

Zeiss Live Imager 2 - Zeiss Axio Observer 7

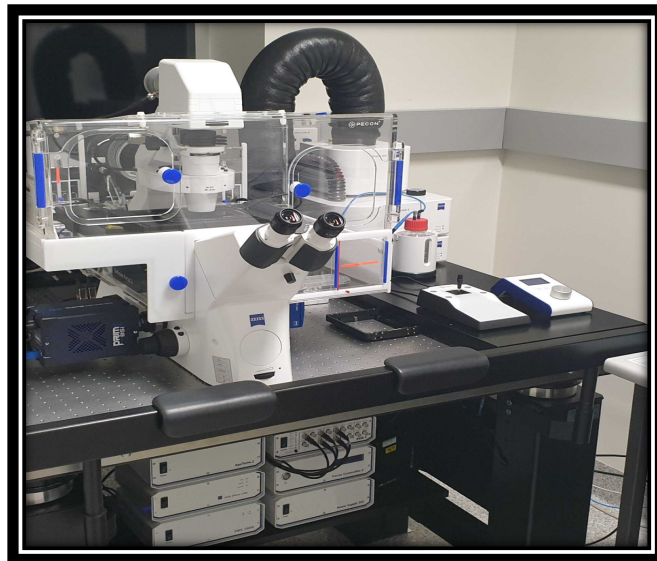
Quick-start Guide

Location: Room 6.025A

(Updated: 19/4/2022)

Acquisition Software:

Zeiss Zen Blue



***Available Objective Lenses:** 5x Air, 10x Air, 20x Air, 40x Air, 40x Oil & 63x Oil

***Available Colibri 7 LED Lines:** 385, 423, 469, 555, 590, 631, 735nm

*Configuration subject to change, always check with microscopy staff and/or the microscopy website for up to date configuration details

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.

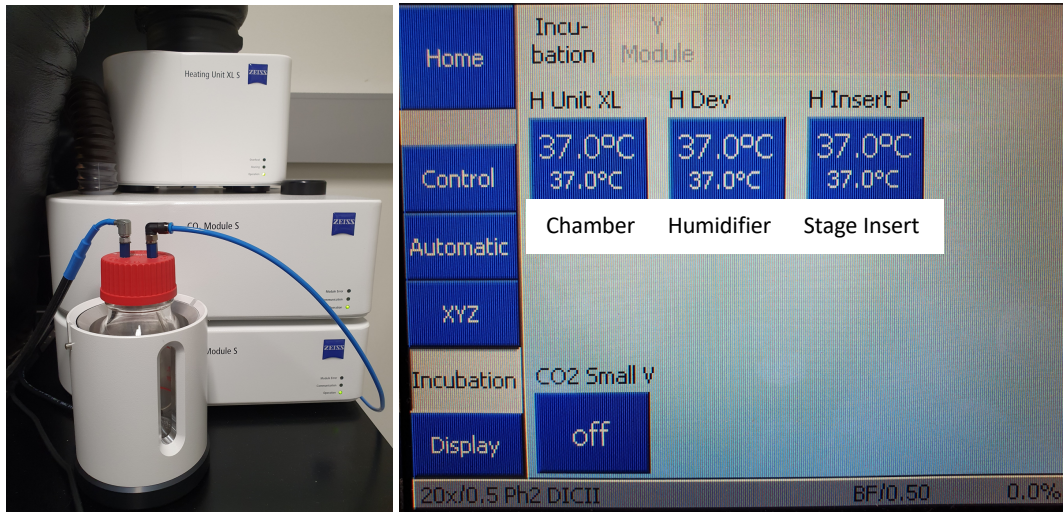
Slide Cleaning and Preparation:

- Make sure your slide is scrupulously clean – all dust, old oil and mounting medium should be removed with a small amount of 70% Ethanol
- If you can use a hard setting mounting media please do so
- **DO NOT** use glitter nail polish to seal your slides as this will scatter the laser light
- Be sure to give your mounting media and sealant at least 8-hours to dry properly

Incubation

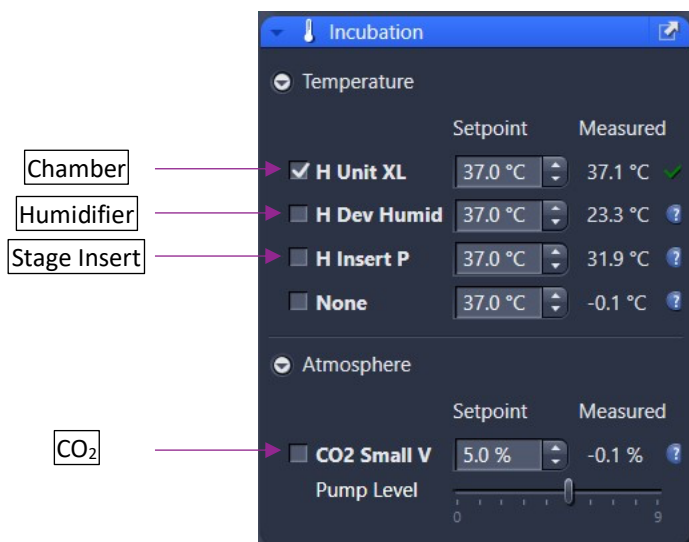
The incubation on the Zeiss Live Imager should always be set at 37°C. If you require a different temperature, the cool-down/warm-up times need to be factored into **YOUR** booking time and you are

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. 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 CO₂, 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 CO₂ **OFF** again once you have finished your session.

The temperature of the components and the CO₂ level can also be adjusted in the “incubation” panel at the bottom right of Zen Blue.



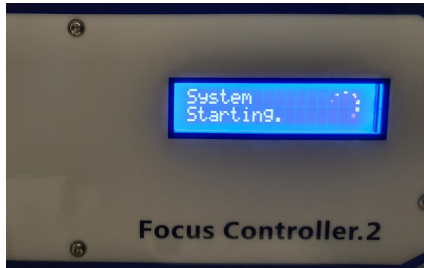
Start-up Procedure

- 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 leaving feedback on the sheets provided.

- Check the eyepiece dioptre settings. If you don't need to make an adjustment for your vision, set both to zero. Set the interpupillary distance for comfortable binocular viewing.
- Log in to the computer, using your IMB username and password.



- Double click 'SCOPE ON' icon on the desktop
- Wait for the Focus Controller Unit under the air table to display 'System Starting' on the LCD



