the middle option in the sub-menu)
- Preferences > Output Trigger Type > 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 > Output Trigger Type > Trigger Mode > Generate Start of Frame AND/OR End of Frame Trigger OR Neither (the last option in the sub-menu)
- Preferences > Output Trigger Type > Start of Frame Trigger
- Preferences > Output Trigger Type > End of Frame Trigger

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, the user can disable both the Start of Frame and the End of Frame triggers, or choose Generate No Trigger from the Trigger Mode 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

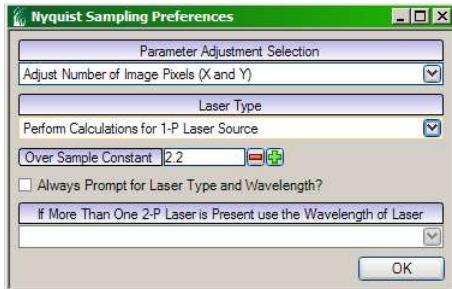
Frame triggers can be generated by the SFC control box and accessed from one or more AUX ports on the front of that box. This is described in the [SFC Settings - Triggers Tab](#) section of this manual.

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.

Tools Menu

[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.

[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 Controls](#): Opens a dialog for controlling motorized components of a Zeiss microscope stand.

[Nikon Ti Controls](#): Opens a dialog for controlling motorized components of a Nikon Ti 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.

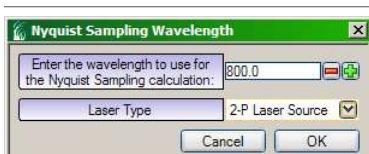
[Calibration/Alignment](#): Options in this sub-menu allow users to set up and modify software-controlled calibrations. Individual options are described elsewhere in this manual.

[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 DODT 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 Prairie 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) = $((0.61 * \lambda) / NA) / OS$

Desired z step size (in nanometers) = $((((0.61 * \lambda) / NA) / OS) * (\pi / NA)$

For a 2-P laser:

Desired x and y pixel size (in nanometers) = $((0.61 * (\lambda / \sqrt{2})) / NA) / OS$

Desired z step size (in nanometers) = $((((0.61 * (\lambda / \sqrt{2})) / NA) / OS) * (\pi / NA)$

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.

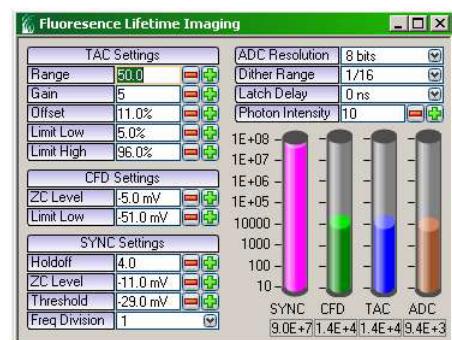
Consider the following example. There is bleed-through of Channel 1 into Channel 2. The goal is to subtract out the Channel 1 signal from Channel 2 and add it back to Channel 1:

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 Onsec for single channel FLIM and 20sec 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](#).

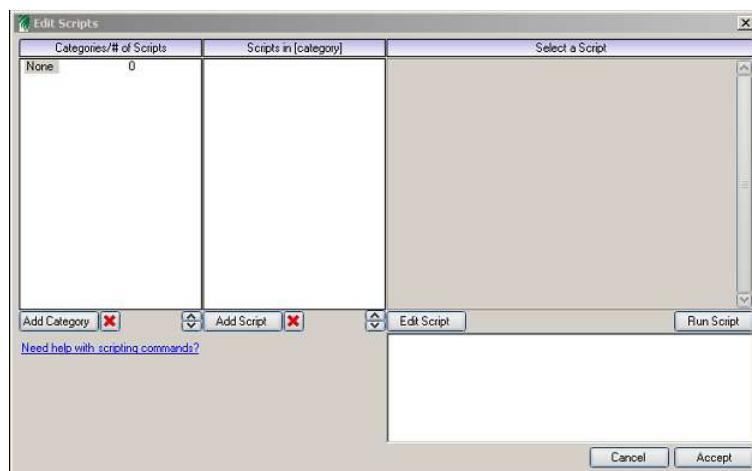
Scripts

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 (Tools > Scripts > Edit 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.

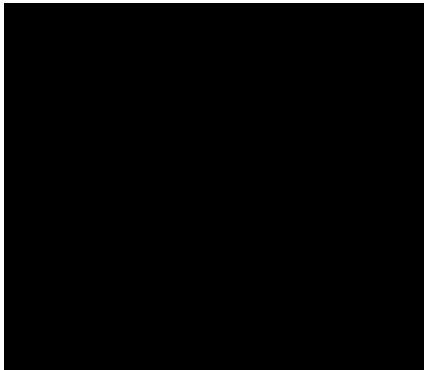
Running a Script

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.

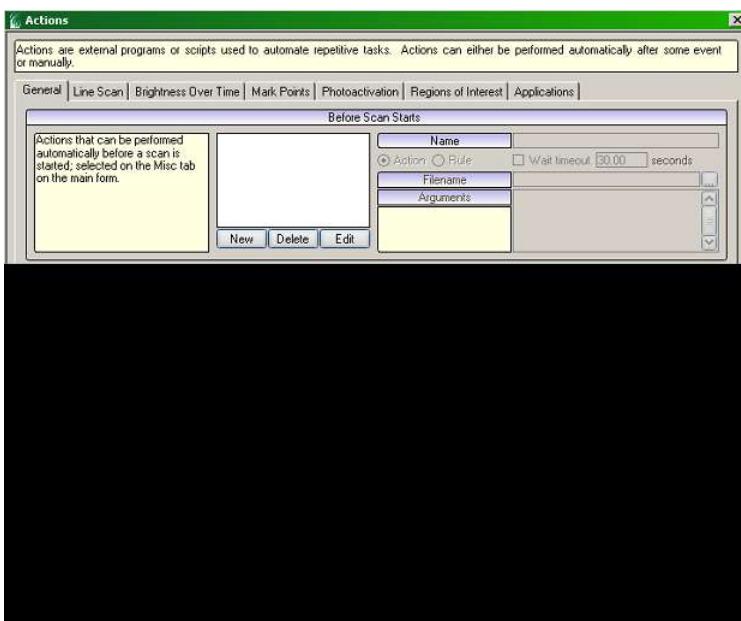


Stopping a Script

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.

