THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
CREATE CHANGE

Confocal 6

ZEISS LSM 990 BIG

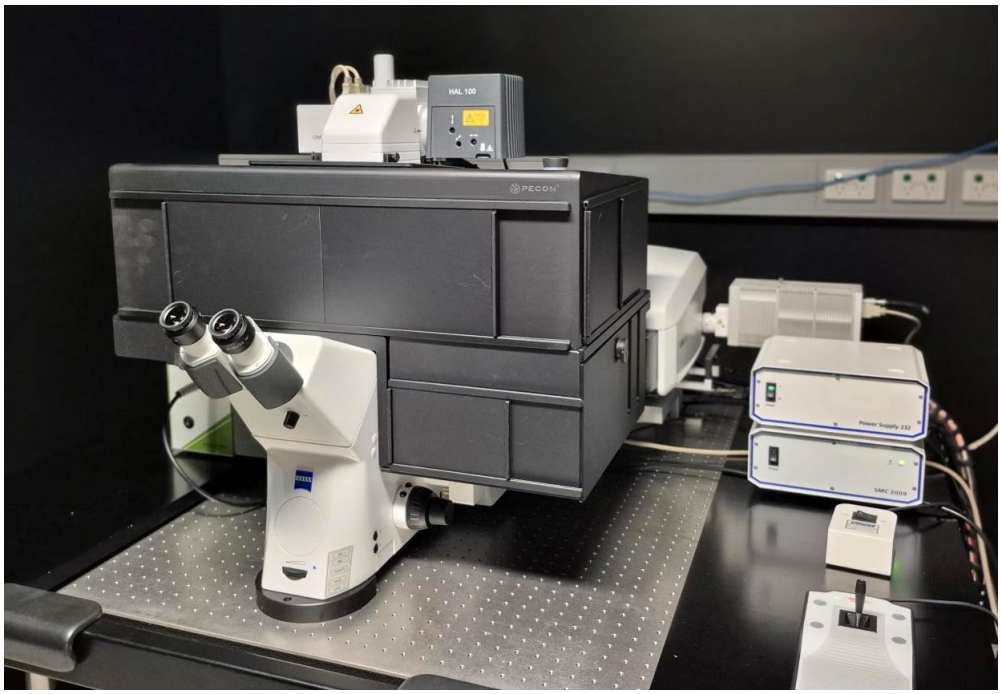
Quick-start Guide

Location:
Training Microscopes
Room 6.019A

(Updated: 25/2/2026)

Acquisition Software:

Zeiss Zen Blue



SYSTEM HARDWARE SUMMARY

Stand - Zeiss Axio Observer
Scanner – LSM 990 with BiG.2 External detectors
Stage – Piezo XY, Nosepiece Z-drive
Lasers – 405, 445, 488, 514, 561, 594 639nm
Detectors – Internal 3 GaAsP detectors, t-PMT, 2x External BiG.2 (GaAsP with photon counting)
Environment – Pecon XL Incubation, Heated insert-P, Heated Humidity, CO_2 control

For more information go to the IMB Microscopy Website (https://imb.uq.edu.au/microscopy)

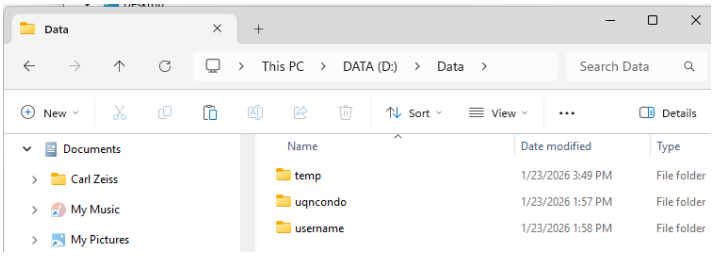
Be mindful of what you are doing at ALL times: The microscope and the lenses are delicate and very expensive and should be treated with care and respect.

This microscope was funded in part by a UQ-RIIS (Research Infrastructure Investment Scheme) in 2025.

SAVING YOUR DATA

Data on the microscope PC is not backed up and is routinely auto deleted with scripts

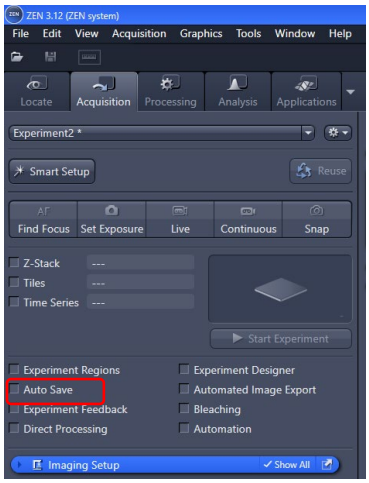
When acquiring data, save it to the following location **D:\Data\<username>**

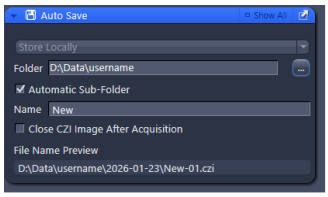


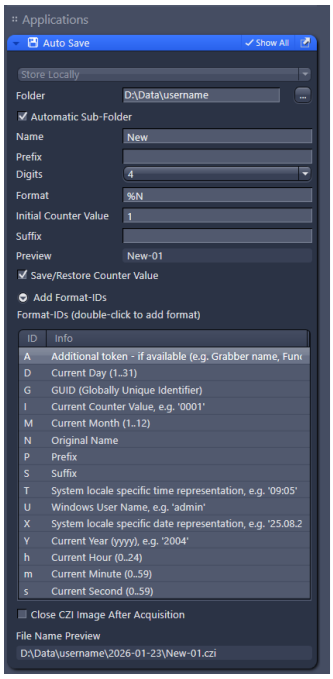
DO NOT save directly to the network, only transfer your files at the end of your session.

DO NOT use external thumb drives or hard drives

We recommend using the **AUTOSAVE** functionality built into Zen Blue. Tick the checkbox for AutoSave to enable the menu under the Applications section.





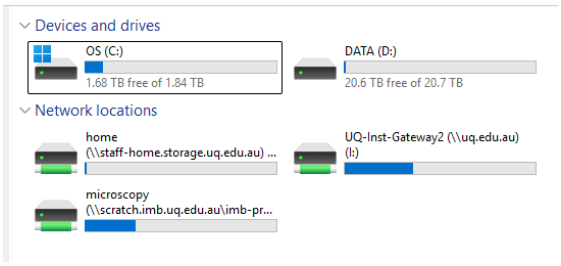


Ensure the Folder path is correct and enable **Automatic Sub-folder** option and choose a suitable file name.

If you check **Show All** additional information can be used to generate your file name as shown on the right.

Connecting to Network attached Storage

- Data you acquire on this device should be backed up to a server for future retrieval and use.
- Note you need UQ-SSO credentials to access data storage drives



UQ RDM Collections

- Can be accessed via the Network location (UQ-Inst-Gateway1/2)
- Can be mapped locally (\\data.imb.uq.edu.au\<shortcode>-Qxxxx)
- Can be mapped from home (\\shares.rdm.uq.edu.au\<shortcode>-Qxxxx)
- Note: it is not advised to use “A” collections, or the cloud.rdm instances

Scratch Drive Options

- IMB Transfers (not backed up/deleted every 7 days) \\transfers.imb.uq.edu.au\transfers
- Microscopy Scratch (not backed up) \\scratch.imb.uq.edu.au\imb-projects\microscopy

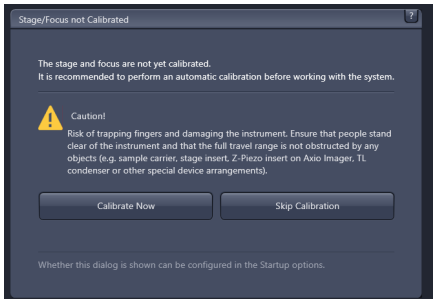
IMB Advanced Microscopy Platform does not recommend using H: drive (Home).