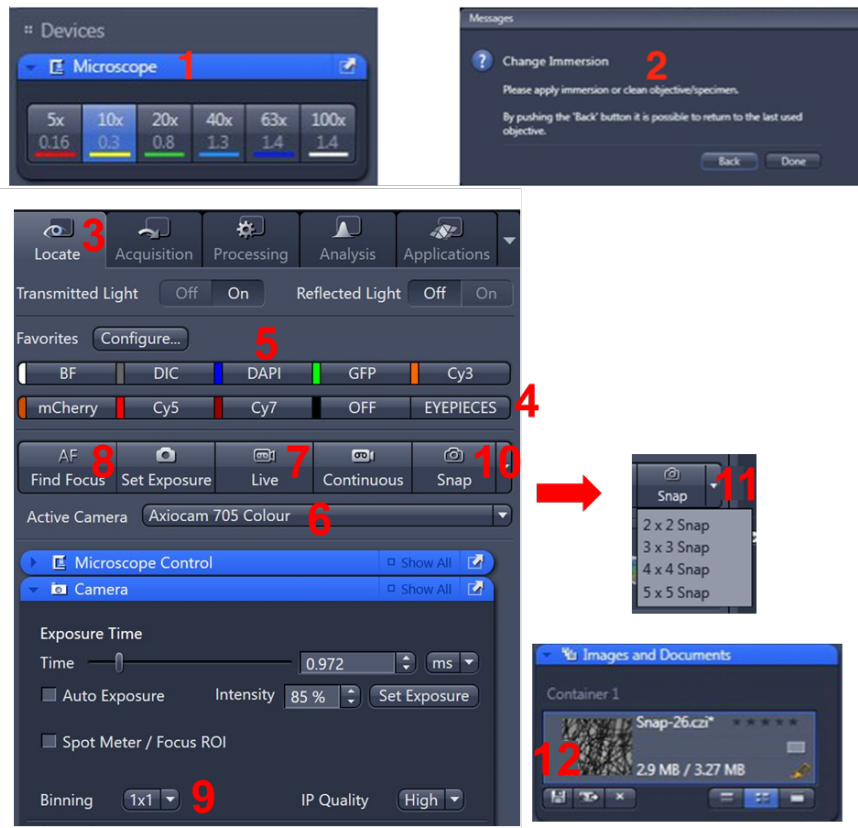
- Then press the soft touch power button at the left hand rear of the microscope body
- Once the controller screen is initialized, you may start the acquisition software, **ZEN**, by double-clicking the ZEN desktop icon.
 - Please be patient: ZEN is a large program and takes a while to load and start. Double-clicking numerous times will not make this process go any faster and may, in fact, prevent it starting. Note that you may also start ZEN by right-clicking on the ZEN icon and selecting *Open* from the resulting menu.
 - If you are using a template to image specific wells, calibrate the stage position.
- Objectives – always inspect and clean oil immersion objectives both **before** and **after** a session. Note: only clean the objectives by using a lens wipe, do not use any solutions or solvents.
- Always double check the LCD touchscreen, ZEN software or the objective itself to confirm which immersion media should be used on each objective, Dry or Oil.
- **Zeiss Deconvolution Microscope has both Dry and Oil Objectives. Note:**
 - Dry (these **never** have media applied): 5x 0.16NA, 10x 0.45NA, 20x 0.5NA, 40x 0.95NA
 - Oil: 40x 1.3NA, 63x 1.4NA,
- **Do not apply too much oil to the slide and objectives; one drop will suffice.** Be sure to clean the oil off the objective between samples. Apply fresh oil for each sample.

Basic Navigation

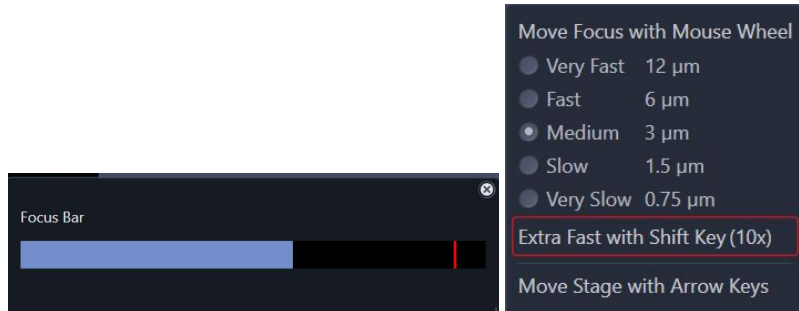
ZEN has four basic interface screens, accessed via tabs toward the top left of the display. **Locate** is used when finding the object of interest through the eyepieces or via the camera system. **Acquisition** provides for set up of imaging provisions, and image capture. These are most used during routine imaging, and will be covered in the user tutorial. The other two screens, **Processing** and **Analysis**, provide various methods of post capture image modification and characterization, respectively. Contact microscopy staff for instruction in these functions.

Locate Tab

- This is used to focus on the sample through the eyepieces.
- Ensure you remove any remaining oil from the objective or sample before changing objectives.
- Select the **10x** objective by clicking on the objective icon **(1)** in the **Microscope** menu on the right-hand side of the screen.
- Follow the prompts if asked to change the immersion oil **(2)**.
- Place your slide on the stage.
- When you open Zen Blue, it will open on the **Locate** tab **(3)**.
- Select the EYEPIECES button **(4)** and then the illumination method, BF (brightfield), DIC, or dye you wish to image, by pressing one of the favourite buttons **(5)** that most closely matches your own dyes emission colour.



- Using the joystick, locate a region of interest in your sample. F1 toggles the XY speed between slow and fast.
- Looking down the eyepieces, carefully bring your sample into focus using both the course and fine focus knobs on either side of the microscope.
- Switch to whichever objective you wish to use.
- Click the active camera drop down (or the macro buttons) to choose the camera **(6)**
- **Note:** the field of view of the BSI (Monochrome) camera is greater than the field of view for the Axiocam (Colour) camera.
- Click the **Live** button **(7)**, to see the live camera image on the computer display.
- In Live mode, there is a focus bar that can help you focus your image, best focus is achieved when the blue bar reaches the right edge of the indicator.
- To change the z position without having to move back to the microscope, click on the image window, and hold down the ctrl key. Roll the mouse wheel to move the z position up and down. If you are really out of focus, hold the ctrl + shift key to move extra fast (10x) the default speed for that objective.



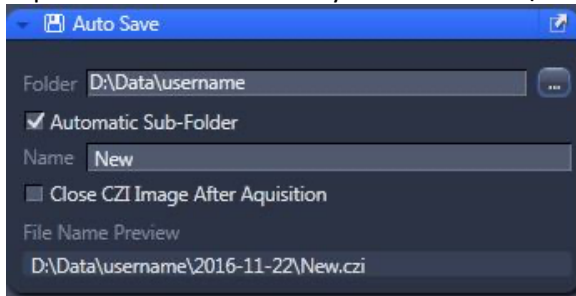
- Alternatively, click the **Find Focus** and **Set Exposure (8)** buttons to adjust the focus and exposure of the image, or adjust the exposure manually using the **Exposure Time** slider.
- Optionally, increase the image resolution by changing the binning to 1x1 **(9)**, then adjusting the exposure again.
- If you are collecting single colour images, you may now simply click the **Snap** button to capture an image **(10)**, or use the dropdown tab to capture a quick tile of your sample **(11)**.
- Click **Save (12)**.

Saving Your Data

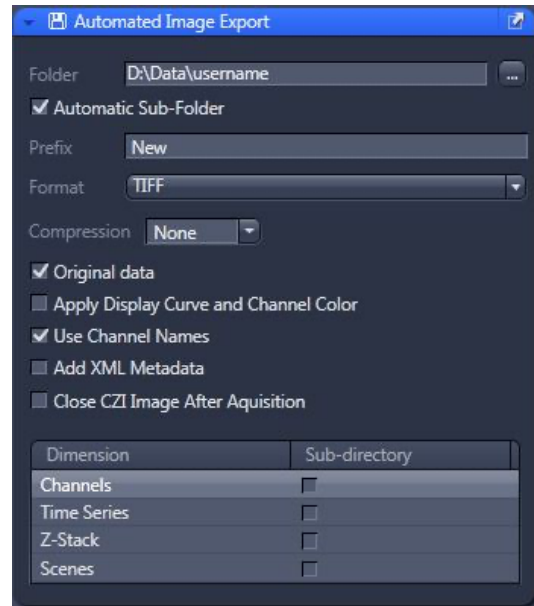
- **Note:** unless you have specifically set up automated saving (as described below) images are not saved as your session proceeds. You must save your images manually, preferably, as you go.
- There are three different places from which to save images:
 - The buttons below the image thumbnails in the **Images and Documents** panel (RHS).
 - The diskette icon above the menu tabs (Top).
 - From the application menu system, under *File* > *Save*.
- Set up a local folder in **My Computer / Data (Drive D:)**. Save all acquired data here, initially. Do not save to the Windows desktop, or to My Documents, etc.
- **Never** perform an initial save over the network: ZEN isn't built for it and may crash.
- Unsaved images have an orange symbol appearing next to their thumbnails in the *Images and Documents* menu.
- When finished imaging, ensure you transfer your images to your network folder. **Note:** data is automatically cleared from the PC when the storage nears capacity.