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).

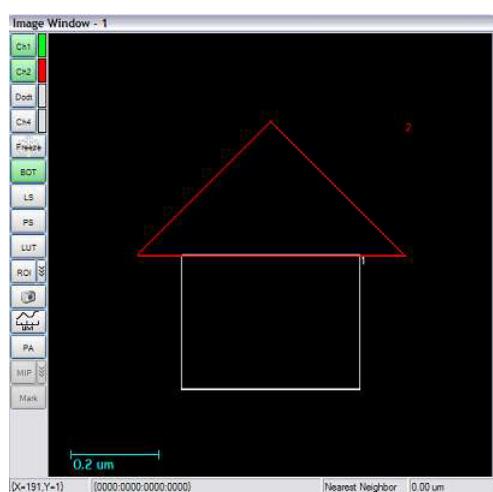
Brightness Over Time Region Generation actions are run manually from the [Brightness Over Time](#) dialog and are used to automatically generate brightness over time regions based on the current image data. There are two tokens available for use in the arguments field:

'<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).

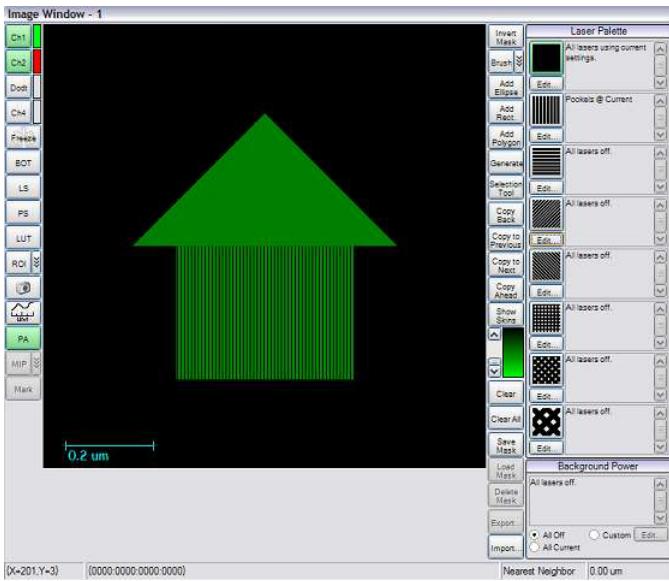
Photoactivation Mask Generation actions are run manually from the image window when the [Photoactivation](#) mask editor is enabled and are used to automatically generate photo activation masks based on the current image data. There are two tokens available for use in the arguments field:

'<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>')" "imageresc(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 Prairie Technologies. 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

This feature is new in Prairie View version 5.0. It replaces some functions formerly carried out by TriggerSync software. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

This dialog contains step-by-step instructions for calibrating photo-stimulation galvo positions to locations in the image.

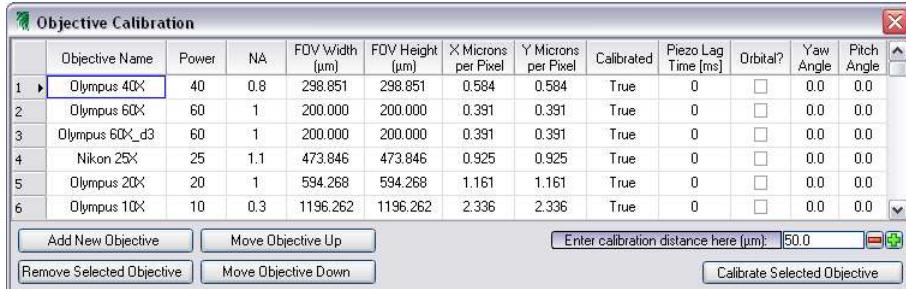
Note that all beam alignment must be done before performing this software calibration.

Based on the hardware present on the system, the user may choose to view the position of the stimulation laser using a **Spot Detector** (Ultima systems with a 2-P laser on the uncaging path), **Fluorescence** (Camera/SFC/confocal systems on a uniformly fluorescent sample), or **Burn Spots** (any system) in a fluorescent sample.

The user can also update the currently active calibration file using the **Quick Offset** option.

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 within the [Mark Points](#) dialog for a fast method by which the user can specify a location on the image at which to direct the photo-stimulation.

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. If the objective will be used on an Orbital Nose Piece, check the box in the Orbital? column.
13. 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.

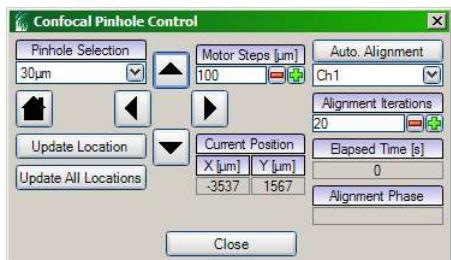
An objective must be calibrated in each Acquisition Mode in which it will be used. For example, the 40x objective will need to be calibrated in both Galvo mode and SFC mode, if both scanners are present on the system. If the objective is calibrated only in Galvo mode, the field of view and pixel size 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 Prairie 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 Prairie 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.