SLIDE CLEANING AND PREPARATION

- Make sure your slide is scrupulously clean – all dust, old oil and mounting media should be removed with a small amount of 70% ethanol.
- If you can use a hard setting mounting media, please do so and DO NOT use glitter nail polish to seal your slides.
- Be sure to give your mounting media and sealant at least 8 hours to dry properly. **Do not bring wet slides to the microscope.**

START-UP PROCEDURE

- **Check the microscope** to be sure it is clean. You are responsible for the condition of the microscope at the close of your session.
  If you find it dirty, make sure microscopy staff know about it; that is, email, phone us or by recording an incident in RIMS/via the control panel on the microscope.
- **Turn on the Main Switch** on the front of the laser rack
- **Turn on the PC** located to the right of the microscope air table
- **Log into the computer** using your UQ username and password
- **Turn on the microscope** using the single power switch on the right shelf.
- Wait at least 1 minute after logging in to allow windows to fully start
- **Open Zen Blue 3.12** software on the desktop
- **Click Start System** when prompted to connect to the microscope.
- If you require tiling or positions perform a **stage calibration**

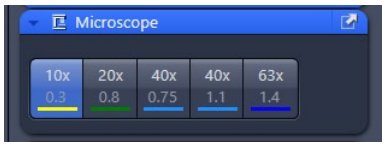


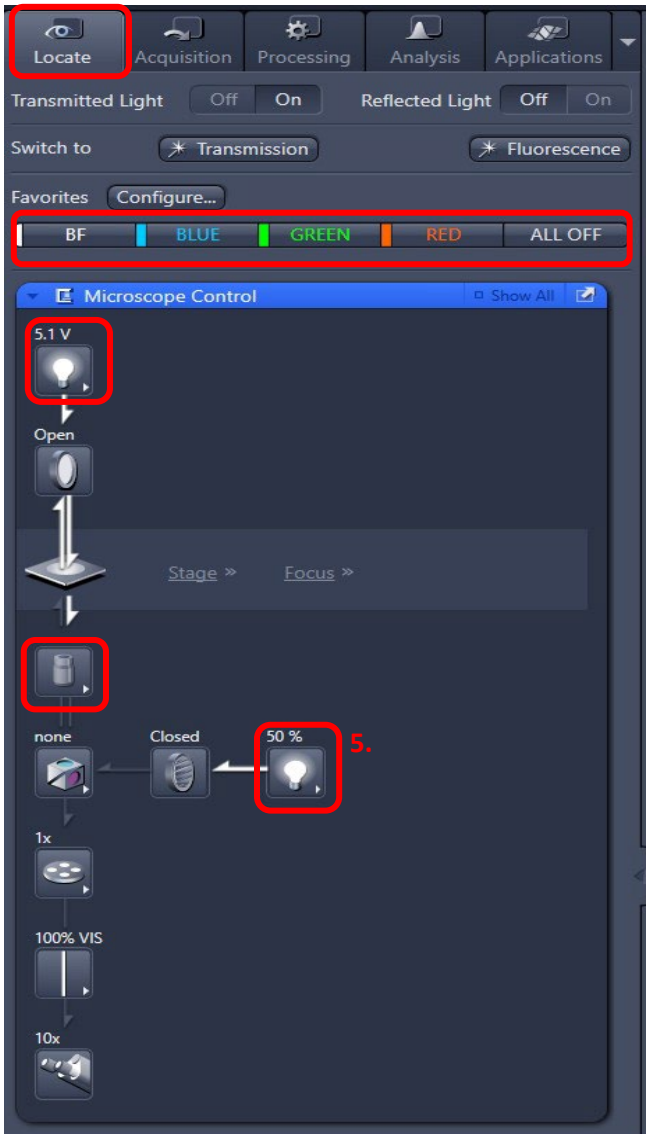
Note: If you perform a stage calibration first ensure the 10x objective is selected and the objective is lowered to the minimum Z-position as this procedure results in high-speed movement of the stage and can result in a collision with an objective.

LOCATE TAB

The **Locate Tab** is used to find your sample with the eye pieces.

1. Click on the **Locate Tab** at the top left of Zen
2. Ensure the Correct Objective is selected either on the **TFT touch screen (under Microscope > Optical > Objectives),** in the **Microscope Control** menu or via the **Objective** menu on the right hand side of Zen.





3. Choose your illumination method by selected one of the buttons under the **Favorites** presets (BF, Blue, Green, Red). To turn off the illumination light, select All Off.
4. If required, the brightness of the **Brightfield** lamp can be adjusted either in software or by adjusting the black dial on the front of the microscope stand.
5. If required, the brightness of the **Fluorescence** lamp can be adjusted in the software.
6. Set the eye pieces for your comfort.
7. Find focus of your sample using the eye pieces, you can use the joystick to move in XY. **F1 button** (top right) on the joystick will toggle between two different speeds for that particular objective.

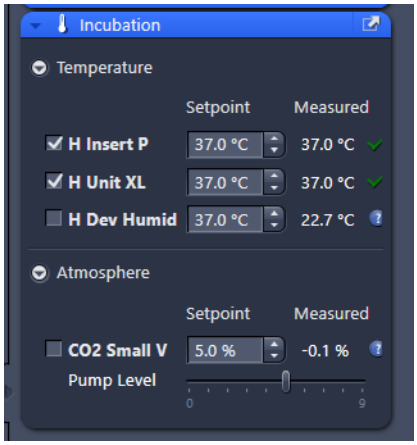
*Note: Do not select the **Switch to Transmission*** or **Fluorescence** buttons at the top of the locate panel.

*Note: No other buttons on the **Microscope Control** window should be touched or changed.*

Incubation

The incubation on **Confocal 6** 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 RIMS.

The humidifier for the chamber has an independent temperature controller which can be controlled both in the software and the touch-pad controls. This can be used to fine tune the heating and humidity of the sample.

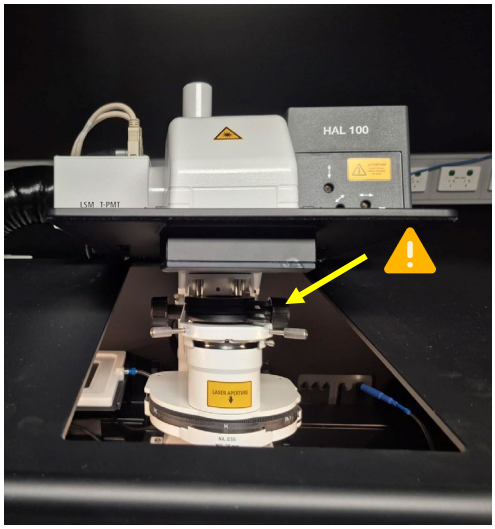


For CO_2, this can be switched **ON** at the start of your session and set to your desired concentration through both the software and the touch-pad controls. Please make sure to turn the CO_2 **OFF** again once you have finished your session.

The temperature of the components and the CO_2 level can also be adjusted in the **Incubation** in Zen, or via the **TFT touch screen (under Microscope > Incubation)**.

Note: If you need to top up the water for the humidity bottle, only use RO-water from the Milli-Q system in the communal instrument room (6.015).

Applying Immersion to an objective



- Open the incubation chamber and tilt the microscope turret back being careful to ensure the swing-in filters are in the correct position and won't strike the incubation chamber.
- Remove the slide and rotate the immersion objective into position with the software
- Place a small drop of the correct immersion very carefully onto the centre of the objective lens
- Replace the slide, coverslip side down, lower the turret and close the chamber back up.
- **DO NOT** use too much immersion fluid on the objective as this will cause the fluid to run down the sides and into the housing of both the objective AND the objective turret.
- To remove the slide simply open the incubation chamber and remove it from the stage insert.
- Clean the objective after use, using only single strokes with clean lens tissue (no ethanol).