Automatic Saving / Exporting

- *AutoSave* enables a user to automatically save the raw .CZI files to a specified location after capture. Users should always save to the D:\Data\ directory and create a folder in their name.



- *AutoExport* performs similarly to AutoSave but converts the raw .CZI file into the desired image format such as TIFF. (This is not recommended, and should only be used on very large tile-scans, contact facility staff for more information)

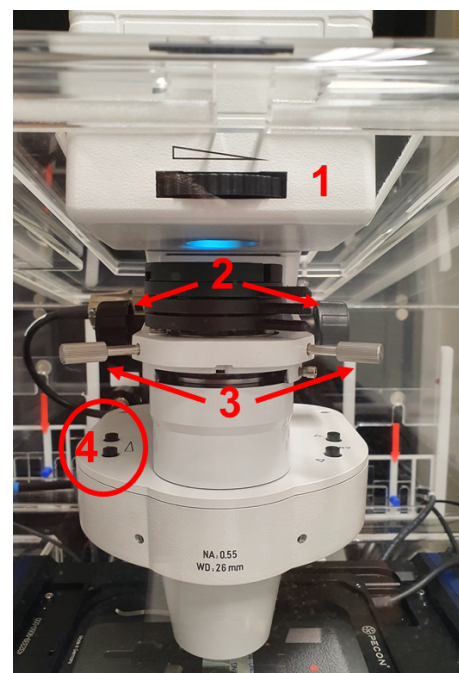


Connecting to Network attached Storage

- Data you acquire on this device should be backed up to a server for future retrieval and use.
- UQ-wide resources:
 - **UQ RDM**
 - Project based shared drive
 - Cached locally (\\data.imb.uq.edu.au\shortcode-Q0000)
 - For more information see rdm.uq.edu.au
 - Note this uses UQ-SSO credentials (username = uq\uq[yourname])

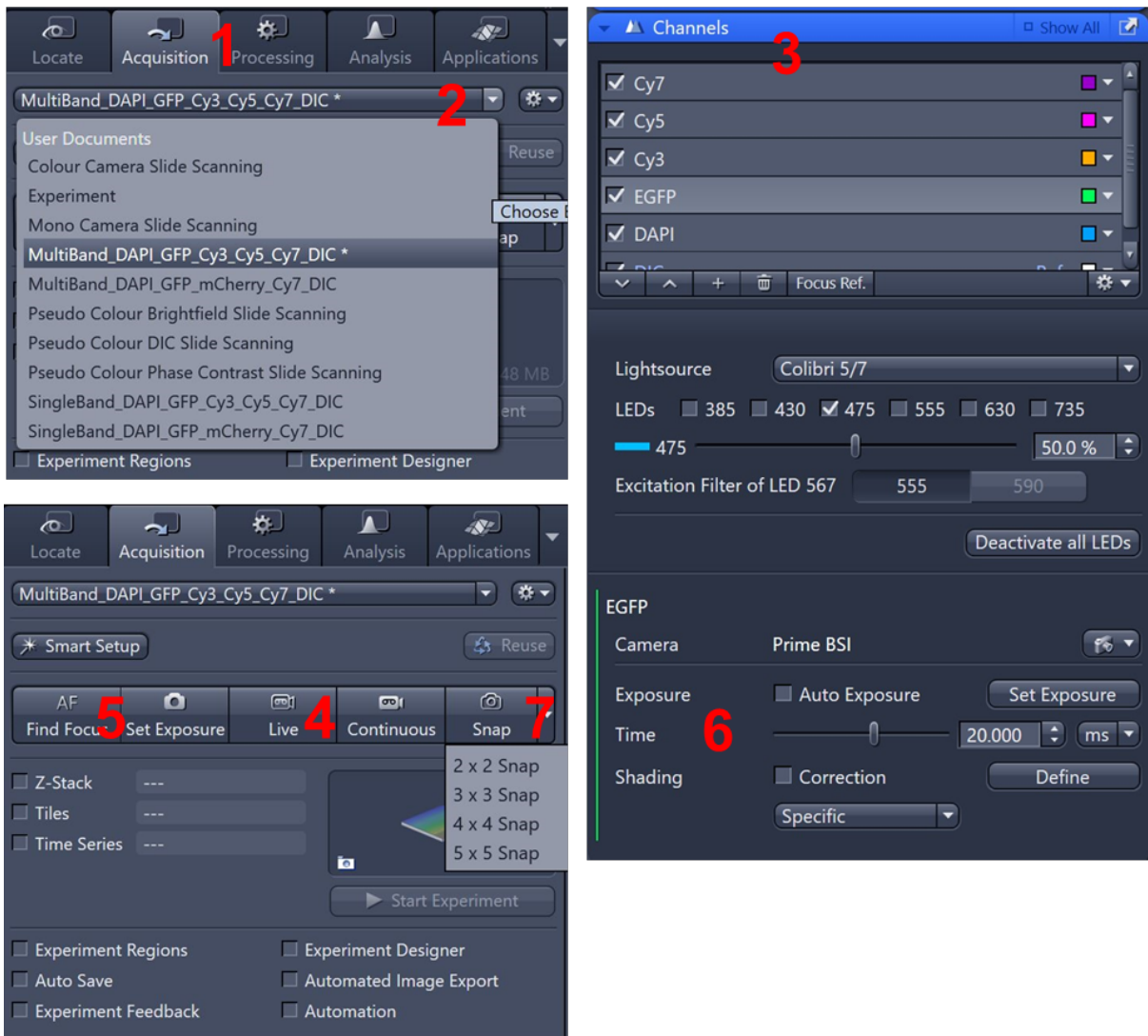
Setting up Kohler Illumination

- Close the **Field Stop (1)** and bring the image of the Field Stop into focus using the **Condenser Focus Wheel (2)**.
 - Centre the image of the Field Stop in the field of view using the sub-stage condenser centring screws **(3)**.
 - Open the Field Stop until it is *just* beyond the field of view.
- Set the **Aperture Stop (4)** to a value of three-quarters of the numerical aperture of the objective ($0.75 \times A_N$, for the mathematically minded).



