

# Fluoro 2 – Zeiss Apotome 2.0

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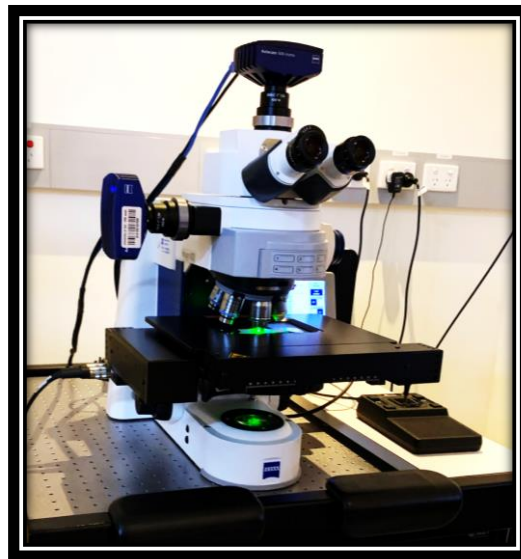
## Quick-start Guide

**Location:** Room 3.021

*(Updated: 10/2/2021)*

### Acquisition Software:

Zeiss Zen Blue



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

**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

## 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 UQ-SSO credentials.
- Press the (grey) power button on the bottom left hand side of the microscope stand and wait for the LCD on the microscope to finish loading.
- 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.
- **Fluoro 2 has both Dry and Oil Objectives. Note:**
  - Dry (these **never** have media applied): 5x, 10x, 20x.
- Oil: 40x, 63x,
- **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.

## Saving Your Data

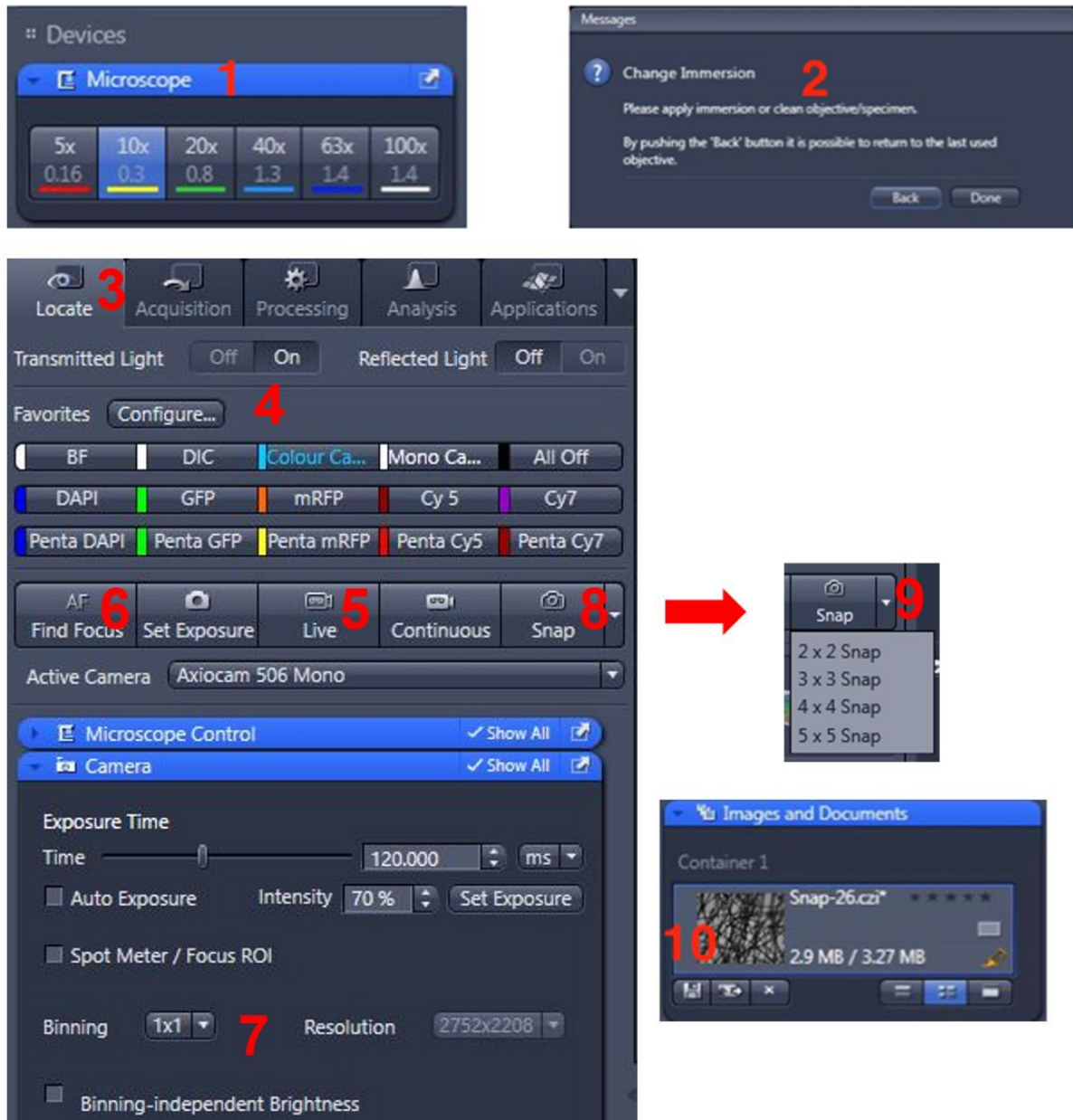
- **Note well;** unless you have specifically set up automated saving (as described below) images are not saved as your session proceeds. You must save your images manually, preferably, as you go.
- There are three different places from which to save:
  - The buttons below the image thumbnails in the **Images and Documents** panel.
  - The diskette icon above the menu tabs.
  - 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 may be cleared from the computer as frequently as once a week.