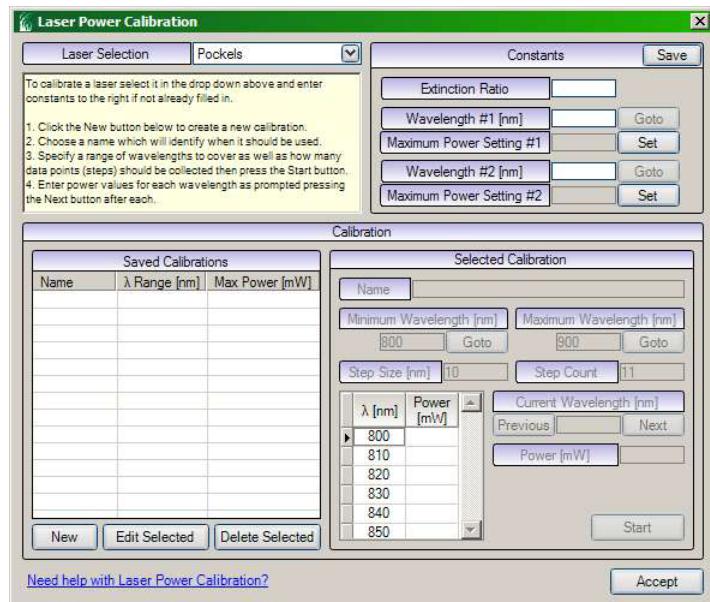
The **Home** button drives the pinhole plate motors to their home positions. This can be used for troubleshooting/diagnostic purposes when working with Bruker Fluorescence Microscopy support personnel. Note that there is unlikely to be a pinhole at the home position.

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.

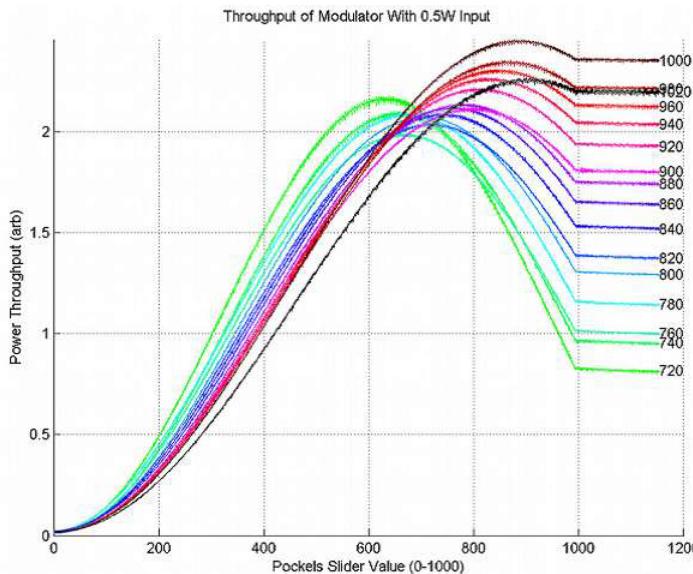
Detailed information about laser calibration can be accessed from within Prairie View by clicking the **Need help with Laser Power Calibration?** link at the bottom of the Laser Power Calibration dialog.

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 to 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.

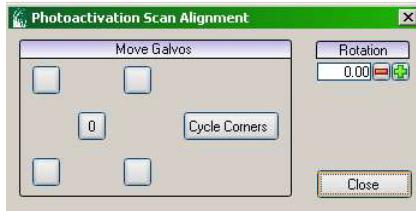
Since the maximum sample power for a given calibration is limited to the 'lowest' measured power through the calibration wavelength range, the operator might wish to make several calibrations for a given objective lens at various wavelength ranges.

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.

Photoactivation Alignment

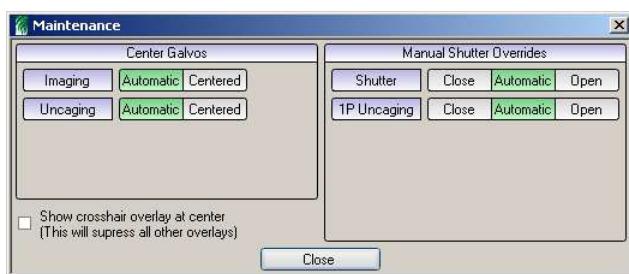
If the system is configured with a Camera or SFC and a photoactivation module, this option opens a dialog to adjust the scan rotation of the photoactivation scan relative to the imaging scan. No adjustments should be made until the user is certain that the camera is properly aligned with the SFC.



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.

Click the **Centered** button for one set of galvos to bring the galvanometers to center.

Click the **Automatic** button to restore galvo control to the Main Control window.

- If the galvos are centered via the Tools menu > Calibration/Alignment > Center Galvos option, the galvos will remain centered
- If the galvos are not centered via the Tools menu option, the galvos will return to their park positions

When the user closes the Maintenance dialog, all controls are returned to the Automatic state.

Manual Shutter Overrides

In this section, the user can closer 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 for one shutter to open the hard and soft shutters for that light path.

Click the **Closed** button for one shutter to open the hard and soft shutters for that light path.

Click the **Automatic** button to restore shutter control to the Main Control window.

When the user closes the Maintenance dialog, all 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 Window](#) button in the [Scanning Controls](#) section of the Prairie View main control window. In earlier version of Prairie View, this option was located under a separate Window menu.

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.