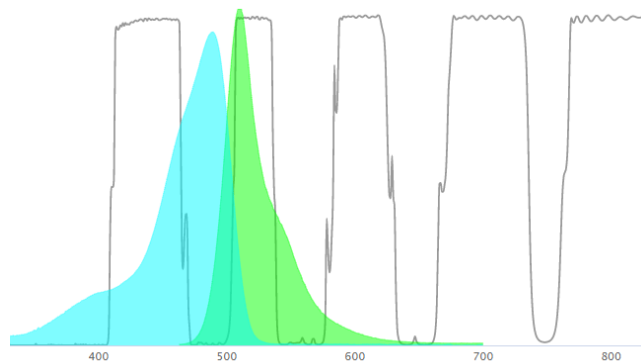
Acquisition Tab

This is used to collect multicolour, z-stack or other complex multi-dimensional datasets.

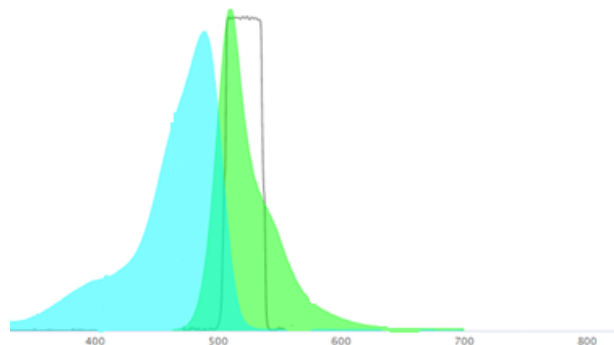


Multicolour Images

- Open the *Acquisition* Tab **(1)**.
- Select the type of experiment from the drop-down list **(2)**.
 - Multiband (DAPI/GFP/mCherry/Cy5/Cy7) are excited by individual LEDs and are detected through a penta-band emission filter, which can result in bleedthrough/crosstalk, when you can see fluorescence from a neighbouring channel in the channel of interest but acquisition is fast.
 - Pseudo colour bright field imaging uses the monochrome camera to collect red, green and blue channel images to be used in a composite colour image.
 - Single band filters are excited by individual LEDs and are detected through individual band-pass emission filters, which results in no bleedthrough but is slower.



Example: GFP
Multi Band filter will collect emission in all 5 bands – there will be bleed through of GFP into the next channel

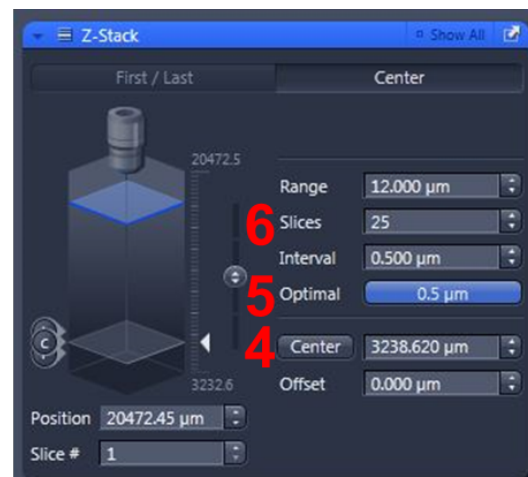
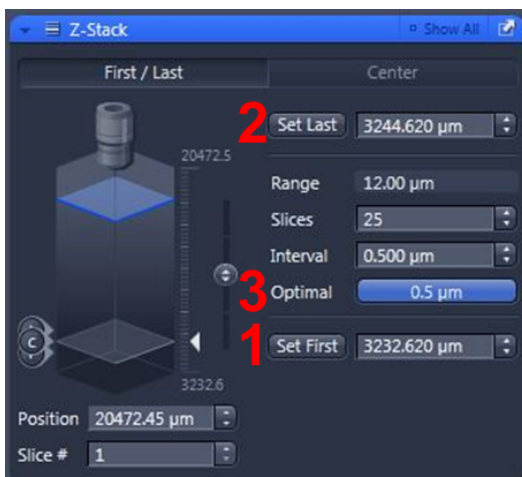


Single Band filter will only collect emission in this narrow range

- Select the tickboxes for the *Channels* you wish to image **(3)**.
- Click the Live button, to see the live camera image on the display **(4)**.
- Click the *Find Focus* and *Set Exposure* buttons **(5)** to adjust the focus and exposure for all of the Channels of the image, or manually click on each Channel to highlight it, then adjust the exposure manually using the *Exposure Time* slider **(6)**.
- By default, the camera captures a 2x2 binned image. You may increase the image resolution if desired by changing the binning to 1x1, then adjust the exposure again.
- Click *Snap* **(7)** to capture a multicolour image or use the dropdown tab to capture a quick multicolour tile of your sample.
- Then click *Save*.

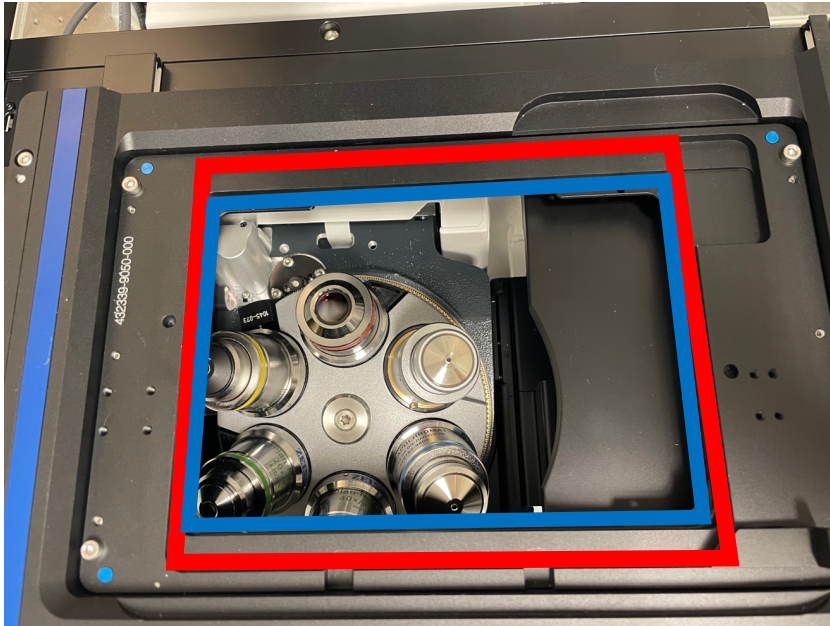
Z-stacks

- Perform the steps in outlined Multicolour images above.
- Click on the *Z-stack* tickbox.
- Highlight a channel with signal throughout the sample to set the range
- Now setup the Z-stack range via one of two methods.
 - **First/Last** – good for quick scans and irregular samples:
 - Using *Live* view, focus up through the sample until the top is reached.
Click *Set First* **(1)**.
 - Then, focus down through the sample until the bottom is reached.
Click *Set Last* **(2)**.
 - Select the interval (distance) between z-sections: the easiest option is to click *Optimal* **(3)**. The software calculates the optical slice thickness and then sets the interval between slices to be less than this; ensuring that you won't miss anything between slices.
 - **Center** – good for tiles scans and multi-position imaging:
 - Bring the sample into focus.
Press the *Center* button **(4)**.
 - Select interval (distance) between z-sections; the easiest option is to click *Optimal* **(5)**.
 - Enter an arbitrary number of Slices (start with about 21) **(6)** or, alternatively, set the Range.
- Note: Use an odd number of slices so that the FOV you are on is included.
- Select all of the Channels you wish to image.
- Click the *Start Experiment* button (below the *Live/Continuous/Snap* buttons) to begin acquisition.