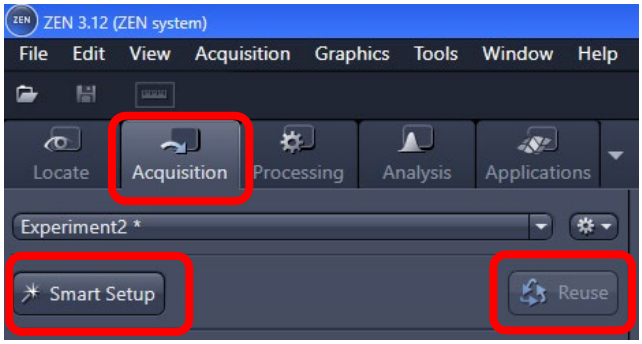
Setting Up Kohler Illumination

- Kohler Illumination is an important setup procedure for optical microscopy giving even illumination and reduced sample heating/bleaching
  - To start with, select the **BF** button on the **Locate tab** to get a brightfield image
  - Fully close the **Field Aperture** (black dial at the top of the turret head)
  - 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
  - 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
  - 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 TAB

The **Acquisition Tab** is used to capture and save images

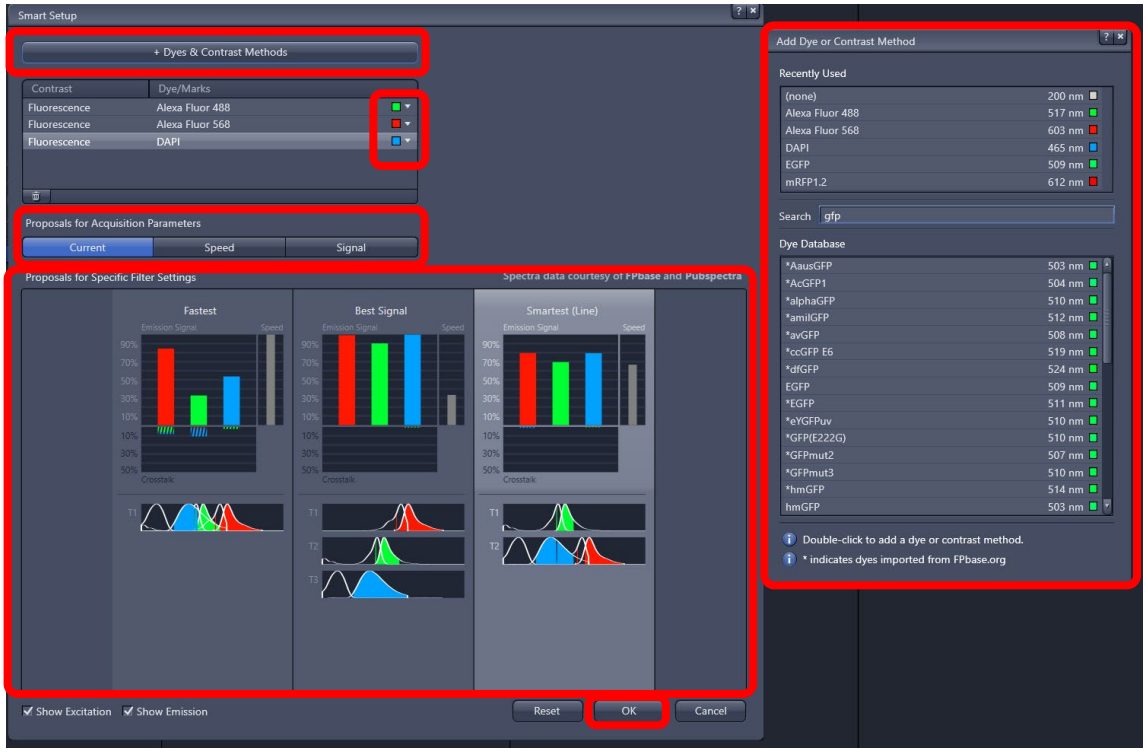
1. Click on the **Acquisition Tab** in the top left of Zen
2. For most users, **Smart Setup** is the best course of action to set up their experiment. Click the **Smart Setup** button at the top of the **Acquisition Tab.** *Note: If you have a file acquired previously on this microscope, you can **Open** the image file and click **Reuse** to re-load the previous settings.*



Smart Setup

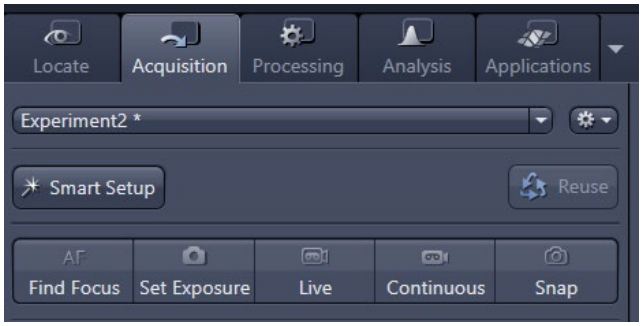
Smart Setup is a tool to help design your experiment.

1. Add your dyes and contrast methods by clicking **+Dyes & Contrast Methods** button
2. From the database of fluorophores select those that best match your experiment from the popup window
   Note: You can choose specific look up tables for each channel by selecting the arrow next to the coloured box
3. If required, select a **Proposal for Acquisition Parameters** such as Current, Speed or Signal.
4. Choose the relevant **Proposal for Specific Filter Settings** such as **Fastest** (all dyes imaged at once), **Best Signal** (all dyes imaged sequentially) or **Smartest (Line)** (scans spectrally close dyes together sequentially, while scanning dyes that are far apart together)
5. Click **OK** at the bottom of the window to apply the chosen proposals.



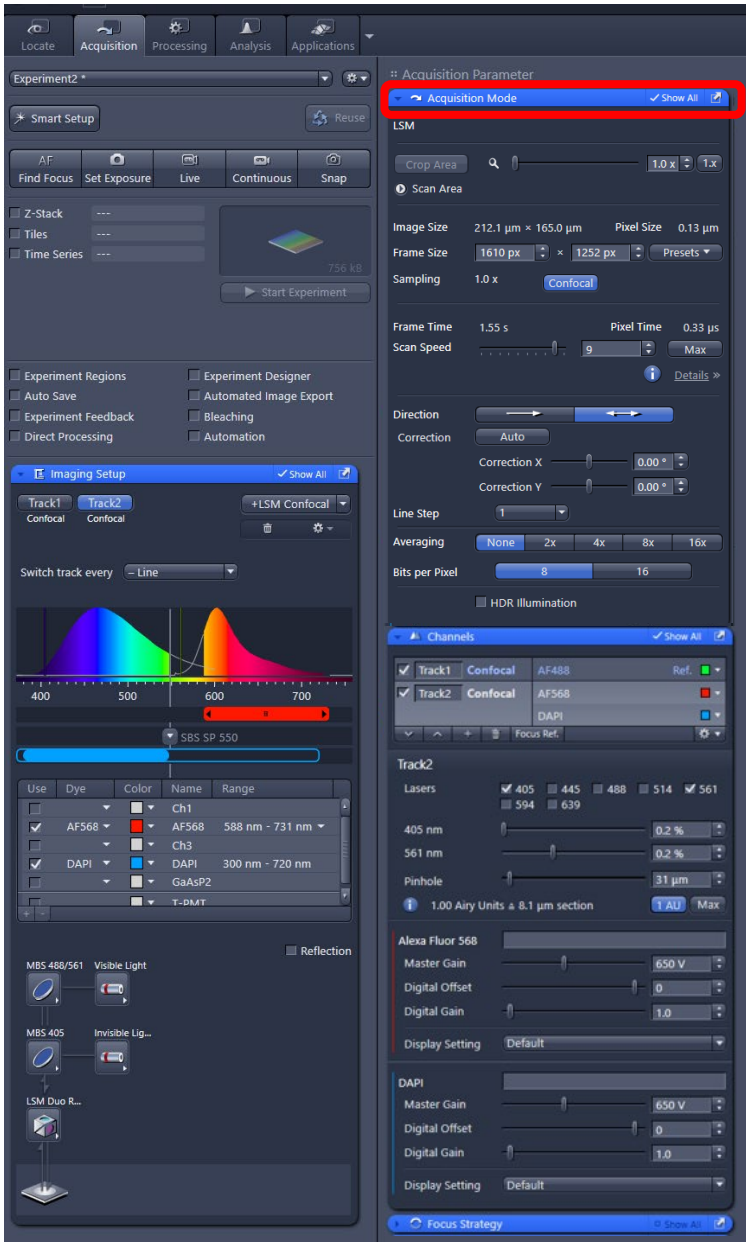
Be aware sometimes smart setup will encourage you to choose a configuration that includes the external GaAsP detectors. Zen has no knowledge of what filter cube is installed and it likely won't work.