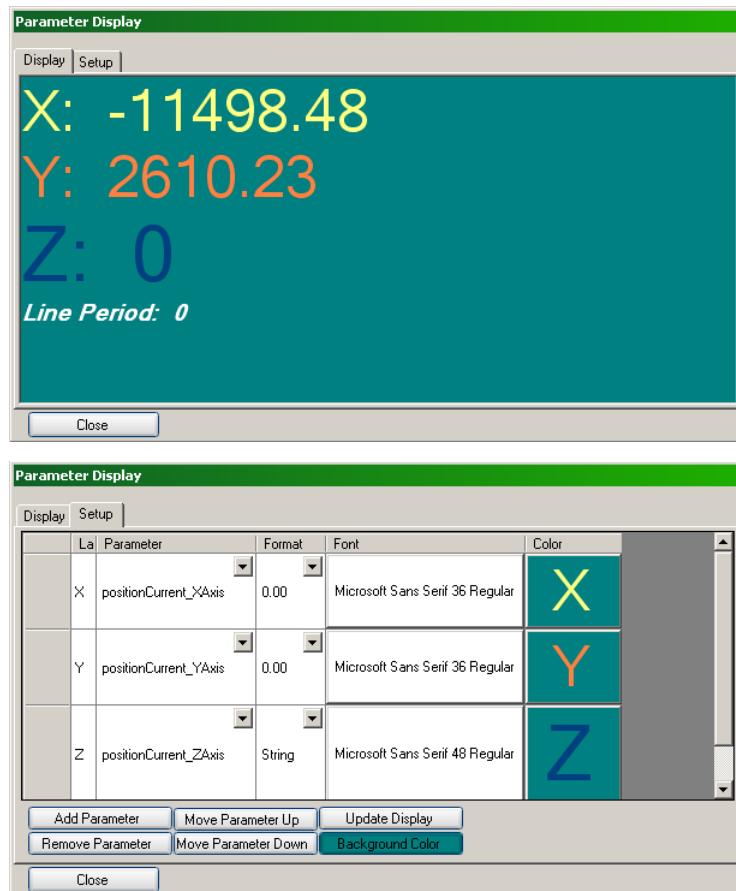
Z-Position Display: For systems configured with multiple Z-devices, this option allows the user to choose whether the Z position displayed at the bottom of the Prairie View main control window is the **Sum of all positions**, **All positions (comma delimited)**, or **Current device position**.

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 will always be zero during a scan or after a single image. 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.

Parameter Display: Opens a dialog in which the user can display any number of system parameters. The Display tab shows the current values of the selected parameters. The Setup tab contains buttons with which the user can edit the list of parameters and 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), **Parameter**, **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.



Applications and Photo-Stimulation 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 application. Each application 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
- [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
- [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

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.

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 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

This feature is new in Prairie View version 5.0. It replaces some functions formerly carried out by TriggerSync software. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

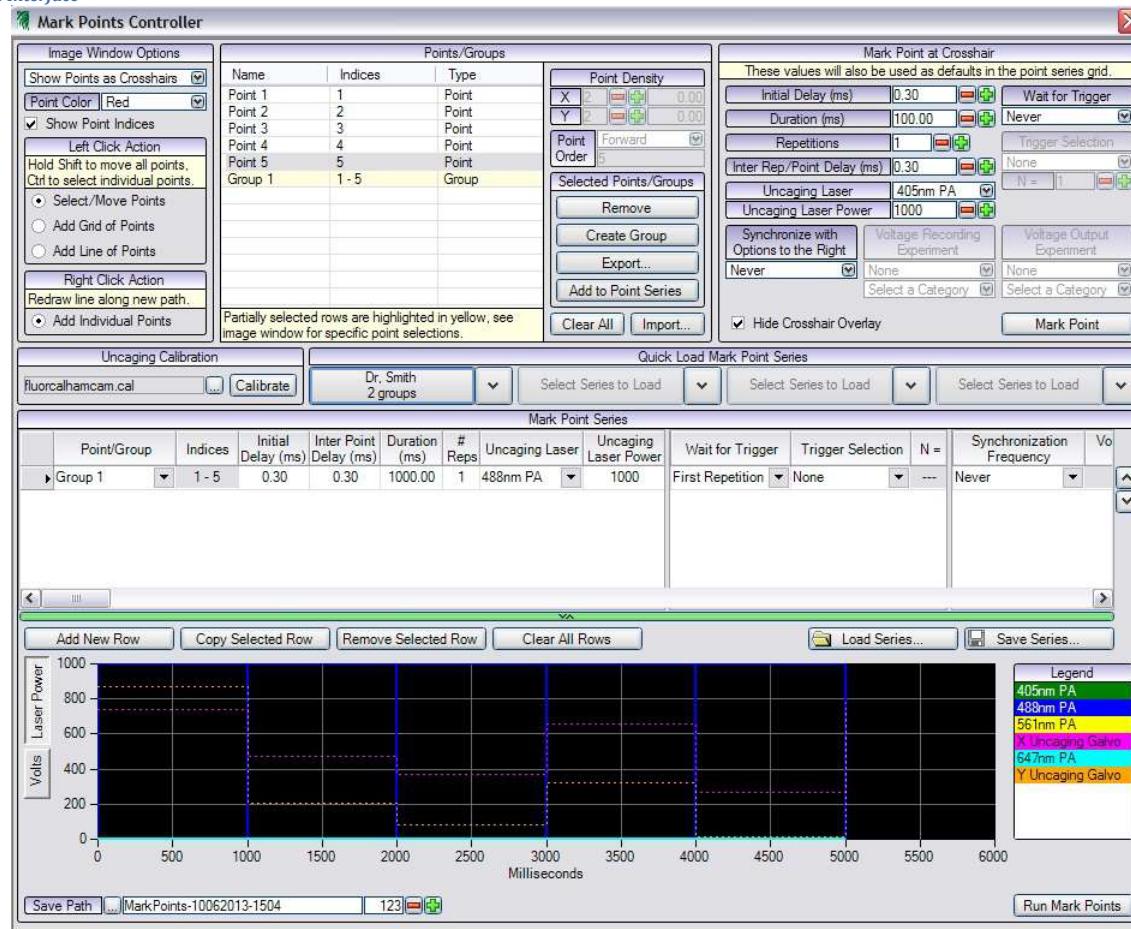
Mark Points

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 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 Voltage Output signals that are used to control electrophysiology data as well as any other devices that can be controlled by analog voltage outputs and TTLs, and Voltage Recording of analog signals from electrophysiology amplifiers or an device generating an appropriate analog signal.