Using the Piezo stage:

- The Piezo stage can be used for fast z-stack acquisition as it can move fast and precisely in z. The stage has a movement range of 500 μm , so cannot be used for z-stack thicker than that. Please take extra care when putting on stage insert. The Piezo stage is highly sensitive and should not be impacted by force.
- The Piezo stage (red rectangle) lays over a motorise stage (blue rectangle). The correct stage insert that sits over the Piezo stage (not only the motorise stage) needs to be used for the Piezo stage to move the sample.



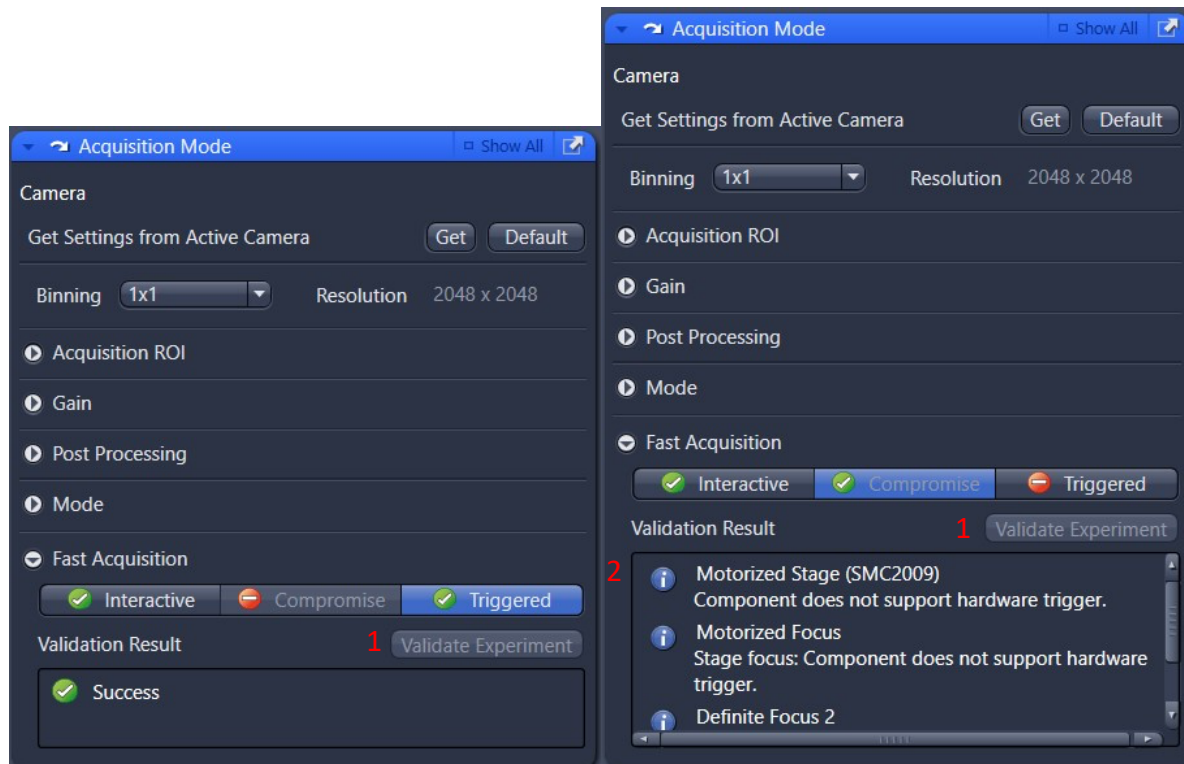
Changing focus with the Piezo stage:

- Turn on the Piezo stage by clicking the middle button of the controller (1).
- Return to the centre of Piezo z-movement range by clicking the left button (2).
- Change the rate of movement by clicking the right button (3).



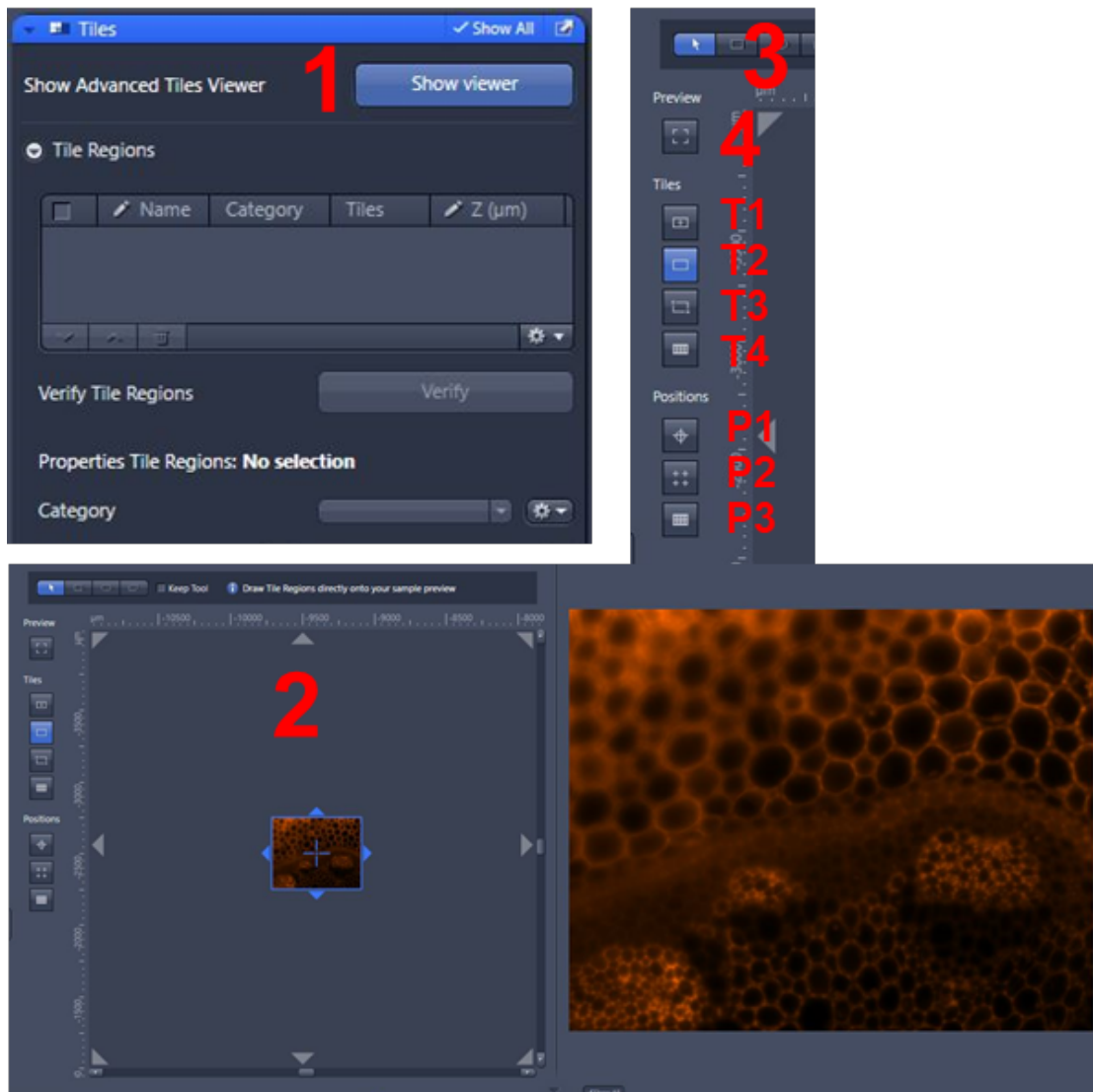
Setting up a triggered experiment to use the Piezo:

- Set up your experiment
- Go to the “Acquisition Mode” and expand the “Fast Acquisition” tab
- Click “Validate Experiment” and the Piezo stage will be used for z-stack acquisition
 - “Interactive” indicates that no component is being triggered
 - “Compromise” indicates that all components that can be triggered is triggered, and the others are controlled as usual. Piezo stage is used in this setting. Components that cannot be triggered is listed below.
 - “Triggered” indicates that all components are triggered, and the Piezo stage is used.



