🌐 Zeiss LSM880 with Fast Airyscan Guide

Condon ND[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
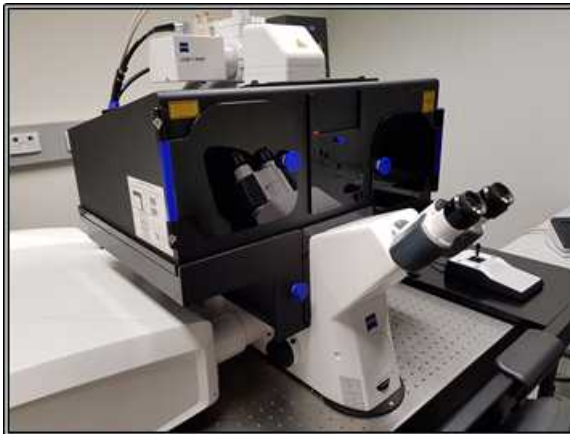
Oct 01, 2021

| 1 | *Works for me* | | ⤳ Share |

This protocol is published without a DOI.

Condon ND

ABSTRACT

PROTOCOL CITATION

Condon ND 2021. Zeiss LSM880 with Fast Airyscan Guide. **protocols.io**
https://protocols.io/view/zeiss-lsm880-with-fast-airyscan-guide-byp9pvr6

CREATED
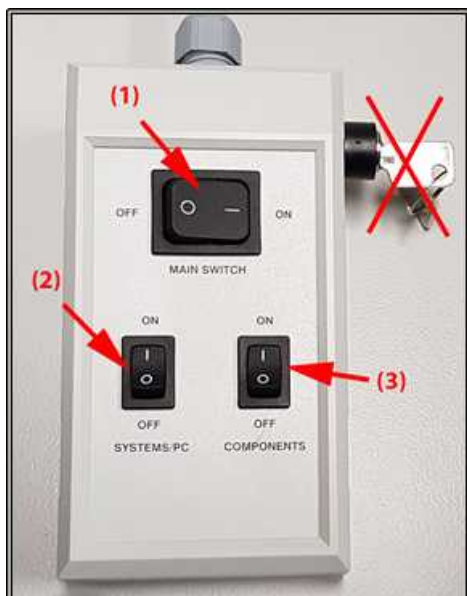
Oct 01, 2021

LAST MODIFIED

Oct 01, 2021

PROTOCOL INTEGER ID

53729

System Start-up

1

1. -Turn the Main switch **ON (1)** You should not have to touch the key since this should never be turned off
2. -Turn the Systems/PC switch **ON (2)**
3. **-** Turn the PC **ON** and wait for it to boot up completely
4. -Log in to the PC using your AD login details
5. -Turn Components **ON (3)**
6. **-** Double-click the ZEN Black icon on the desktop
7. -Click the Start System button and wait for the software to load

Incubation

1   The incubation on the 880 should always be set at 37°C.If you require a different temperature, the cool-down/warm-up times need to be factored into **YOUR** booking time and you are responsible for ensuring the temperature is back at 37°C **AND** stable before the next user is booked on.A booking for a different temperature can be logged while creating your session booking in PPMS.

For CO_2, this can be switched **ON** at the start of your session and set to your desired concentration through both the software and the touch-pad controls.Please make sure to turn the CO_2 **OFF** again once you have finished your session.
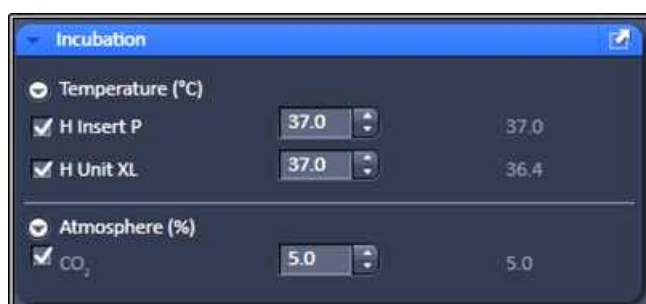
- Incubation controls can alter temperature for both the chamber as well as the heated sample insert
- The controls can also turn the CO_2 ON/OFF and alter the levels for the concentration