Mark Points Interface

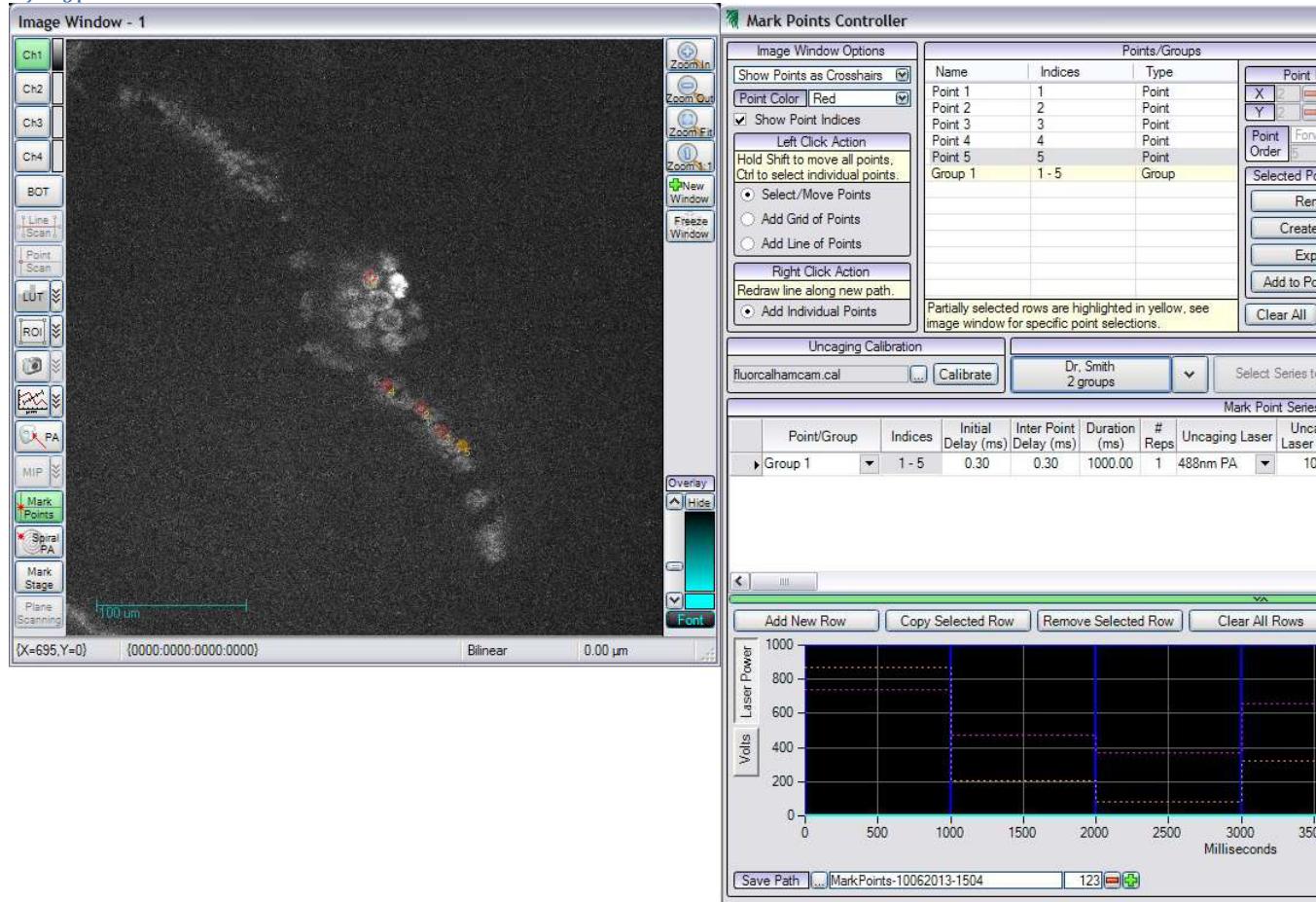


Left click on the **Mark Points** on the Image window or select Mark Points from the Application menu to launch the Mark Points interface.

The Mark Points interface is divided into following sections:

- Image window options: Sets display of graphic overlays as well as control of the mouse pointer when defining and editing point setup
- Point Groups: Lists points and groups defined on the image window. Allows user create groups of points as well as delete points, lines and groups. Point density of lines and grids can be defined. Custom point order can be defined. Sets of points, groups, grids and lines can be saved and loaded.
- Mark Points at Crosshair: Allows configuration of parameters for protocols using the interactive cross hair. These parameters are the same as those used in a Mark Point Series; see below for descriptions. The settings in this section also define the default values for parameters when points, groups, lines and grids are added to the Point Series table.
- Point Series Table: Defines the protocol to be run. Points, lines of points, groups of points and grids are added to the table and selected laser, laser intensity, pulse duration, intervals, triggering and synchronization are defined.

Defining points



After acquiring a reference image, the user selects Mark Points from the Image window or Application menu to launch the Mark Points interface.

Individual points (which can later be grouped), lines of points or grids of points can be defined. Select the type of points to be defined in the Left Click Action of the Image Windows Options.