Advanced Tiling

- Click the *Tiles* checkbox, situated below the z-stack checkbox mentioned previously. 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.



Tiling

T1: Predefined Grid

- For a set number of regions, set the X and Y number to the number of tiles required
- Click the + button to add this tile to the tile list
- 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
- The centre of the tile will be located at your current position
- You can use the stamp option to place multiple tiled regions across your slide

T2: Contour

- 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
- 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

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

T4: Predefined carrier

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)

Calibrate the stage for the chosen sample carrier (below is for multi-well plate setup)

- Select the Calibrate button, unless you did it on startup
- 1/6 Set up the bright-field view so you can see the camera, click Next
- 2/6 To perform automatic calibration click the Calibrate button otherwise click Next to proceed to the manual (and more accurate) calibration
- 3/6 Select the method to use for your calibration (choose the 7 points option for most accuracy)
- 4/6 Move the stage so the cross-hair 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)
 - Repeat the process for the top, right and bottom edges of the well
 - 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
 - Click next when all 4 calibration points have been set
- 5/6 Repeat the calibration process in step 5 for the upper right well e.g. A12
 - Note you only require the left and top edges to be set for this well
 - Click Next when finished
- 6/6 Finally set the top edge only for the Lower Right well e.g. H12 and click Finish
- 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 this well
- Please avoid using the outermost wells as trying to image these could cause the objective to crash into the stage
- Setting up your imaging of the Carrier
 - Select the wells you wish image from the template
 - You can select individually by clicking on the well in the Setup Viewer
 - Select by clicking individual wells while holding down Shift or alternatively by left-clicking and dragging a selection area around the wells of choice
 - The selected wells should turn Blue
 - To set up Tile regions of these wells select the Tile Region Setup tab and highlight the Carrier option

- Set how much of the well to tile with the Fill Factor option
- Click the + Create button to create tiles
- Tile regions should appear in red on the Advanced Setup window for each well that was selected
- In the Tile Regions setup on the left you will also see each of these tiles appear in the list view
- 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
- 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
- To remove a tile from the list:
 - Highlight the tile you wish to delete
 - Click the rubbish bin icon at the bottom of the list view
 - You may also right-click on the one to delete and select the Delete Selected option
 - Alternatively, right-click on the list and select the Delete All option to clear the whole tile-list

Positions

P1: Individual positions

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

P2: Positions distributed in a predefined grid

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

P3: Predefined carrier

- To set up multi-positions acquisition within a carrier
 - Set the number of positions you wish to add
 - Set to either have set layout or random selection of positions
 - When choosing random positions, you can also set a bias towards the edge or centre of the well
 - Click Distribute button at add the positions
 - Click Remove positions to delete these positions
 - The positions will now appear under the Position Array list where each Array will correspond to a well with multiple positions within it
 - Set up Support Points by selecting the Support Points

- Set the number of points per well to add
- Click Distribute to add these points within the selected wells
- Follow the instructions in the Focus Surface section to set your focus map
- Using the Verify Positions option
 - Once all positions have been selected you can re-check focus before starting experiment
 - Click the Verify Positions button to get the pop-up window
 - 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
 - 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

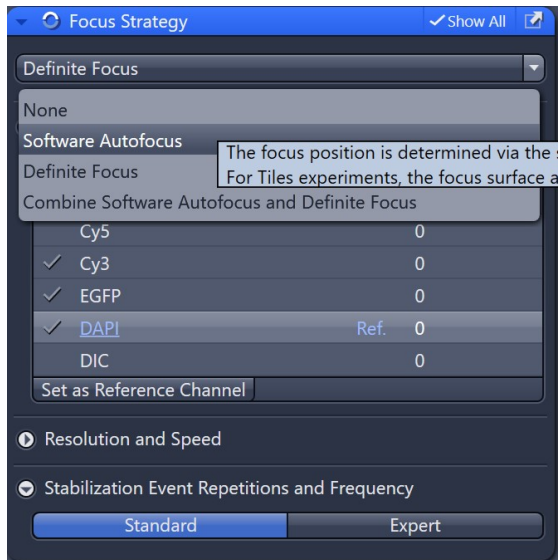
Using a Focus Surface

- 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 or definite focus 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

Setting a Focus Strategy

- It is important to always check the Focus Strategy the system is going to use for acquisition after setting up Tiles or Multi-Position acquisition

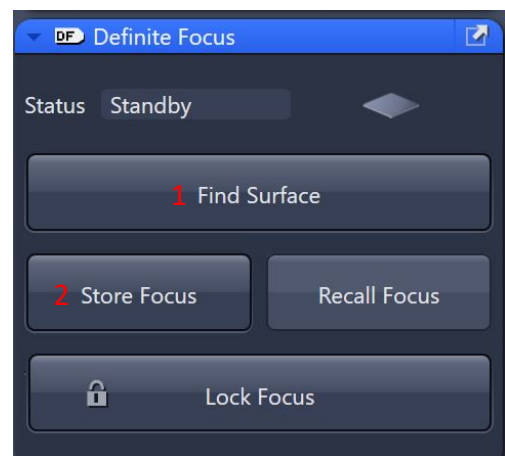


- None will use the current Z position of the stage
- Software Autofocus will go through a z range and determine the best focal plane
- Definite Focus uses laser to determine the position of the coverslip and the focal plane is relative to the position of the glass. Faster method than software autofocus.
- Combine software autofocus and definite focus will use software autofocus/definite focus as reference and adjust focus with the other method.

Definite Focus:

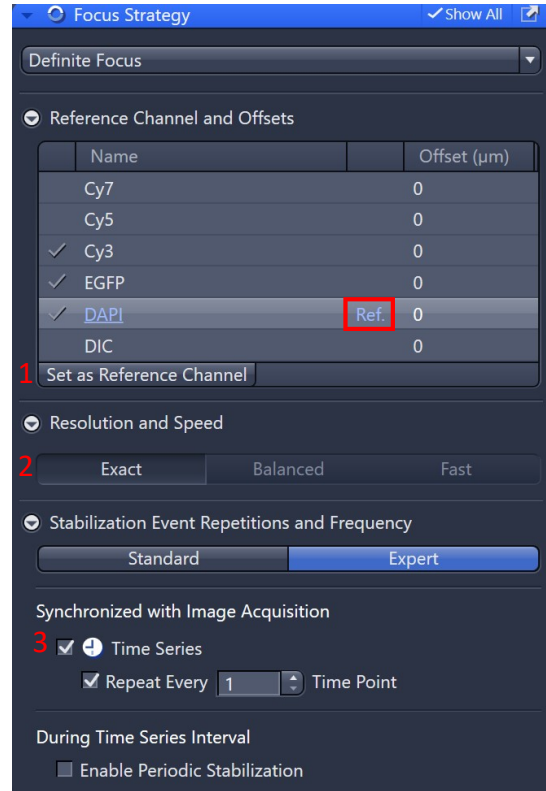
Definite Focus uses laser to determine the position of the coverslip and the focal plane is relative to the position of the glass.