## 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
    - Easiest and fastest way to view your automatically populated RDMs at IMB: Windows Explorer ➡ This PC ➡ UQ-Inst-Gateway2
    - 
    - Cached locally (\\data.imb.uq.edu.au\shortcode-Q0000)
    - For more information see [rdm.uq.edu.au](http://rdm.uq.edu.au)

## 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.

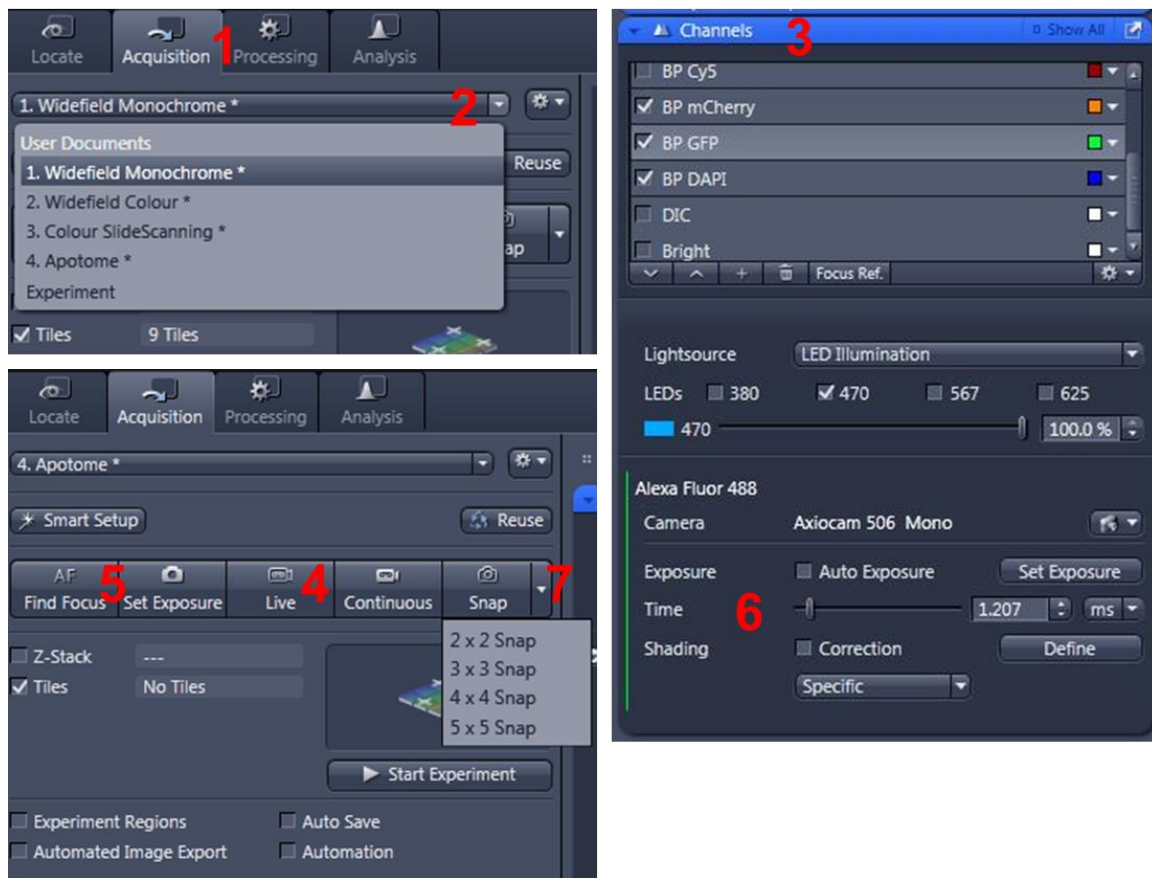
- Select the **10x** objective by clicking on the objective icon **(1)** in the **Microscope** menu on the right-hand side of the screen.
- Follow the prompts if asked to change the immersion oil **(2)**.
- Place your slide on the stage. Note: the edges of the slide must be slid under the silver retention clips.
- Move the **eyepiece/camera slider** on the right side of the microscope head into the eyepiece position (“in”).
- Click on the **Locate** tab **(3)**.
- Select the illumination method, brightfield, DIC, or dye you wish to image, by pressing one of the favourite buttons **(4)** that most closely matches your own dyes emission colour.
  - Quad DAPI/GFP/mCherry/Cy5 are excited by individual LEDs and are detected through a quad-band emission filter, which can result in bleedthrough/crosstalk, when you can see fluorescence from a neighboring channel in the channel of interest but acquisition is fast.
  - BP DAPI/GFP/mCherry/Cy5 are excited by individual LEDs and are detected through individual band-pass emission filters, which results in no bleedthrough but is slower.
- Using the joystick, locate a region of interest in your sample. F1 toggles the XY speed between slow and fast.
- Carefully bring your sample into focus using both the course and fine focus nobs on either side of the microscope.