To select individual points, right click in the desired locations on the image in the image window. Note: the image window can be zoomed when creating points or sets of points. To move a point, left click in the point and drag to a new location. Points can be moved after a Mark Points experiment is run. This feature is useful in evaluating location based on response.

To add a line of points, select **Add Line of Points**. To add a free hand line, point the mouse cursor to the beginning location, hold down the left mouse button, draw the desired line, and release the mouse button. The point density can then be set using the X point density controls to the top right of Points/Groups. To draw a straight line, hold down the shift key while drawing the line. To move a line, point at a point on the line, and while holding down the left mouse button move the line. To rotate the line, place the mouse cursor over an endpoint of the line, and while holding down the left mouse button rotate the line.

To add a grid of points select **Add Grid of Points**. Place the mouse cursor over the desired location of the upper left hand corner of the grid, hold down the left mouse button, then drag down to the desired lower right hand corner of the grid and release the mouse button. To move or resize the grid, place the mouse cursor on a point on the interior of the grid, hold down the left mouse button, and move the grid. To resize, place the mouse cursor on a point on the edge of the grid and drag. Placing the mouse cursor on the upper right corner allows the grid to be rotated. X and Y density are set with the controls to the upper right of Points/Groups.

There are two ways to create groups of existing points. One way is to set Left Click Action to **Select/Move Points** and while holding the left mouse button drag the cursor over the area containing the desired groups. The other way to create a group is to select the points in the Points/Group list by using control+click or shift+click. Then click the **Create Group** button on the right side of Points/Groups.

Defining protocols

Protocols are defined in the Point Series table. To add points, groups, lines or grids to the Point Series table, click on the desired entries in the Points/Group list and click the **Add to Point**

Series button. The selected items will now appear in the Point Series table.

Note: If the parameters for selected laser, laser intensity, pulse width, etc. will be the same for all entries in the Point Series table, it is convenient to set those values in the **Mark Point at Crosshair** section as they will then be the defaults when entering items to the Point Series table.

Mark Point Series												
	Point/Group	Indices	Initial Delay (ms)	Inter Point Delay (ms)	Duration (ms)	# Reps	Uncaging Laser	Uncaging Laser Power	Wait for Trigger	Trigger Selection	N =	Synchronization Frequency
Group 1	▼	1 - 3	1.00	1.00	10.00	1	405nm (Uncag)	1000	Never	---	---	Never
Group 2	▼	4 - 6	1.00	1.00	10.00	1	405nm (Uncag)	1000	Never	---	---	Never
► Group 3	▼	7 - 9	1.00	1.00	10.00	1	405nm (Uncag)	1000	Never	---	---	Never

The Mark Points variables to be set for any experiment are:

- Initial Delay is the time until the first point in a group, line or grid is illuminated with the selected laser. Note: The shortest possible initial delay is the move time set for the galvanometers in the scan settings defined for the system; this parameter is set by Bruker Fluorescence Microscopy personnel during installation of the system.
- Inter Point Delay is the time between points in the group, line or grid. In the case of individual points this variable is used only if there are repetitions of that point.
- Duration is the length of time the laser is on at each point.
- # Reps is the number of times the group, line or point is repeated.
- Uncaging Laser is the laser to be used for that line in the Point series table. Note that different lasers can be selected for different lines. The lasers available for selection depend on system configuration.
- Uncaging Laser Power is the power of the laser when turned on as defined by the scaling set in the system configuration. This power setting uses the same scale as the laser power slider in the main control window.

At the bottom of the Point Series table is a graph showing the timing of the protocol. This graph can be hidden or displayed by clicking the green bar at the bottom of the Point Series table.

Running Mark Points

Mark Points protocols can be run in a number of ways, typically synchronized with imaging, in conjunction with electrophysiology recording, or both.

Stand-Alone Mark Points Experiment

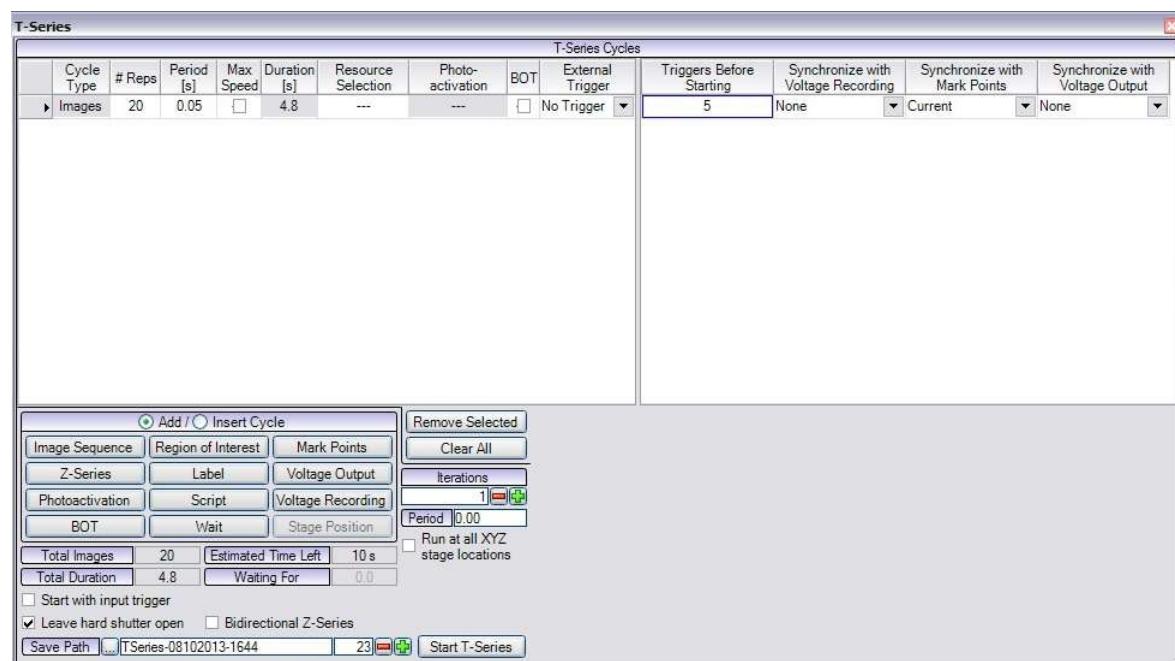
Mark Points can be run alone, which is typically done to verify that protocols are operating properly by using a marker slide or a slide with caged fluorescein. Simply click the **Run Mark Points** button at the bottom right corner of the Mark Points interface.