Microscope Controls

2
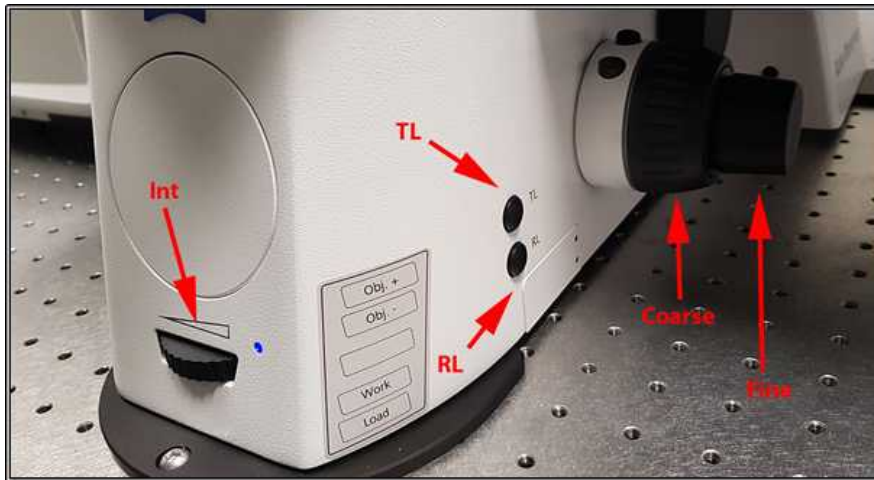
- Brightfield light source can be adjusted manually by rotating the wheel (**Int)**
- Open and close the shutters using the **TL** (Transmitted Light) and **RL** (Reflected Fluorescence Light)
- Focus the objective using the **Coarse** or **Fine** focus knobs
- Controls are repeated on both the microscope stand itself as well as the remote touch-pad
- Select the "Microscope" option on the left hand side to enter hardware control
- Controls for the hardware can then be accessed through the software
- The touchpad controls can be used to change between the objective lenses automatically

Warning messages will pop up if changing between lenses of differing immersion media (e.g. between oil and water lenses)

- The touchpad also contains Reflector turret options allowing to rotate between fluorescent filter cubes such as DAPI, GFP or DsRed
- Incubation controls can also be accessed on the left hand column of options
- Press the Load Position to lower the objectives away from your sample at any time

Finding your sample with the Eyepieces

3   Make sure you have the **Locate** tab selected
Click on the relevant short-cut button for the fluorescence channel you need (**arrow**)
- Select the BF button for bright-field
- The colours refer to the wavelength range of the fluorophores

- Find your sample and focus using the eye-pieces, coarse/fine focus and joystick
- The top-right button labelled F1 on the joystick pad switches between coarse and fine stage movement
- Once you have finished looking at your sample ALWAYS turn your shutters OFF to stop any sample bleaching

- Despite having the shortcut buttons, there are also controls for individual components
  -**Obj** will allow users to change to a different objective lens
  -**Filter** will allow users to manually select the desired fluorescent filter cube
  -**Shutters** can be used to manually open and close both the brightfield and fluorescent light source shutters

Applying Oil for a Slide

4
1. Open the incubation chamber and tilt the microscope turret back
2. Remove the slide and rotate the oil objective into position with the software
3. Place a small drop of the correct oil onto the middle of the slide or very carefully place a small drop onto the centre of the objective lens
4. Replace the slide, coverslip side down, lower the turret and close the chamber back up
5. **DO NOT** over oil the objective as this will cause oil to run down the sides and into the housing of both the objective AND the objective turret
6. To remove the slide simply open the incubation chamber and remove it from the stage insert
It is recommended to wipe away excess oil with the lens tissue (NOT Kim-wipes) provided and apply fresh oil between each sample

When finished make sure to clean the objective before leaving

Setting Up Kohler Illumination

5
Kohler Illumination is an important setup procedure for optical microscopy giving even illumination and reduced sample heating/bleaching

1. To start with, select the BF button on the Locate tab to get a brightfield image
2. Fully close the Field Aperture (at the top of the turret head)

3. Focus the light source using the Condenser Focus knobs until you can see a sharp outside ring of the field aperture while looking down the eyepieces
4. Ensure the light is in the middle of the field of view by adjusting the two silver centring screws at the front of the condenser
5. Once centred, open the Field Aperture until it just fills the whole field of view but no more

This should be repeated when you change objective magnification as it is often different between lenses