- Switch to whichever objective you wish to use.
- Move the eyepiece/camera slider on the microscope to the camera position (“out”).
- Click the **Live** button **(5)**, to see the live camera image on the computer display.
- Click the **Find Focus** and **Set Exposure (6)** 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 **(7)**, then adjusting the exposure again.
- If you are collecting single colour images, you may now simply click the **Snap** button to capture an image **(8)**, or use the dropdown tab to capture a quick tile of your sample **(9)**.
- Click **Save (10)**.

### Setting up Kohler Illumination

- Close the **Field Stop** (two small buttons on the right-hand side along the base of the stand) and bring the image of the Field Stop into focus using the **Condenser Focus Wheel** (found *below* the stage but *above* the Specimen Focus Wheel).
  - Centre the image of the Field Stop in the field of view using the sub-stage condenser centering screws.
  - Open the Field Stop until it is *just* beyond the field of view.
- Set the **Aperture Stop** 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).
  - Widefield Monochrome for fluorescence
  - Widefield Colour for brightfield and DIC images
  - Colour SlideScanning for fast, brightfield acquisition of whole slide (shutter open)
  - Apotome for optical sectioning
- Select the tickboxes for the *Channels* you wish to image (3).
- Move the eyepiece/camera slider on the microscope out to the camera position.
- 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, if required.
- 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).