If more than one line is present in the Mark Points table, and no triggers are set, the entire table will run to completion.

To run a single point as a stand-alone experiment, use the Mark Point at Crosshair controls.

Triggering Mark Points with Imaging or Line Scans

Mark Points is often run with imaging, either full frame or ROI images or line scans. Simultaneous execution of Mark Points with imaging requires that the system have separate light paths (galvanometers and lasers) for the two operations. On systems with only one set of galvanometers, imaging and Mark Points must take place sequentially, rather than simultaneously.



When running Mark Points as part of a T-Series of images, the user can select on the T-Series line on which frame the Mark Points protocol should run. Scrolling to the right in the T-Series Cycles table will show a field for Synchronize with Mark Points. Selecting this field will open a drop down with the options to use the current Mark Points settings or a saved Mark Points protocol. This selection is only available on systems configured with the hardware needed for simultaneous Mark Points with imaging. To the left of the Mark Points synchronization field is the Triggers Before Starting column, in which the user enters the frame on which they want the Mark Points protocols to run. Frame triggers are discussed [here](#).

A Mark Points experiment can also be added to a T-Series as a separate cycle, by clicking the **Mark Points** button to add that type of cycle.

More information about T-Series setup is available [here](#).

In the Linescan Control window, Mark points is synchronized by selecting a Synchronize With option and then selecting Mark Points, with options to run the current configuration or a saved protocol. Note that with Line Scan the Synchronize with option gives two choices, Once at Start or Each Repetition. Once at Start will run the Mark Points protocol once when the **Start Linescan(s)** button is clicked, even if Line Scan is configured to run multiple repetitions. If Each Repetition is selected, Mark Points will run once for each repetition.

More information about line scan acquisition is available [here](#).

Triggering and Synchronization in Mark Points

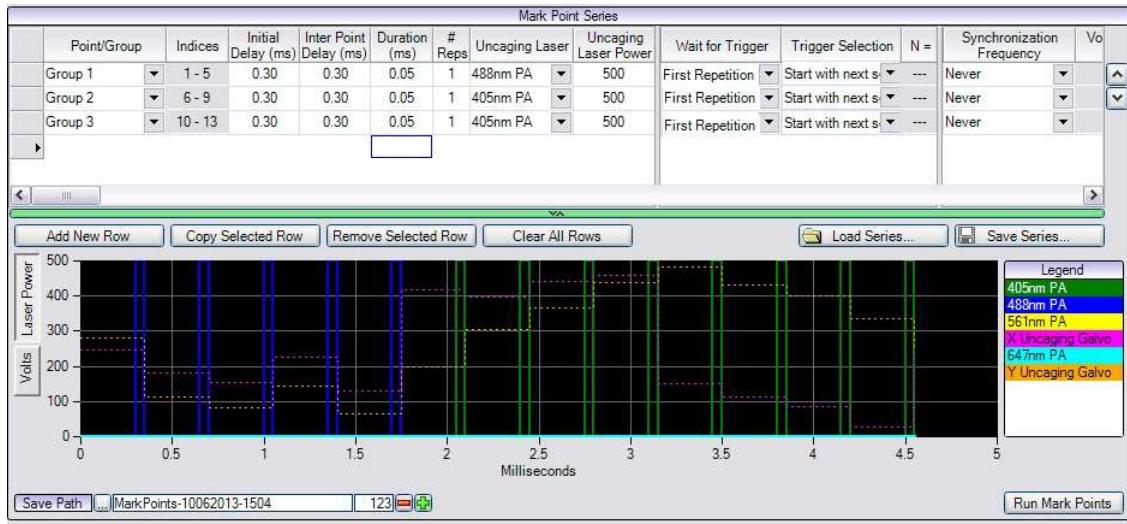


Mark Points has internal trigger variable that can be set to run Mark Points when an external trigger is received, or for more complex experiments involving multiple internal image triggers.

Scrolling to the right in the Point Series table will show the triggering section. Mark points can be triggered in two ways in conjunction with internal image triggers as well as being triggered by external triggers such as those that can be generated by an electrophysiology recording system.

Image triggers are PFI 0 (start of next scan) and PFI 8 (a specific frame). Frame triggers are discussed in more detail [here](#). These triggers are not available if the system is configured to use the same galvanometers for both imaging and Mark Points functions. If PFI 0 is selected for a line, that line will start when the next scan is started, a scan being defined as a T Series line, Live Scan, Single Scan, or a line scan. PFI 0 would be used when individual Point Series lines are to be matched up with individual T Series lines or line scan repetitions.

As an example, a user wants to do an experiment where they want to evaluate the effect of three different sets of points on eliciting a rise in calcium as measured by a calcium indicator. They define 3 groups of points, put each group on a line in the Mark Points Point Series table, and define the laser and timing variables. They set Wait for Trigger in Mark Points for each line to First Repetition and select the PFI 0 trigger.