The main buttons along the top of the **Acquisition Tab** which will be used frequently are:

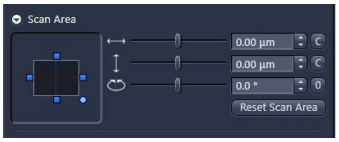


- **Find Focus** – This will conduct a software autofocus
- **Set Exposure** – This will adjust gain settings to achieve an image
- **Live** – This scans a fast preview scan that is of similar intensity to the acquired image
- **Continuous** –Scans at the given Acquisition parameters continuously
- **Snap** – Acquires a 2D image of the current settings and plane. *Note: Snaps are not auto saved.*

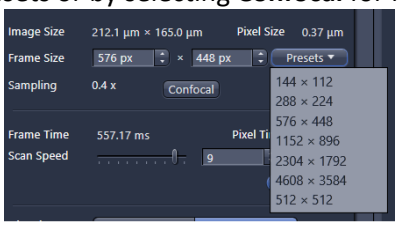
Acquiring an image and basic fine tuning



1. After applying the **Smart Setup** you will have one or more **Tracks** listed under the **Channels** Acquisition Parameters menu.
2. Expand the **Acquisition Mode** menu.
3. If required, expand the **Scan Area** menu for scan field rotation or XY adjustments

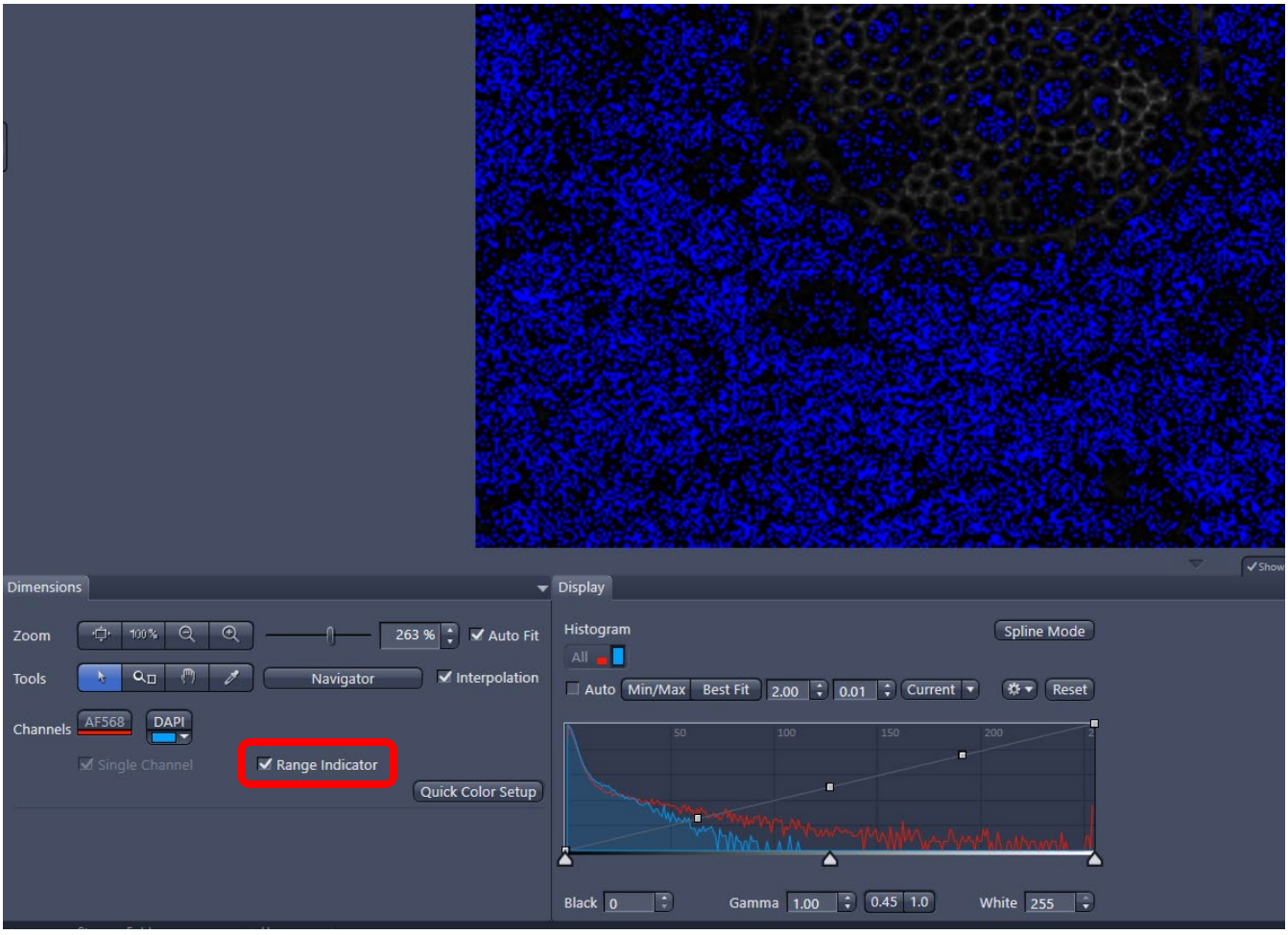


4. Choose the **Image Size** by using on of the presets or by selecting **Confocal** for Nyquist.



5. Adjust the **Scan Speed** as required. *Note the faster the scan speed results in more noise.*
6. Choose either **Single Direction or Bi-Directional** scan modes.
7. Choose any **Averaging** if required. *Note the more averages will reduce noise.*
8. Choose the **Bits per Pixel** required.

9. Highlight one of the tracks by clicking on it either in the **Imaging Setup** or **Channels** menu. This will update the various menus with context for this track (i.e. Imaging Setup, lasers and detectors used for this track).
10. Adjust the individual **Laser Powers** to 2-5% initially.
11. Ensure one **Airy Unit** is being used to achieve true confocal imaging
12. Turn on **Range Indicator** by checking the box below the image in the **Dimensions** panel.
    *Note: There is no split mode in Zen Blue when running Live, instead use the checkbox **Single Channel** and choose the relevant Channel while adjusting parameters.*
    *Note: The **Range Indicator** is a special look up table where saturated pixels are displayed as Red and the underexposed pixels are Blue.*
13. Adjust either **Laser Power** or **Master Gain** for the channel of interest to achieve suitable illumination/detection of your sample. Increase **Digital Offset** to remove any large areas of Blue.
14. If required, adjust **Scan Speed** or **Averaging**.
15. Repeat for all Tracks and Channels.
16. Click Snap to acquire a 2D image.
17. Experiment settings can be stored by selecting the **Cog Icon** at the top of the **Acquisition Tab** and choosing Save As... button.

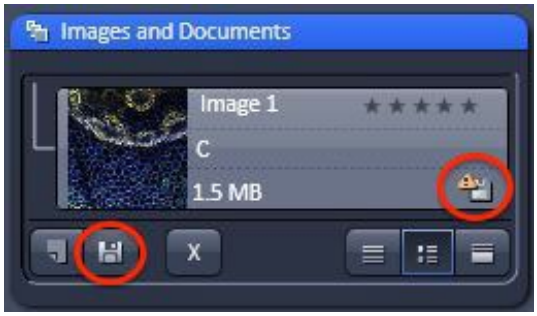


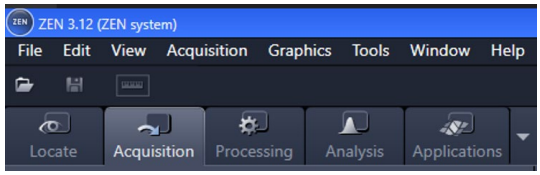
Saving individual files manually

Unsaved images are represented by the small warning icon in the lower right of the **Images and Documents** menu on the right-hand size of Zen.

You can right click on the specific image and choose **Save…** or highlight the image file and click the **Save icon** at the bottom of this menu.