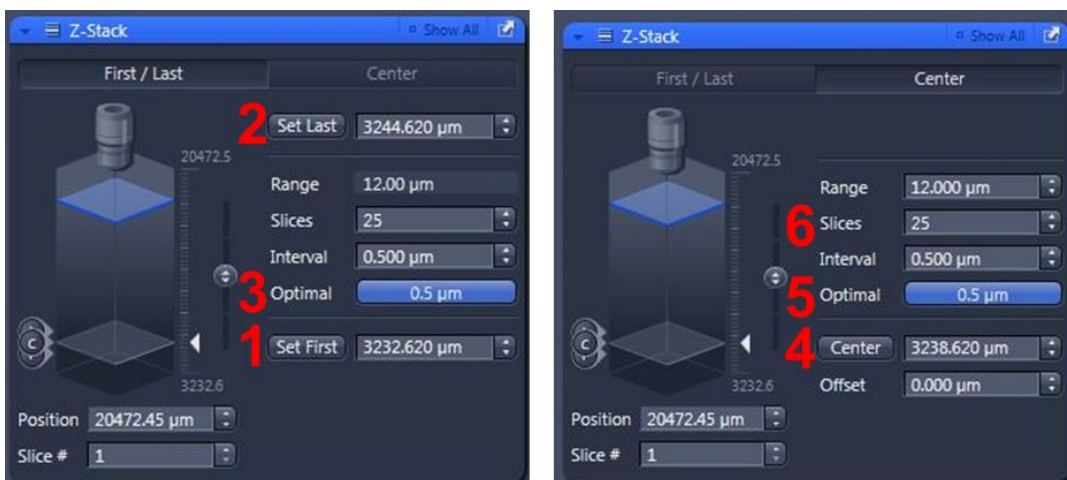
Then, focus down through the sample until the bottom is reached.  
Click *Set Last* (2).

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).

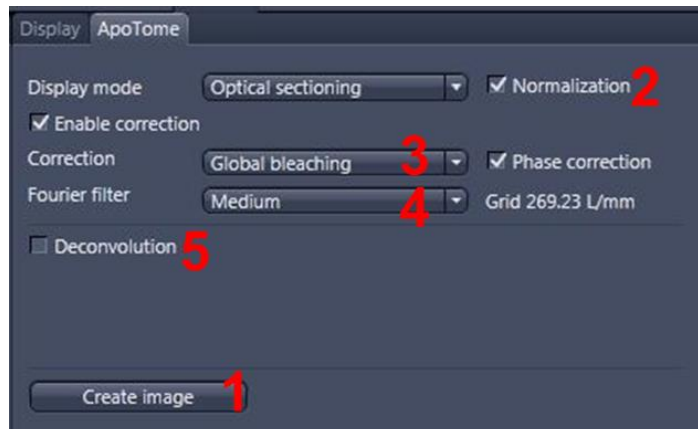
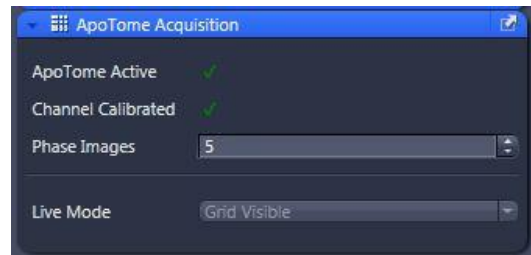
Select interval (distance) between z-sections; the easiest option is to click *Optimal* (5).

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.
- Save your data.



## 2D Apotome

- **Carefully and gently** slide the Apotome module into the side of the microscope body. The microscope will beep when it is in place.
- Select **Apotome** from the experiment dropdown list.
- 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.
- Select the tickboxes for Channels you wish to image.
- Move the eyepiece/camera slider on the microscope out to the camera position.
- Click the *Live* button, to see the live camera image on the display.
  - Note the Apotome blocks a lot of light and you will need to re-set exposure values when using this method.
- 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.
- Increase the image resolution if desired by changing the binning to 1x1, then adjust the exposure again.
- Click *Snap*.
- **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.
- Save your data.