Acquisition Mode

6   Click on the Acquisition Tab to change the light path setting to the confocal scan head

- If not already done so, click the Show All Tools tick box to show the Light path and laser settings – turn the Argon laser **ON** if it is required and allow it to warm up
*The Show All tick box in the top right of each settings window can be used at any time to display more advanced features. You can also go to the View option in the top menu and select Show All Global to activate this feature for all windows at once*

Turn **ON** any other lasers that you will require for your imaging session

Customising Your Workspace
- Much of the screen layout of ZEN can be customised to some extent:
- You can click and drag on a tool group (the grey headings between the blue tool menu windows) to different columns
- Individual tool menu windows (i.e. Z-stack or Tile Scan) can be repositioned anywhere in the screen and sent back to the original position using the diagonal arrow in the top right-hand corner of the tool window
- Change the display size by sliding the Workplace Zoom tool in the top right corner of the ZEN software window
- Once you are happy with your layout you can save this using the Workplace Configuration save button in the top right corner of the ZEN window, just below the workplace Zoom slider
- You can then load your pre-configured layouts at the beginning of your session

Smart Setup for Basic Acquisition
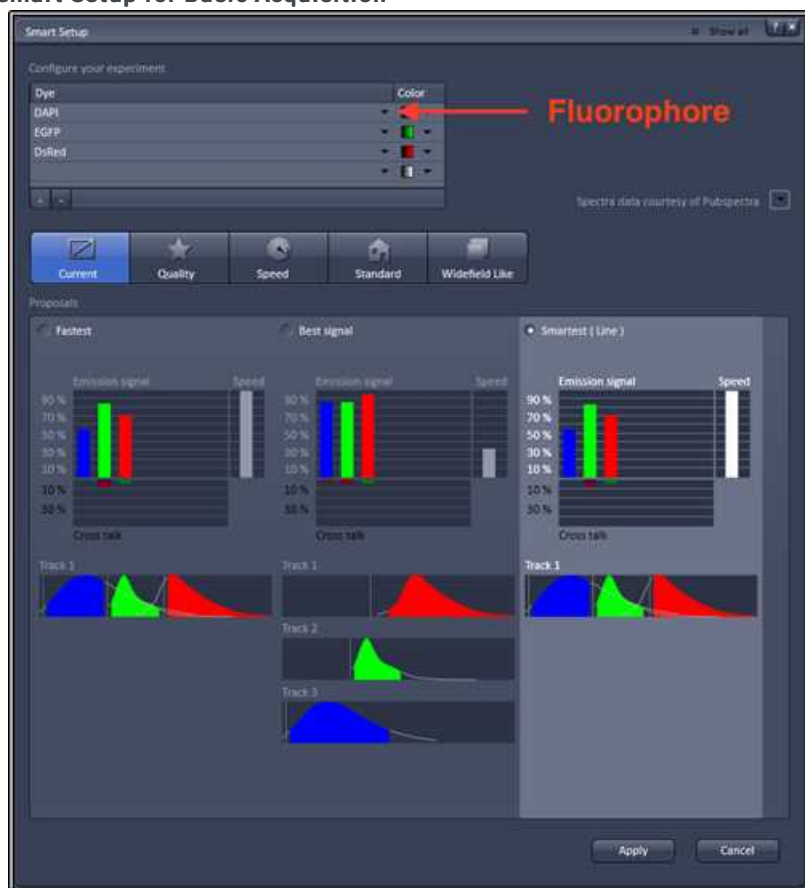
Smart setup allows users to perform an auto-setup by simply selecting their desired fluorophores and the mode of imaging required

1. Click the Smart Setup button to enter a guided setup procedure (A new window will open containing option for fluorophores and types of setup)
2. Choose your first fluorophore from the drop-down list (**Fluorophore**)
3. Add any other fluorophores you have, up to a maximum of 4
4. Adjust the assigned colour of each fluorophore if desired
5. Choose the correct mode of imaging between Fastest, Best Signal or Smartest (Line)

  Fastest will image all dyes at the same time but has the highest cross-talk

  Best Signal creates separate tracks for each colour - slower but the best separation

  Smartest (Line) uses line switching between tracks - good dye separation while still showing all colours at once BUT does have some compromises
6. Click **Apply** when done and the software will automatically set up the imaging channels

Manual Setup for Basic Acquisition

To add a new track to your experiment, click the "+" button on either the Channels or Imaging Setup tool windows (**New Track**)