Images can also be saved by selecting **File > Save…**

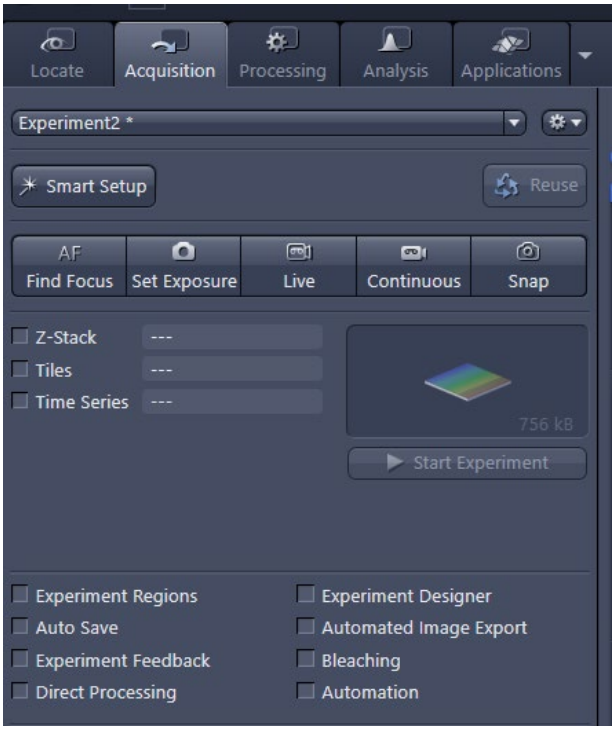




Note: As noted previously, IMB Advanced Microscopy Platform recommends using the Auto save functionality in Zen and all files should be saved locally before being copied to a relevant backed up file location.

Advanced Imaging Modes

You can combine any of the advanced setup options together, such **as Z-Stacks**, **Tiles** or **Time-Series** 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.



Additional complex imaging modes are also available:

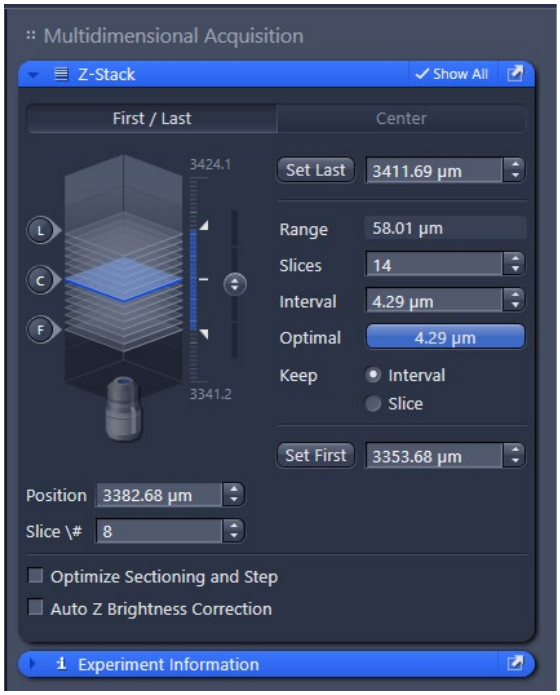
- **Experiment Regions** – Enables small user-defined ROIs to be acquired at the specified image size. These are also utilised in Bleaching.
- **Auto Save –** Enables user-definable saving parameters to automatically save experiments following their acquisition.
- **Experiment Feedback** – Enables the user to create macros where the outputs can guide the acquisition.
- **Direct Processing** – Enables automatic tasks to run following acquisition (such as Deconvolution or Stitching).
- **Experiment Designer** – Allows for acquisition 'blocks' to be developed and strung together.
- **Automated Image Export** – Not advised to be used
- **Bleaching** – Allows for user defined areas to be bleached
- **Automation** – Enables scripting for the acquisition.

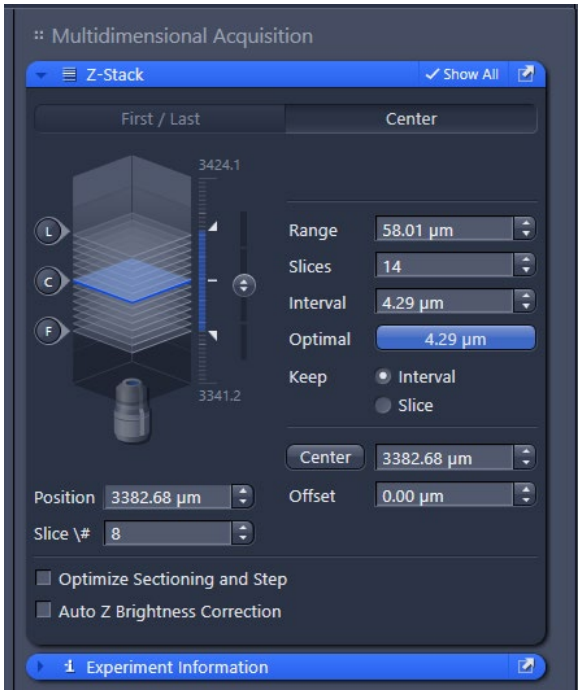
Z-stacks

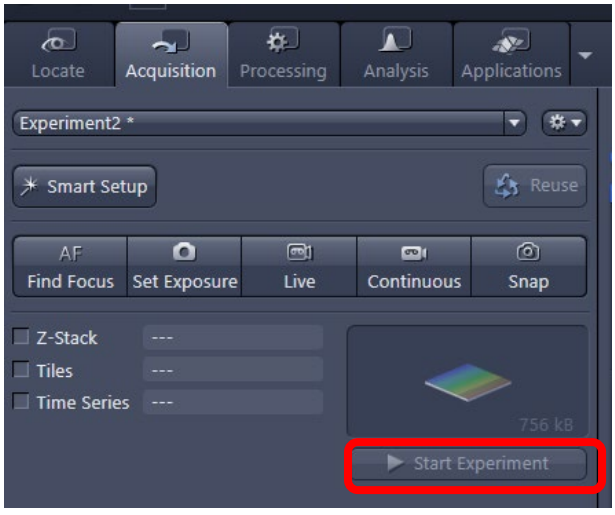
- Click the **Live** button to get an updating view to assist in focussing
- Select the range of your 3D Z-Stack
    - For **First/Last** method, start by focussing to the top of your object and click the Set First button, next focus to the bottom of the object and click Set Last
    - For **Center** 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
- Click **Stop (Live)** to halt the Live View
- 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 4.29µm – 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
- Click **Start Experiment** to begin acquisition (NOT Snap as before), the experiment will capture all channels set with all 3D slices.