In T series they set up three T Series lines, in this each with the same number of frames and frame interval. They DO NOT select triggering or synchronization in the T Series line, as the triggering is being set in Mark Points.

To start the experiment, they first click on **Run Mark Points** in the Mark Points window. Mark Points is now waiting to be triggered. They then click **Start T Series**, and the T Series starts to run. The start of each T Series line triggers Mark Points to run the next line.

In another variant of the above experiment, the user wants to have a single T Series, and have three different groups of points run after a certain number of frames. They again set up 3 groups in Mark Points in the Points Series table. In this case they select as a trigger PFI 8, and select the number of frames to wait until the line in Points Series table runs. In this case they are going to run a T Series of 100 frames. They want to wait 25 frames before triggering the first line of the Point Series, and every 25 frames thereafter. They put 25 in as the number of frames for each line in the Point Series table. They click **Run Mark Points** so that it is waiting to be triggered, and then start the T Series.

Using Mark Points triggering with line scans is usually done with repetitions of line scans where a different line in the Point Series table is to be run with each repetition. For example, three groups of points, each to be run with a repetition of line scan. PFI 0 is used for the trigger for each line, and in line scan synchronization with Mark Point is not selected. Line scan would be set to run 3 repetitions. Mark Points would be started, then line scan started. At the beginning of each line scan a line from the Mark Points Point series would run.

Triggering by an external device can also be done from within Mark Points. Again, Wait for Trigger is selected, and the trigger source is selected. The trigger source can be PFI 1, which is the PFI 1 connector on the front of the GPIO box, or Trigger In, which is one of the 8 Trigger In connections on the front of the GPIO box. Again, **Run Mark Points** is clicked to put Mark Points in a state where it is waiting to run.

If there are multiple lines in the Mark Points Point Series, but only the first has a trigger set, the entire Points Series will run when the trigger is received. It is possible to set up protocols where there are multiple lines with triggers and lines without triggers. For example, a user wants to create 9 groups and run them 3 at a time with a line scan. They would create the 9 groups and enter them in the Point Series such that lines 1-3 were the groups for the first line scan, 4-6 were for the second line scan and 7-9 were for the 3rd line scan. On lines 1, 4 and 7 they would set the trigger to PFI 0. They would set line scan to 3 repetitions, click **Start Mark Points** and then start line scan. The first three lines would run with the first line scan, line 4 would wait for the next start of scan trigger from line scan, etc.

Synchronizing with Voltage Output and Voltage Recording



Lines in the Point Series can be set up to synchronize with defined Voltage Output and Voltage Recording. This type of synchronization can provide full control and recording of data for experiments where optical stimulation is being used in conjunction with electrophysiology recording. Voltage outputs can also be used as triggers for external devices, and Voltage Recording can record any type of -10 to +10 V signal.

In order to use Voltage Output or Voltage Recording, Voltage Output and/or Voltage Recording must be configured, or have saved experiments present (see Voltage Output, Voltage Recording).

To use Voltage Output or Voltage Recording, select First Repetition, Each Repetition or Each Point from the Synchronization dropdown. Then select Current or Choose Experiment from the Voltage Output and/or Voltage Recording experiment.

When using Voltage Output through Mark Points synchronization, the waveforms being generated are displayed in the graph below the Point Series. Voltage Recording time is displayed in the graph as a red overlay.

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

Voltage Recording

This feature is new in Prairie View version 5.0. It replaces some functions formerly carried out by TriggerSync software. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

Seal Test

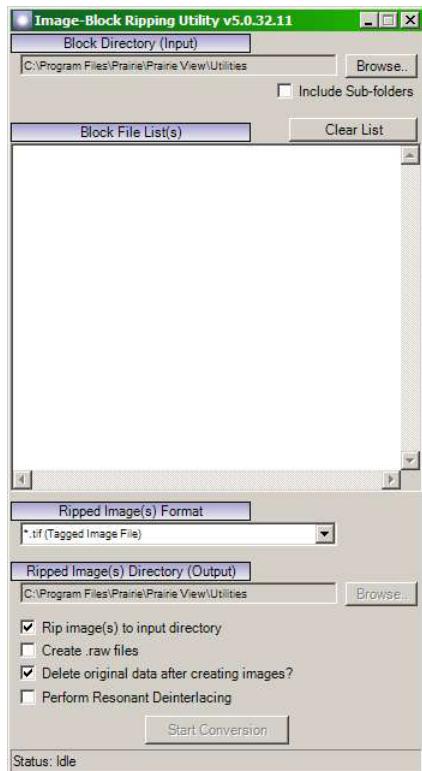
This feature is new in Prairie View version 5.0. It replaces some functions formerly carried out by TriggerSync software. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

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.

Spiral Activation

This feature is new in Prairie View version 5.0. This feature is fully implemented, but the Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

Functional Mapping

This feature is new in Prairie View version 5.0. The Help file description has not been completed. Please contact Bruker Fluorescence Microscopy support personnel with questions about this feature.

Help Menu

Keyboard Shortcuts: Opens a dialog to display the list of keyboard shortcuts available on the system

Luis & Neumann Technical Note: Provides information specific to systems configured with a stage manufactured by Luis & 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 FM personnel to remotely access the computer for service and support; ensure an internet connection is established and [contact](#) Bruker FM support personnel to set up a session

About: Provides information about system device firmware and the Prairie View license agreement

Multi-User Systems

By creating separate user profiles in Windows, Prairie View can keep track of usage and settings for multiple users. By default, a number of settings are saved at the user level including labels. Other settings will retain the same values as the last time Prairie View was run. It is possible to customize which settings are user specific and which are not in the 'CustomUserKeys.txt' file found in the 'Configuration' folder.

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.

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