1. Select the detector you wish to use by ticking the Use box in the line of Ch1, ChS1 or Ch2 in the Imaging Setup window

  -Ch1 and Ch2 are GaAsP PMTs

  -ChS1 is the 32 GaAsP Spectral array

  -ChS1 can be used as a spectral detector or split and combined into separate detector tracks (at a compromise of detector gain flexibility)
2. Adjust the detector range (the white bar above the detector selection) to select specific emission wavelength ranges

If you need assistance in knowing where to place this select the appropriate fluorophore from the Dye dropdown list next to the detector you have ticked – a spectral profile graphic will then appear showing the peak emission wavelength range you need

You can alter the track name under the Tracks column and can change the track colour at the right side of the track, both in the Channels window

3. Tick the desired laser wavelength in the Channels window to add this laser to the track

4. The laser power slider will appear – set this to 2% power

5. Select the appropriate dichroic mirror for the laser you are using (**Dichroics**) in the Imaging Setup window

6. Adjust the pinhole to the correct size for the selected wavelength by clicking the 1AU button in the Channels window

You may find that the smart setup uses a larger pinhole as a starting point, this allows for easier sample location and you can be done for your manual setup too, though optimal pinhole will always be 1AU for your confocal imaging

7. Set the Gain (Master) to around 500 as a starting point

8. To add further tracks, repeat the process above for each additional fluorophore you wish to use as many times as you need for the fluorophores you have on your sample

   -Highlight the individual track in the Channels window to edit settings for this specific track

   -It is possible to have multiple channels on a single track using more than one detector at the same time

   -When using a single colour, it is recommended to use greyscale due to the higher sensitivity of viewing this combination, though for multiple tracks assign the desired colour to each channel individually using the LUT dropdown list at the right side of the Channels list

   -When setting up multiple tracks keep cross-talk/bleed through in mind

   -Separate out channels that may have crosstalk into separate tracks (for example, DAPI will tend to show up in a GFP channels if both lasers are on so should be placed in separate tracks)

   -Colours that have closely overlapping spectra may be difficult to separate out without more complex spectral un-mixing tools

Acquisition Mode

Once you have set up your channels go to the Acquisition Mode window and set up the image settings

1. Set the Pixel resolution of your image
2. 512 x 512 and 1024 x 1024 are the most common options for routine imaging
   -Be aware that the more lines = slower scanning
   -Optimal uses "Nyquist" sampling
3. Click Max for scan speed (if you have a noisy image you can drop the speed one or two levels to help improve signal : noise
4. Set the bit-depth of your final image
   -8-bit = 256 colours; 12-bit = 4096 colours
   -16-bit has 65,535 colour values but is **NOT** recommended
5. Adjust the amount of averaging you wish to use for your images
   -Averaging (be it frame or line) helps to improve your signal : noise by imaging your sample multiple times and taking the average intensity of each pixel across all those images
   -You can swap between Line and Frame and even change to Accumulation (adding the values rather than averaging them) by clicking the Show All button in the top right of the Acquisition Mode window, revealing further options
6. Bi-directional Scan mode can be revealed with the Show All button, this allows scanning in both forward AND backward scan directions, increasing scan speed, be careful to note any misalignment in the scan directions when they are interlaced together and adjust the calibration directions if required

By clicking the Show All button, you will also gain access to a number of translational and rotation options in the Scan Area section, allowing you to move the area to be imaged and rotate it

7. Zoom will zoom in to smaller areas, effectively changing the resolution of your image without increasing your pixel number at the cost of a smaller field of view
   -A 2x zoom is equivalent to changing from 512 pixels to 1024 pixels
   -The Reset All button can be used to reset all the translational and rotation changes, as well as the zoom factor, back to their default values