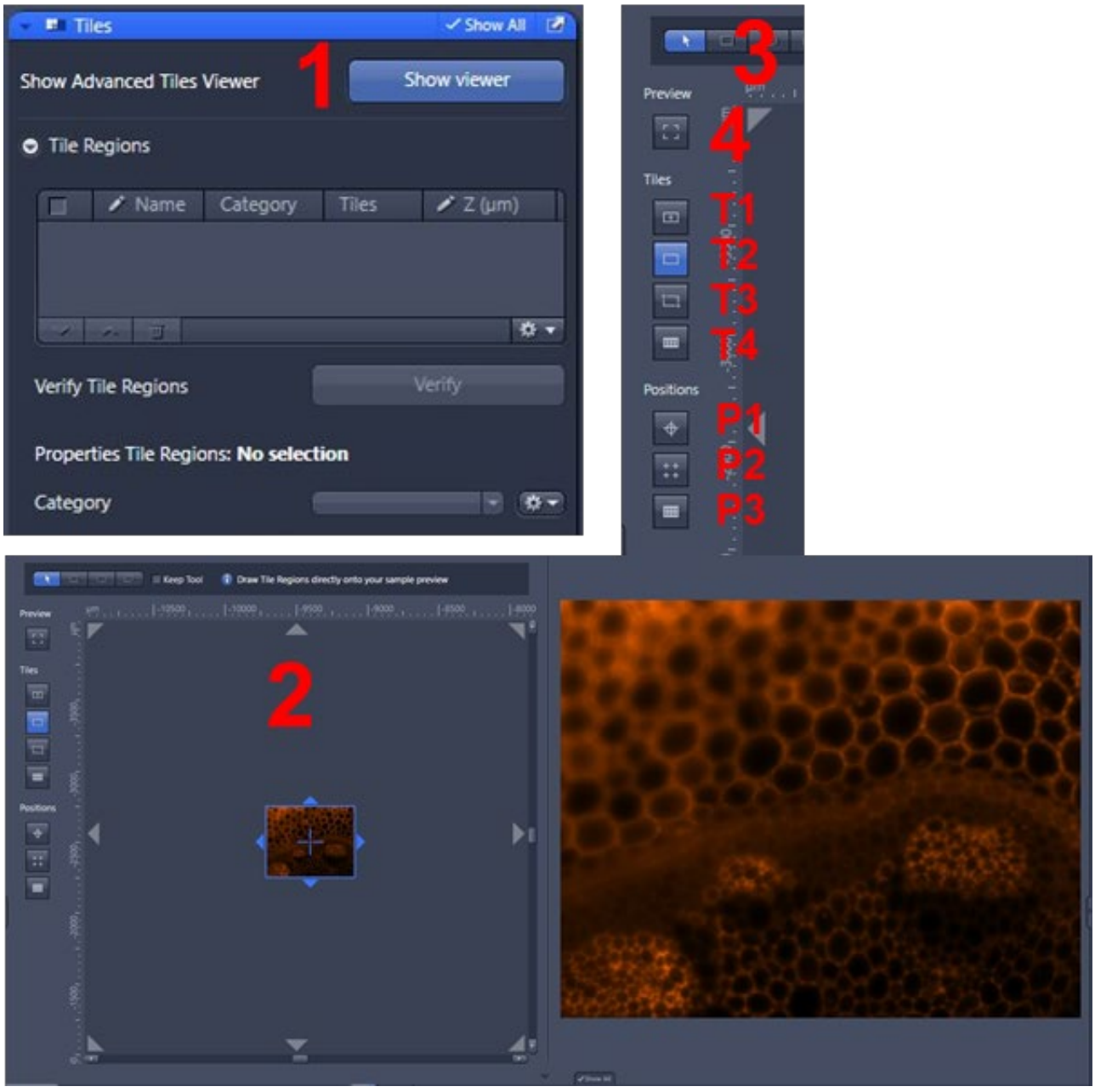






Tiles

- Click the **Tiles** checkbox, a Tiles parameters panel will open.
- In the *Tile Regions* section **(1)**, click on the Show Viewer button to invoke an interactive display which shows the live image captured in relation to the sample carrier.
- Use the *interactive viewer* **(2)** to define XYZ points around which you can capture multicolour, z-stack, and tiled datasets, if required
- Setup options appear at the top **(3)**
- It is recommended to capture a preview scan at lower magnification **(4) and** then identify and mark regions to tile at the desired magnification.
- Do not setup experiment that contains both tiles and positions, as the software cannot adjust z-stack accordingly.



Tiles

T1: Predefined Grid

For a set number of regions, set the X and Y number to the number of tiles required

a. Click the + button to add this tile to the tile list
b. The area of your tile will be shown in the window, and you can click around in live mode to make sure it encompasses the area of interest
c. The centre of the tile will be located at your current position
d. You can use the stamp option to place multiple tiled regions across your slide

T2: Contour

a) Set your shape to Square, Circle or Ellipsoid and encompass the area of interest and the software will automatically fill in the number of tiles required for that area
b) Click the + button to add this tile to the tile list

T3: Stake the boundaries

To set an area in reference to specific points of interest

a) Find your first area and click the + button to add your first point
b) Move the stage to the next area and click the + button to add this point
c) Repeat this process for the whole area you wish to image
d) You will see the area of the tile as you add new points
e) Once you have finished click the Done button to complete your setup procedure and add the tile to the tile list

T4: Predefined carrier

1. Select the appropriate Template from the list
   - If you have a different sample holder, please speak with a member of the Imaging Facility staff to help set up a custom template
   - You should see a visual representation of the chosen carrier in the Advanced Setup window (both in the stage view and at the bottom on the setup window)
2. Calibrate the stage for the chosen sample carrier (if not completed at start-up)
   a. Lower objective to Z-Minimum
   b. Select the Calibrate button
   c. Set up the bright-field view so you can see the camera feed, click Next
   d. To perform automatic calibration, click the Calibrate button otherwise, click Next to proceed to the manual (and more accurate) calibration
   e. Select the method to use for your calibration (choose the “7 points” option for highest accuracy)
   f. Move the stage so the crosshair on the image window is lined up with the left-most edge of well A1 and click the corresponding reference button on the calibration wizard (a tick should appear at the point to denote it has been set)
      i. Repeat the process for the top, right and bottom edges of the well
      ii. You may select to move the stage automatically between positions, but this can sometimes cause problems, so it is advised to do at least the first well by hand
      iii. Click next when all 4 calibration points have been set
   g. Repeat the calibration process in step 5 for the upper right well e.g. A12

i. Note you only require the left and top edges to be set for this well
ii. Click Next when finished

h. Finally set the top edge only for the Lower Right well e.g. H12 and click Finish
i. The Sample Carrier should now be set up and ready to use
- Double click on a specific well and the stage should automatically move to the centre of this well

Please avoid using the outermost wells as trying to image these could cause the objective to crash into the stage

3. Setting up your imaging of the Carrier
   a. Select the wells you wish image from the template
      i. You can select individually by clicking on the well in the Setup Viewer
      ii. Select by clicking individual wells while holding down Shift or alternatively by left clicking and dragging a selection area around the wells of choice
      iii. The selected wells should turn Blue
   b. To set up Tile regions of these wells select the Tile Region Setup tab and highlight the Carrier option
      i. Set how much of the well to tile with the Fill Factor option
      ii. Click the + Create button to create tiles
      iii. Tile regions should appear in red on the Advanced Setup window for each well that was selected
      iv. In the Tile Regions setup on the left you will also see each of these tiles appear in the list view
      v. To delete the tiles, click the – Remove button while the wells are still active (Blue) – Note that should you deselect the wells you will need to highlight them again before removing/editing the setup options
4. You are able to add multiple independent tile areas using these features and they will be listed in the list view of the Tile Regions section
   a. To remove a tile from the list:
      i. Highlight the tile you wish to delete
      ii. Click the rubbish bin icon at the bottom of the list view
      iii. You may also right-click on the one to delete and select the Delete Selected option
      iv. Alternatively, right-click on the list and select the Delete All option to clear the whole tile-list

Positions

P1: Individual positions

1. Add the current position by clicking on the + button
   i. The current position will then appear in the list window under Single Positions (see Tile Scanning Setup for the Position Array feature)
   ii. Each position will be recorded with X, Y and Z coordinates
2. Move the stage to the next position and add this by clicking on the + button again
3. Repeat the process for the desired number of positions
4. Remove positions from the list by highlighting them and clicking the rubbish bin icon
   a. Alternatively, you can delete by right-clicking and selecting Delete option
5. To clear the whole list, right-click and select the Delete All option

P2: Positions distributed in a predefined grid

1. For a set number of positions, set the X and Y number to the number of tiles required
2. Click the + button to add this tile to the tile list

P3: Predefined carrier

3. To set up multi-positions acquisition within a carrier
    v. Set the number of positions you wish to add
    vi. Set to either have set layout or random selection of positions
    vii. When choosing random positions, you can also set a bias towards the edge or centre of the well
    viii. Click Distribute button at add the positions
    ix. Click Remove positions to delete these positions
    x. The positions will now appear under the Position Array list where each Array will correspond to a well with multiple positions within it
    xi. Set up Support Points by selecting the Support Points
    xii. Set the number of points per well to add
    xiii. Click Distribute to add these points within the selected wells
    xiv. Follow the instructions in the Focus Surface section to set your focus map

- Using the Verify Positions option

4. Once all positions have been selected you can re-check focus before starting experiment
5. Click the Verify Positions button to get the pop-up window
6. Click Move to Current Point to move to the first position in the list
7. Refocus if necessary and click the Set Current Z button to update the position
8. Click Move to Next point to go to the next position in the list
9. Refocus and click Set Current Z to update this position
10. Repeat the process for all positions in the list
11. All positions should have a green tick next to them to indicate they have been verified
12. Click Close when done