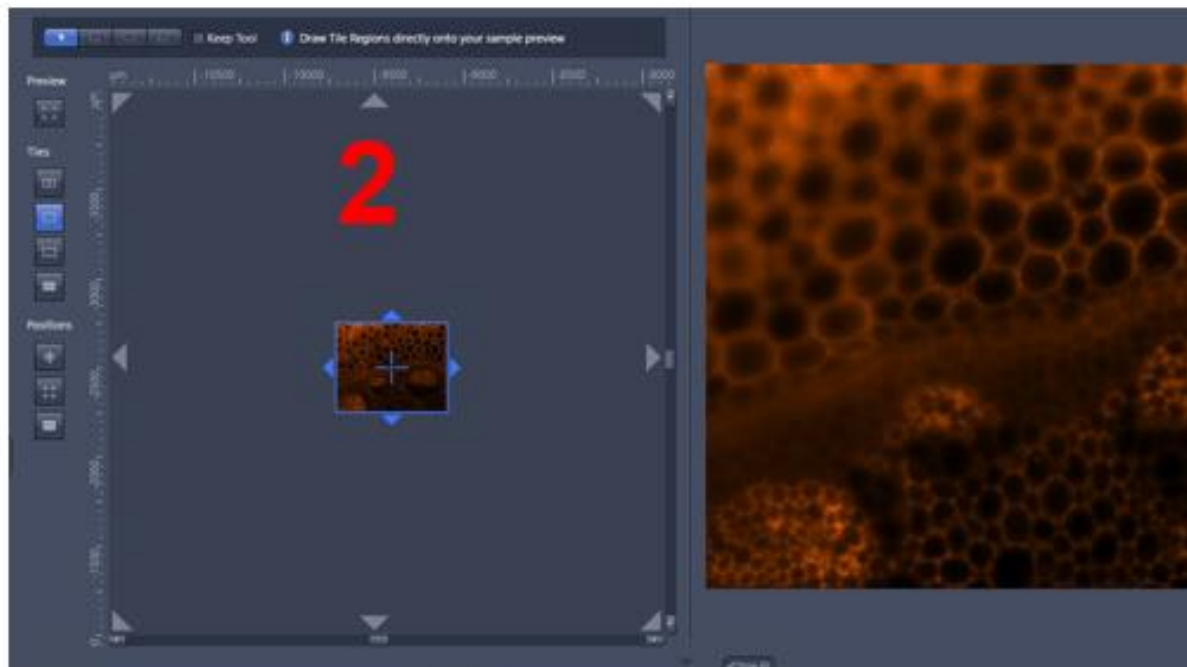
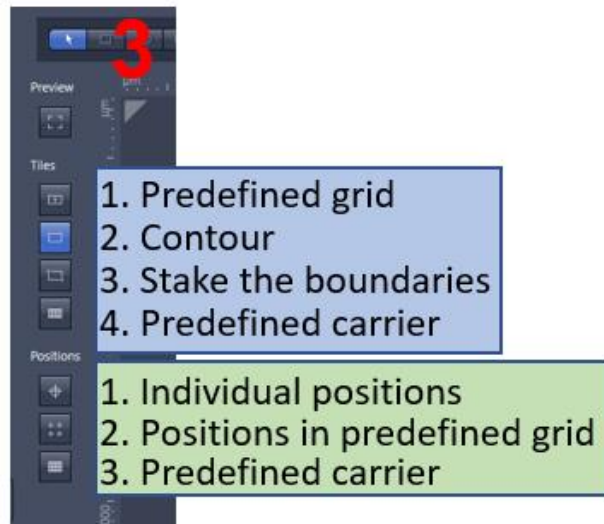
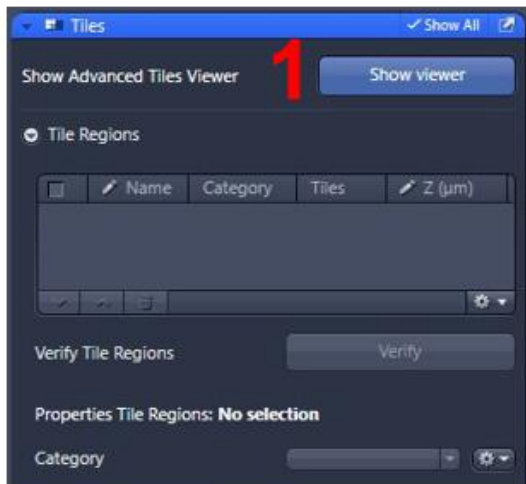
## 3D Apotome

- **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.