Click “Find Surface” (1) to look for the coverslip, then manually adjust the focus and click “Store Focus” (2) to store the distance of the coverslip to the focal plane.



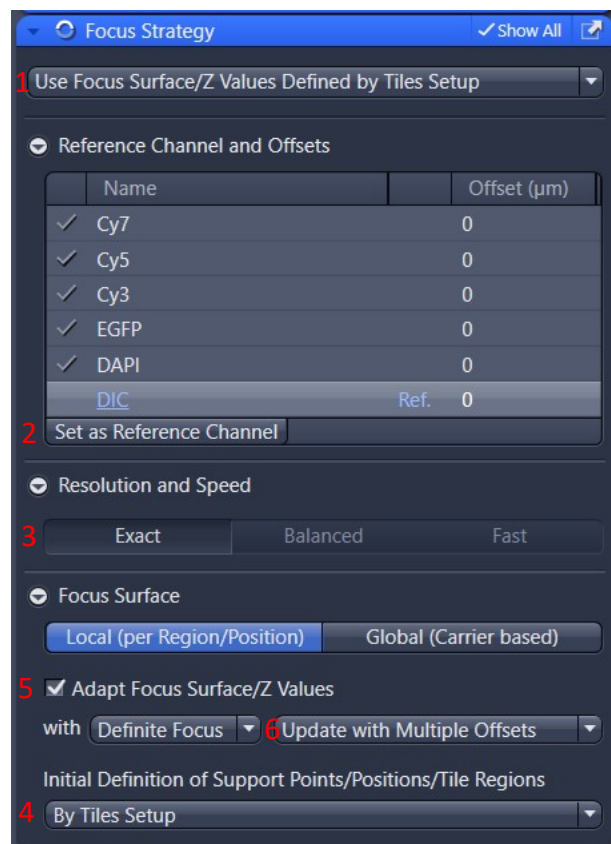
Single-point time lapse:

- From the channels in your experiment, choose a strong signal for your reference channel
- Highlight the desired channel and click “Set as Reference Channel” (1), ensure that the reference channel is set (red rectangle)
- Adjust the resolution and speed of Definite Focus according to your imaging need (2)
- For a time series experiment, the focus strategy will add time to the acquisition, so define when the focus strategy is executed to minimise the effect on the experiment (3).



Multi-point time lapse:

- Drop down (1) and select “Use Focus Surface/Z Values Defined by Tile Setup”
- Set the appropriate reference channel (2)
- Adjust the resolution and speed of Definite Focus according to your imaging need (3)
- Choose initial definition of support point/positions/tile regions to be “By Tile Setup” (4). That will use the saved z-position in your tile setup.
- Check “Adapt Focus Surface/Z Values” (5) so that focus will be adjusted using software autofocus or definite focus
- Select “Update with Multiple Offsets” (6) so that the z position is adjusted for each position.
- A pop-up will ask you to verify the individual offset once the experiment is started.

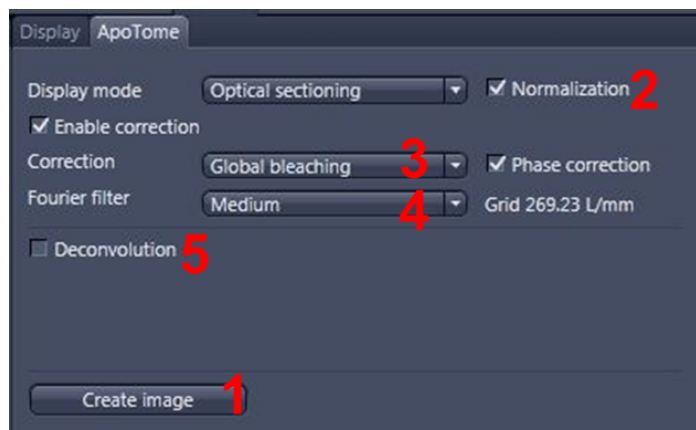
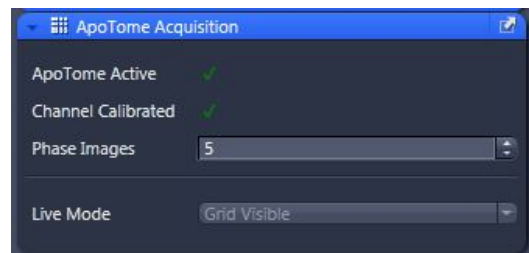


Stitching Tile Scan Data

- Once your tile has finished acquiring go to the Processing tab
 - o Alternatively, you may also open a previously saved image that you need to stitch
- Select Stitching from the Method menu
 - o If used previously it should be in the Recently Used options
- Click Select on the Stitching window to set the processing to the desired image data
- Adjust any settings you need to change
 - o It is recommended selecting to Create New Image in case you need to change anything after it is done
- Click Apply to start the stitching process
- Save out your stitched image once the processing has finished
- There are also offline versions of ZEN on our VM's that can stitch your tile scans

2D Apotome

- o **Carefully and gently slide the Apotome module into the side of the microscope body.** The microscope will beep when it is in place.
- o Select **Apotome** from the experiment dropdown list.
- o Check that the Apotome is Active, If there is a red X, make sure Apotome is in position. If there is a red X next to Channel Calibrated, contact microscopy facility staff.
- o Select the tickboxes for Channels you wish to image.
- o Move the eyepiece/camera slider on the microscope out to the camera position.
- o Click the *Live* button, to see the live camera image on the display.
 - o Note the Apotome blocks a lot of light and you will need to re-set exposure values when using this method.
- o Click the *Find Focus* and *Set Exposure* buttons to adjust the focus and exposure for all of the Channels of the image, or manually click on each Channel to highlight it, then adjust the exposure manually using the *Exposure Time* slider.
- o Increase the image resolution if desired by changing the binning to 1x1, then adjust the exposure again.
- o Click *Snap*.
- o **Important:** In the ApoTome tab (under the image, next to the display tab), Click *Create Image* (1) to process the image file into a single, resolution enhanced image in the display settings area below your image.
- o Save your data.