Using a Focus Surface

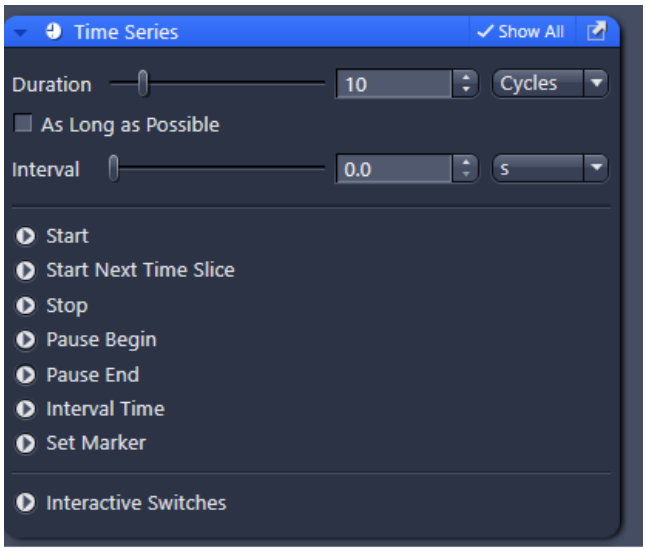
- A focus surface allows you to plot a map of the surface of your sample area after you have verified positions.
    - This allows the software to adjust the focus of your image automatically without the need for every position to be set independently
- Highlight the Tile or Wells of interest and move down to the Support Points section
    - Select the number of support points to add and click Distribute to add them
        - Onion skin starts from middle of the image and places support points in an outward spiral
        - Grid distributes points in a grid
    - Click off the tile area or well, and then select a support point to move it onto the tissue or cells of interest, don't focus a blank part of your slide.
- Under the Focus Surface setup click Verify Positions to begin setting these
    - Click Move to Current Point to move to the first position in the list
    - Refocus if necessary and click the Set Current Z button to update the position
    - Click Move to Next point to go to the next position in the list
    - Refocus and click Set Current Z to update this position. You can choose autofocus to find the z position
    - Repeat the process for all positions in the list
    - All positions should have a green tick next to them to indicate they have been verified
    - Click Close when done
- Change the Interpolation Degree setting to a desired setting
    - Note the more positions you set the more accurate the focus map will be

- When using a minimum of 9 support points use the Parabolic Surface option which is the most accurate for larger area tiles or for large sample carriers

- Set up your Z-Stack and Time Series as normal
- Click Start Experiment to begin acquisition

Time Series

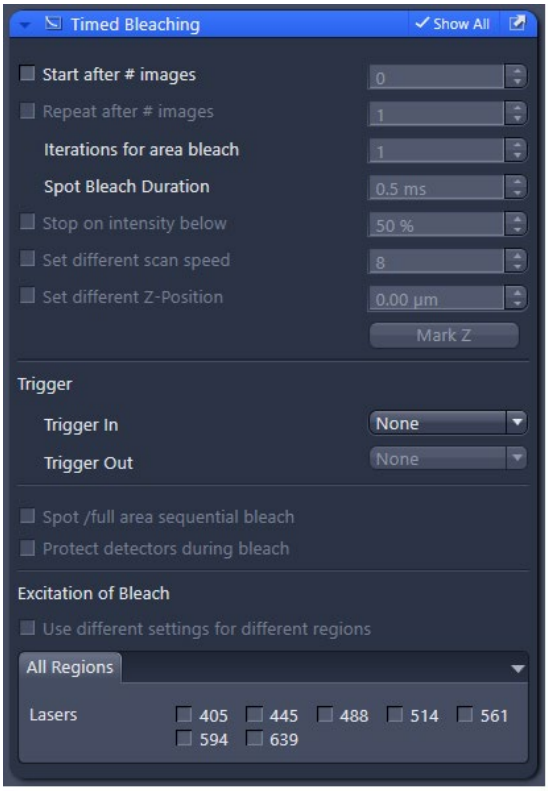
- Activating the Time Series option allows users to set up for time-lapse imaging to capture movies of live events
- Set the Duration (this is the number of times the acquisition setup will repeat)
- Set the Interval (this is the time the system will wait between capturing one image and starting the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



Bleaching (FRAP and FRET)

Timed Bleaching

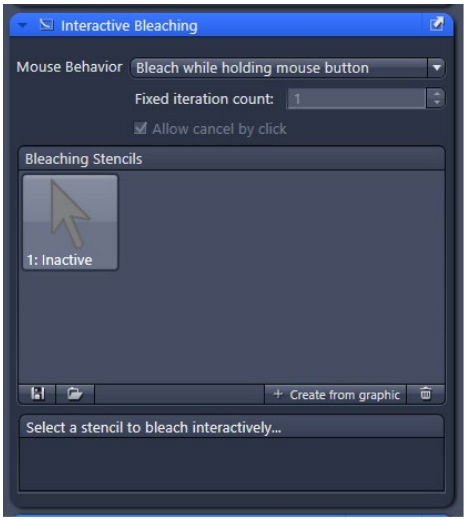
This tool permits setting the bleach parameters for a bleaching experiment in combination with a timeseries. Bleaching is done between acquisition frames.



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

- 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
  - *Note: The region can be used for bleaching a specific area but can also be used for analysis of that area too*
- Use the **Start Bleaching after # images** to capture a set number of images before the bleach step (a "before" snapshot)
- You may also **Repeat the bleach after # images**
- Set the number of **Iterations for the bleach area**
  - *Note: More iterations mean a longer bleach step which may be required for samples that you are struggling to bleach effectively*
- **Spot Bleach Duration** (only visible if spot region chosen) defines the time to perform the bleach
- Use the **Set 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
- You may also choose to **Set different Z position** to perform the bleach at a different z-level, enter the difference in microns into the input field.
- **Spot/Full area sequential bleach** when activated all regions (and spots) are bleached sequentially performing all bleach iterations for each region first before moving to the next. The scan field is limited to each bleach region.
- **Protect detectors during bleach** when activated will temporarily set all PMT gain to 0V during the bleaching step. This will slow down the acquisition speed, note that for small ROIs will not be causing critically high signal for the detectors, however if not activates the detectors can enter their safe states and shut down.
- Tick the **Laser line** you wish you use to ON and set the power, typically use 100% for the bleach laser
- 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
- Click Start Experiment to begin the bleach experiment
- 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

Interactive Bleaching

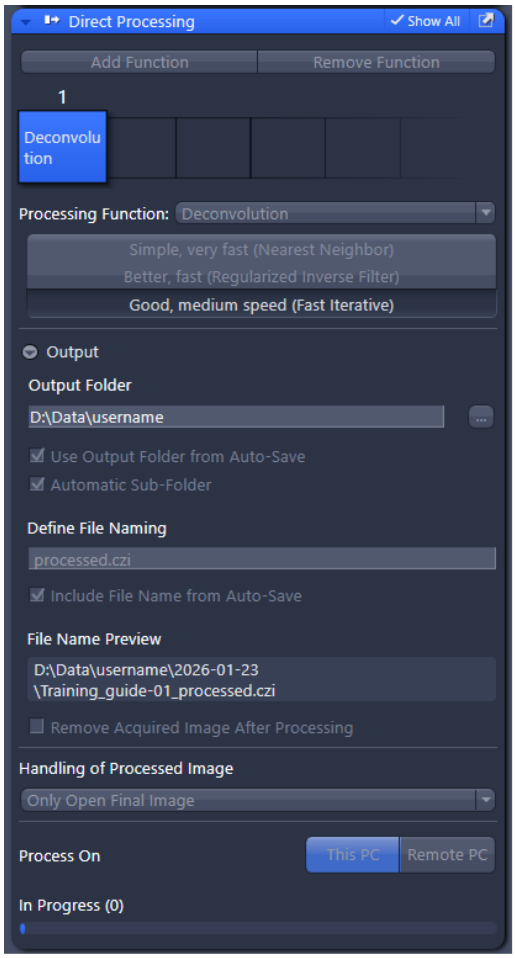


This tool allows to bleach interactively during a **Continuous** scan or during a **Time Series** experiment while image acquisition is performed. The bleach region is determined by pointing the mouse onto the position in the image.

- **Mouse Behavior** changes the display of the selected clipping plane using the dropdown list to the right.
  Bleach while the mouse button is pressed means the bleaching is continued while the mouse button is pressed
  Bleach fixed number of iterations means the bleaching process is continued for a fixed number of times after the mouse is pressed. The number of iterations can be defined.
- **Fixed iteration count** determines the number of iterations.
- **Allow cancel by click** if activated, the bleaching process can be stopped before the fixed number of iterations is accomplished. Simply click on the left mouse button again to stop the bleaching process.
- **Bleaching Stencils** are equivalent to ROIs for timed bleaching. To create a custom stencil, using the **Graphics** tool tab to first draw it, then using the **+ Create from graphic** button. Stencils can be saved, loaded or deleted.