Capturing your first image

1. Click the Live button to begin a scan of your sample
   -This will give you a fast, repeating frame of the image as per the channel setup
   -In line switching mode all channels will be included in the scan immediately
   -If you have set up multi-track but are using frame switching, this will capture a single track before switching the settings and capturing the next track, this can be clucky and it is recommended to untick all but one track and adjust track settings individually
2. Adjust your focus so that the brightest part of your sample is in focus, remember the confocal will block out of focus light so the sample needs to be in the focal plane correctly before continuing
3. Adjust the Gain (Master) and Laser power to increase the brightness of your signal
   -Find a balance between the two; too much laser power increases bleaching while too high a gain increases background noise (try and stay below 850 gain)
   -Avoid going too high – overexposed pixels are lost information! Use the Range Indicator tool to check for overexposed pixels that will show up in red
4. Adjust the Offset to set the background cut-off – again be careful not to go too far or you could remove real signal if it is weaker, (use the range indicator here as well to show 'Zero' pixels in blue)
5. If setting up for multi-track, repeat the process for each channel in turn
6. Set the image acquisition settings such as pixel count, averaging, speed etc.
7. Stop the Live scan and then click Snap to acquire your image
8. Save the image out using the save icon or through the File > Save options in the top menu
   -To preserve hardware information and scaling save as the ***.CZI** file format

Advanced Imaging Setups

7   You can combine any of the advanced setup options together, such as Z-Stacks, Time-Series or Tile Scan to create more complex experiments. To activate any of these options simply tick the setting you wish to have added under the Acquisition Tab at the left-hand side.

Z-stacks
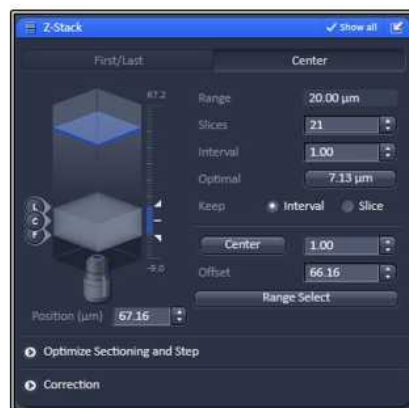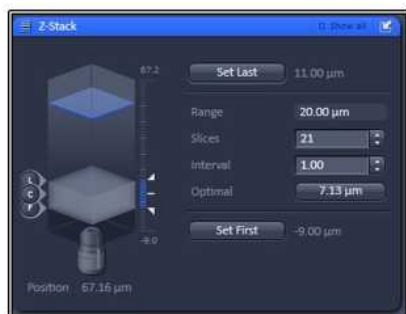
1. Click the Live button to get an updating view to assist in focussing
2. elect the range of your 3d Z-Stack
   -For First/Last method, start by focussing to the top of your object and click the Set First button, next focus to the bottom of the object and click Set Last
   -For Center method, focus to the middle of your object and click the Center button
   Note that the Center method is only available once the Show All box is ticked
   Center method is ideal for multi-position acquisition where the Center of your Z-Stack will be taken as the Z co-ordinate of your position
   *Range Select will perform an X-Z scan, showing the sample from a side-view with three lines; two red lines represent the top and bottom of the stack while a green line represents the centre of the scan – these lines can then be moved to*

adjust the Z-Stack settings

3. Click Stop to halt the Live View

4. Set the Z slice thickness by either manually entering the Interval size in microns or by clicking on the Optimal Button
   -In the example shown, the optimal size is set to 7.13um – this is approximately Nyquist sampling, but should you wish high accuracy it is recommended to use the Nyquist calculator to find the exact distance required
   -You may choose to use the Slice number rather than Interval size

5. Click Start Experiment to begin acquisition (**NOT** Snap as before), the experiment will capture all channels set with all 3d slices

NOTE: You can also select to capture all channels for each slice or a more efficient way is to capture a full Z-Stack for a single colour before proceeding to the next track

Time-Series
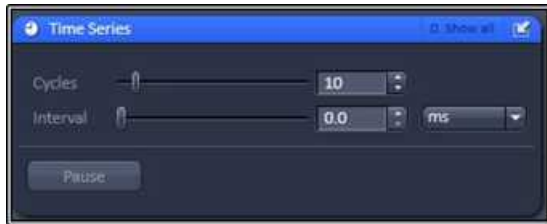
1. Activating the Time Series option allows users to set up for time-lapse imaging to capture movies of live events
2. Set the Cycle number (this is the number of times the acquisition setup will repeat)
3. Set the Interval (this is the time the system will wait between capturing one image and starting the next)
   -Use 0.0 as your value if you wish to image as fast as possible
4. Click Start Experiment to begin the time-lapse capture