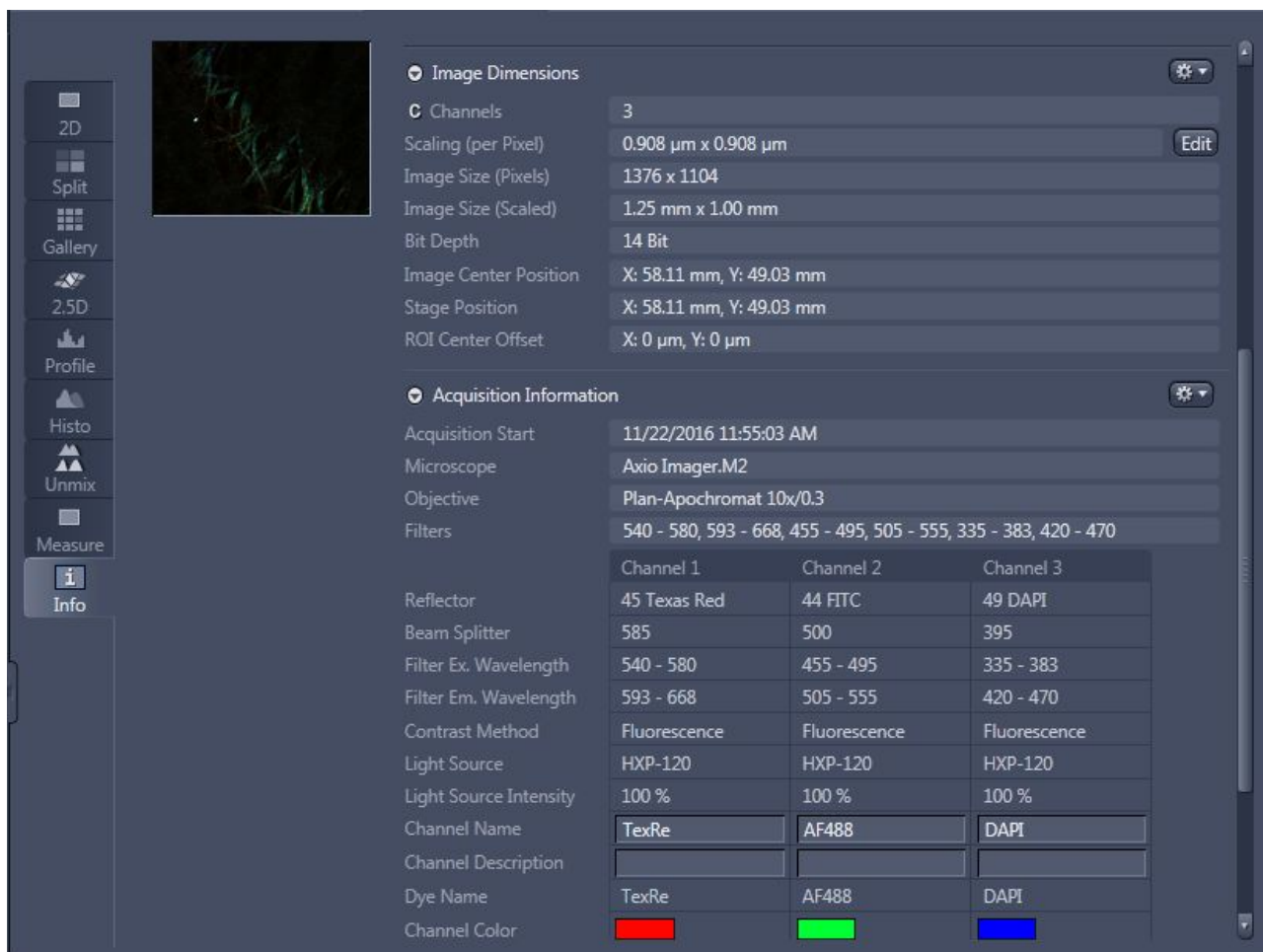


3D Apotome

- **Carefully and gently** slide in the Apotome module, select “Apotome” from the experiment dropdown list, and select desired Channels, as described above.
- Adjust exposure, resolution (if desired), and setup a z-stack as previously discussed.
- Click *Start Experiment*.
- Select Normalization **(2)**.
- Select Phase correction and set to *Global bleaching* to remove any lines visible in the final image **(3)**.
- Set Fourier filter to *Medium* **(4)**.
- Deconvolve the image **(5)**, if desired.
- Click *Create Image* **(1)**, as above.
- Save your data.

CZI image acquisition information

- *Image Info*, accessed from the tabs on the left of the image window, displays all of the settings used to capture the image, including pixel size and emission wavelength.



The screenshot displays the 'Info' tab in the software interface, showing the following acquisition parameters:

- Image Dimensions:** Channels: 3; Scaling (per Pixel): 0.908 μm x 0.908 μm ; Image Size (Pixels): 1376 x 1104; Image Size (Scaled): 1.25 mm x 1.00 mm; Bit Depth: 14 Bit; Image Center Position: X: 58.11 mm, Y: 49.03 mm; Stage Position: X: 58.11 mm, Y: 49.03 mm; ROI Center Offset: X: 0 μm , Y: 0 μm .
- Acquisition Information:** Acquisition Start: 11/22/2016 11:55:03 AM; Microscope: Axio Imager.M2; Objective: Plan-Apochromat 10x/0.3; Filters: 540 - 580, 593 - 668, 455 - 495, 505 - 555, 335 - 383, 420 - 470.

	Channel 1	Channel 2	Channel 3
Reflector	45 Texas Red	44 FITC	49 DAPI
Beam Splitter	585	500	395
Filter Ex. Wavelength	540 - 580	455 - 495	335 - 383
Filter Em. Wavelength	593 - 668	505 - 555	420 - 470
Contrast Method	Fluorescence	Fluorescence	Fluorescence
Light Source	HXP-120	HXP-120	HXP-120
Light Source Intensity	100 %	100 %	100 %
Channel Name	TexRe	AF488	DAPI
Channel Description			
Dye Name	TexRe	AF488	DAPI
Channel Color	█	█	█

Shut Down Procedure

- **Cleaning up the microscope**
 - Carefully clean the oil immersion objectives, if used, using the lens tissues provided, in the manner demonstrated by the microscopy staff. Do **not** use Kimwipes. If a dry (air interface) objective is oil contaminated do not attempt to clean it; notify microscopy staff.
 - Wipe up *any* spills you may have made. Note well; You, the last user, are responsible for the condition of the microscope and ancillary equipment. If the equipment was found dirty, notify microscopy staff.
 - Wipe up any shards of glass you might have produced: nobody likes being jabbed by little slivers of glass in a biology lab.
- Make sure all of your images are saved.
- **Exit the ZEN software.**
- Transfer your files from the local computer to the network storage.
- Wait sixty seconds after quitting ZEN, before powering down the microscope. Use this time to clean around the microscope and make sure you have left a comment.
- Click “Power off” on the desktop – it will shut down in 5 mins, you can walk away.
- **Log off the computer (Do not Shut down):**

Please remember that the optics on these microscopes are **very** delicate, please treat the microscopes gently and with respect. Think of them not as pieces of lab equipment but as **very, very expensive** lenses for very specialised cameras – a really nice lens for up close work for your camera can run around \$2000 and you wouldn't grind pieces of glass into that, microscope lenses can cost much more than that and so why would you grind slides into them???

System Hardware Summary

Microscope Stand: Zeiss Axio Observer 7 Inverted Stand with Apotome 2

Objectives:

Magnification	Type	N.A.	WD (mm)	Immersion
5x	EC Plan NeoFluar	0.16	18.5	Air
10x	Plan Aplanochromat	0.45	2	Air
20x	EC Plan NeoFluar	0.5	0.55	Air
40x	EC Plan NeoFluar	0.75	0.21	Air
40x	Plan Aplanochromat	1.3	0.14	Oil
63x	Plan Aplanochromat	1.4	0.17	Oil

Cameras: (1) Prime BSI monochrome, (2) AxioCam 705 colour

Light Source: Colibri 7 lightsource

Fluorescent Filters:

Position	Name	Excitation	Dichroic	Emission	Example Fluorophores
1	DAPI 5060C	377	409	447/60	DAPI, Hoechst
2	FITC 44	475	500	530/50	EGFP, Alexa Fluor 488
3	Cy3 63 He	572	590	629/62	Cy3
4	Cy5 50	640	660	690/50	Cy5, A647
5	Penta Band	Penta Band	Penta Band	Penta Band	DAPI, FITC, TRITC, Cy5, Cy7
6	Polariser	-	-	-	Brightfield