Direct Processing

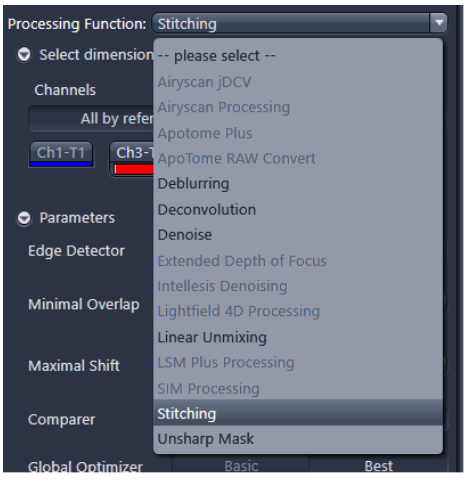
Introduces functionalities directed to increase speed, usability and reduce costs for processing. With **Direct Processing** you can select a processing function which is then directly executed as images are acquired.



Multiple functions can be added together which will be performed in sequence.

Ensure **Auto Save** or **Output Folder** locations are defined correctly prior to starting an acquisition.

*Note if you abort the acquisition, **Direct processing** will not run.*



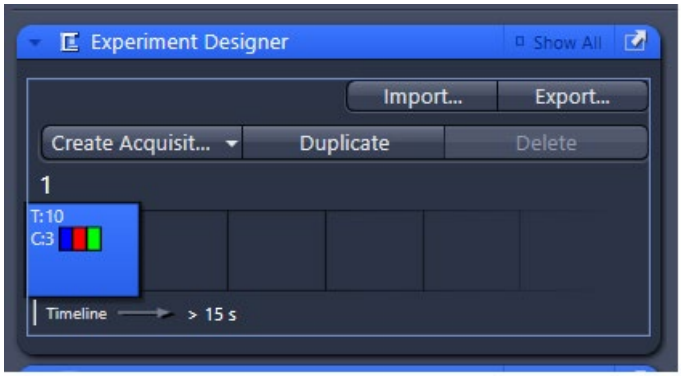
Experiment Designer

In the **Experiment Designer** you can create experiments for multidimensional acquisition. Experiments can consist of any number of components. A component is referred to as an experiment block. Each block has a distinct number (shown).

Focus strategies are block specific.

Blocks can be acquisitions, delays, waits or executions.

Delay Blocks are a pause that can be defined in the dialogue. If **Sychronize with preceding blocks** checkbox is activated, the duration of the delay block is reduced by the measured execution time of the preceding blocks.



Wait Blocks adds a wait to the experimental timeline. The wait block holds the experiment idle as long as clicking on the **Continue Experiment** button in the message box. This can be used for adding a solution to the specimen. Custom text can be used for the wait block.

Execute blocks will execute a selected hardware setting. You can change the blocks properties by clicking on the corresponding block. If the **Sequential** checkbox is activated the experiment will continue while the hardware setting is executed. If it is unchecked, the experiment will wait until the hardware setting was executed. Clicking the **Go!** Button will activate the hardware setting immediately.

Manual track creation

Depending on your acquisition you may need to configure the **Imaging Setup** manually without using **Smart Setup**. To do this, first click the **+LSM Confocal** button to add a **Track.**

Detectors available on the system are listed in the table below the spectra graph. Ch1-3 are Multialkaline PMT detectors, GaAsP1/2 are external detectors and T-PMT is a Multialkaline PMT for brightfield-like images.

Switch track every Line/Frame/Frame Fast/Z-stack are all options. **Line & Frame Fast** options do not allow hardware configuration changes, while **Frame** and **Z-stack** do.

Colour in the table to right represents the LUT that is applied to that detectors output in the image file.

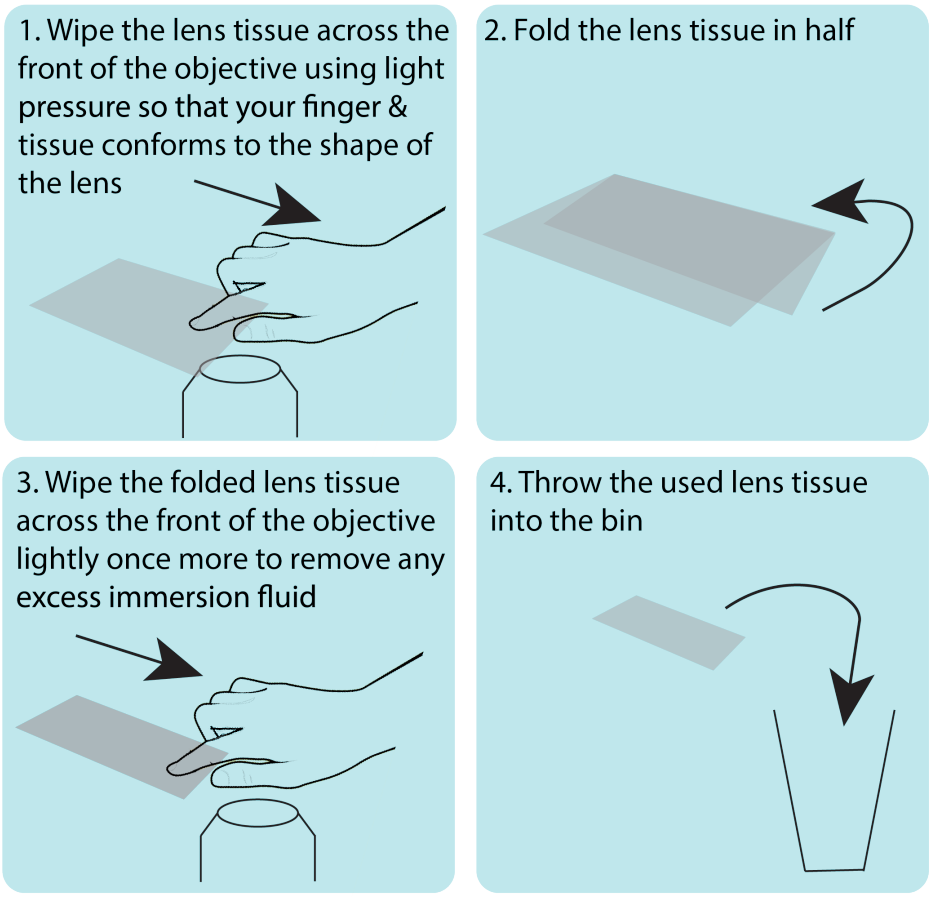
The **Secondary Beam Splitter** (SBS) in the image to the right is SBS SP 550 allows light to be directed to the output port for the external GaAsP detectors. Use **Mirror** to direct all light to the internal channels.

The **Main Beam Splitter** (MBS) is used to bring laser light into the system. Choose a filter that corresponds to your chosen laser lines.

HOW TO USE AN IMMERSION OBJECTIVE

- Find your focus using a low-powered objective first (10x or 20x) to confirm visibility of your sample
- Using the Focus knobs, move the objective away (Z-Axis) from the sample, then rotate the objective turret to the objective you need
- On an upright microscope, place a small drop of immersion fluid on your sample, on an inverted microscope, place a small drop of immersion fluid on the objective
- **BE CAREFUL** not to get immersion fluids on an air objective
- Use the Focus knobs to move the objective until it touches the immersion fluid on an upright, or until the immersion fluid touches the coverslip on an inverted. Look for the flash, then use fine focus knob to focus on your sample.

HOW TO CLEAN AN IMMERSION OBJECTIVE



Note: Only move the lens tissue in one motion (pass) over the objective never back and forth, and never touch the middle of the lens tissue.

SHUT DOWN PROCEDURE

When you are finished acquiring data you will need to complete the following in the order as described.

1. Clean any objectives used and remove your sample(s) and any media you bought with you.
2. Return the microscope to the 10x Objective and lower the nosepiece to the Minimum Z-limit.
3. Close Zen software,
   You will be prompted to save any unsaved acquisitions/files.
   You will be prompted to turn off any lasers used during the session. **Unless the next booking is waiting at the microscope with you turn the lasers to OFF.**
4. Copy your data off the acquisition PC to another backed up location.
5. Logout/Shutdown the PC
6. Wait one minute after PC shutdown to turn off the main switch.
7. Ensure the room is left tidy and report any issues that occurred during the session with facility staff via RIMS (Log an incident).