Positions
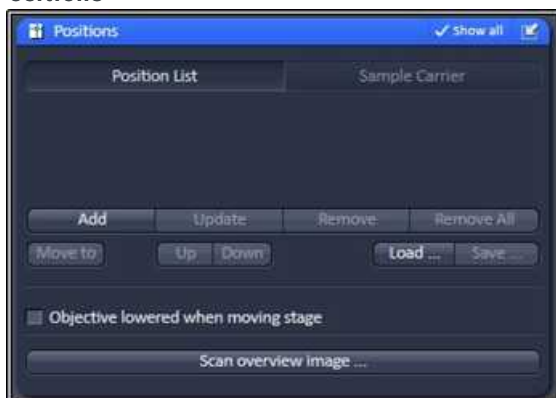
Adding multi-point coordinates allows for more efficient time-lapse imaging, granting multiple data points for the same time spent on the microscope

1. Activate the Positions option
2. Move to the cell or sample area of interest and focus using the Live button
3. Click the Add button to add the X, Y and Z coordinates of this are to the Position List
4. Repeat the process for any subsequent areas you wish to image
5. Should you wish to change any position, click on the point in the list, click the Move To button and readjust the position, click Update to overwrite this position with the new coordinates
6. To remove a single position, click on the position in the list and then click the Remove button to delete it from the list
7. Remove All will delete the entire list of positions
8. Use the Save button to save the coordinate list and Load button to import and reuse a previously saved positon list
9. Scan Overview Image allows the user to scan a large area at low resolution and utilise this as a quick template scan that can be used to more easily identify positions of interest
The Sample Carrier section allows templates to be used to assist navigation

Tile Scans
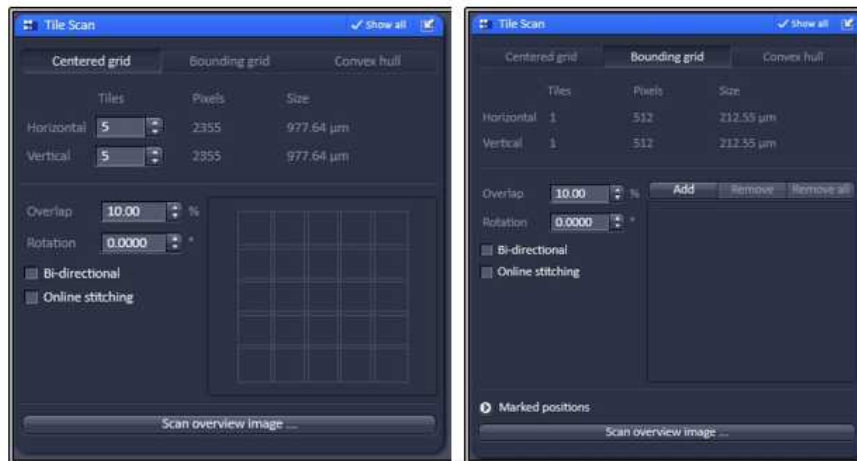
This feature allows multiple smaller fields of view to be stitched together to form one large image, giving users the ability to have a much broader overview of their sample while maintaining the resolution of the current objective.

1. Select the Tile Scan option
2. Use the Centered Grid tab to create set size mosaics
   -Set the number of horizontal and vertical tiles required – these do not have to be symmetrical
   -The software will use the current stage position as the centre of the tile
   -Bounding Grid allows the user to set specific X-Y coordinates to include in the final tile scan
3. Move to the first point of interest to include in the tile and click the Add button
   -Repeat this move and Add procedure for all remaining points of interest to include
   -The software automatically creates a larger tile to encompass all of these coordinates
4. Convex Hull works in the same way as Bounding Grid but will exclude any tiles the software deems not required, saving time by avoiding the need to scan blank areas or areas of no interest
5. Set your Overlap size to 10% minimum, this allows the stitching software to match the repeating patterns to provide a "pixel perfect" final image
   -Avoid using Online stitching, this sometimes fails so it is better to manually stitch post acquisition
6. Click Start Experiment to begin the tile scan acquisition
7. Once completed go to the Processing tab, click on the Stitching method, select the current image and click Apply to create a stitched version
   -You may wish to also select the parameter to create a New Output for the stitched image rather than replace the raw data

Bleaching (FRET & FRAP)

Activating the Bleaching module will also activate Regions and Time Series as well since these will be required also

To deactivate Bleaching you will still need to untick all of the other modules individually

1. Use the regions tool to select a specific area of the image, you may select any size or shape you require and even add multiple regions
   -The region can be used for bleaching a specific area but can also be used for analysis of that area too
2. Use the Start Bleaching after # scans to capture a set number of images before the bleach step (a "before" snapshot)
3. You may also repeat the bleach after a certain number of images
4. Set the number of iterations for the bleach step
   -More iterations mean a longer bleach step which may be required for samples that you are struggling to bleach effectively
5. Use the Different Scan Speed option to slow down the bleach step
   -Slower speed means longer bleach step and longer pixel dwell time, resulting in a more effective bleach step
   -Safe bleach for GaAsP allows for extra protection for the more sensitive detectors
6. You may also choose to bleach in a different Z position
7. Use Zoom bleach if you want a faster bleach
   -Note this is a rough scan and therefore won't be as accurate to your ROI of choice
8. Tick the laser you wish you use to ON and set the power, typically use 100% for the bleach laser
9. You can also choose to assign different lasers to different ROI's
10. Set the Time Series up to a number of repetitions to suit your experiment, this needs to be set to 3 or more for a successful "before" and "after" image experiment
    -Failure to set this properly will result in a single bleach step but no other imaging
11. Click Start Experiment to begin the bleach experiment
12. Use the Mean ROI tool in the Image Window to display an analysis of the ROI over the time course of the experiment
    -For typical FRAP studies you should see an initial level of signal for the "before" images followed by the bleach step causing a decrease in signal, then a recovery of the fluorescence over time
    -Note the recovery will never reach 100% and instead plateaus after a while at a lower intensity than the original
    -You may also have a FRET analysis tool available that will analyse the "before" and "after" intensities and perform a ratio-metric study of Acceptor Bleaching FRET

Saving and Transfering Files

8   -Any files that are unsaved will show on the right-hand side image list with a small yellow exclamation mark sign next to it
    -If you close all windows or close ZEN, the software will warn you of any unsaved files and give you one last chance to save them (ensure they are ticked and you will be presented with a save window)
    -To manually save a file:
       1. Ensure the file is the active window (double click the file on the right-hand side list or click the tab of that file in the image window tabs
       2. Click the save icon on the right-hand side or alternatively use the menu option File > Save or File > Save As
       3. Enter a suitable name for the file and select the location to save it to and click OK
    DO NOT use spaces or non-alphanumeric characters in your filenames
    NEVER save to the C: drive – always save locally to the Data drive
    Make sure to give your file a meaningful name
    Write dates in filenames as YYMMDD (i.e. 190129) to assist in chronological ordering under Windows
    Always save as a *.CZI file where possible; **NEVER** save as a JPEG

    Once you have finished your imaging session, transfer all your recent files to the network drive of choice

    Remember, the acquisition computer is **NOT** backed up and are regularly wiped of old files so make sure you copy the files off **IMMEDIATELY**

Restoring Previous Settings

9   It is vitally important when wanting to have any quantitative analysis performed on your data sets that you maintain the same settings consistent across images

    Saving out as a *.CZI file format creates a file containing a lot of metadata associated with the hardware of the system
    To reuse settings using this metadata, load in your saved image and click the Reuse button in the ZEN software
    -This will write all the settings to the software and you can use the system as if you were still in the same imaging session

-Be aware that these settings are not transferable between microscopes as they may have variations on hardware configurations

You can also save out your settings to the drop-down experiment settings folder at the top of the ZEN settings window to avoid needing access to saved images
-This will be saved for your profile so not available to other users

Shutdown

10

1. Turn the lasers **OFF** in the software
-Wait until the cooling fans stop for the Argon laser (up to 5 min) before turning the rest of the hardware off
-Save your files and transfer them to the network drive while you are waiting
2. Exit the software and wait for 1 min for all background processes to finish closing
3. Remove the sample and carefully clean the objective lens of any oil using the lens tissue provided (please **DO NOT** use Kim-Wipes for this)
4. Shut down the PC once the lasers have cooled
5. You should hear an audible drop in the noise of the Lasos Argon laser fan
6. Turn the Components switch **OFF**
7. Turn the Systems/PC switch **OFF**
8. Turn the Main Switch **OFF**
   DO NOT turn off the Lasos Argon laser (both the key and the power switch should be left on)
-   **DO NO**T turn off the laser interlock key

Please remember that the optics on these microscopes are very delicate, please treat the microscopes gently and with respect – they are **VERY** expensive to replace and may require significant down-time of systems as well as high cost to the facility so please **BE CAREFUL!**