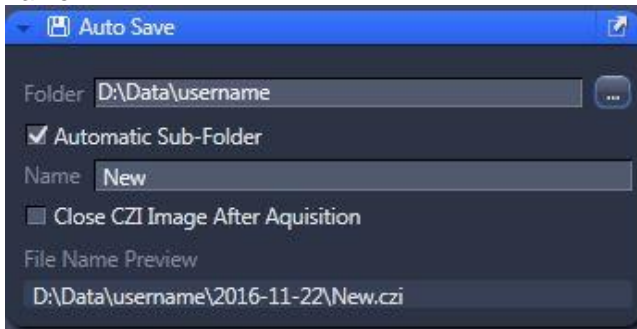
## Advanced Tiling

- Click the *Tiles* checkbox, situated below the z-stack checkbox mentioned previously. A Tiles parameters panel will open.
- In the *Tile Regions* section, Show viewer **(1)**, opens a window that allows you to define a region in Live mode **(2)**.
- The viewer hosts all the controls to define the tiled region, as well as the ability to define individual positions for you acquisition **(3)**. All regions to be defined with carriers are also accessible.

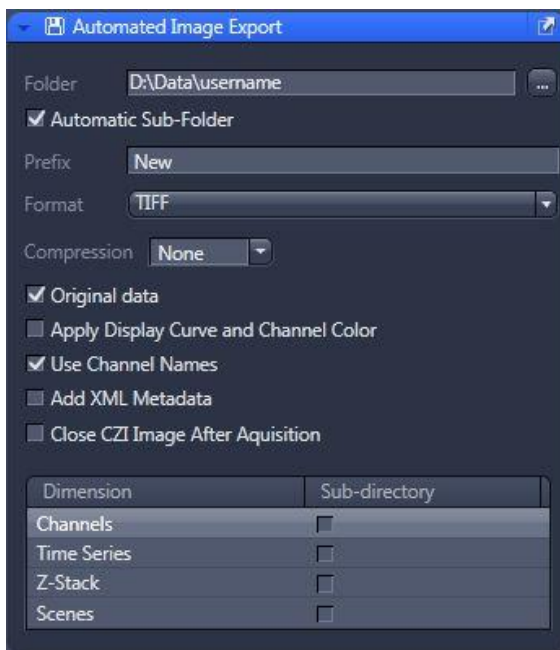
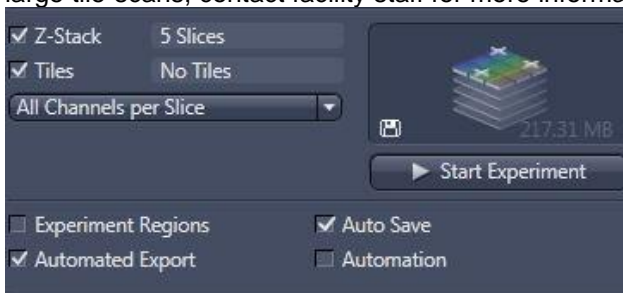


## Other Options

- *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)



- *Image Info (1)*, 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.

**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	<span style="color: red;">█</span>	<span style="color: green;">█</span>	<span style="color: blue;">█</span>

## Shut Down Procedure

- **Lower the stage and remove your sample**
- **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.
- **Log off the computer (Do not Shut down):**
  - Wait sixty seconds after quitting ZEN. Microscope will automatically turn off.

Please remember that the optics on these microscopes are **very** delicate, please treat the microscopes gently and with respect. Think of them not at 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 AxioImage M2.m Upright Stand with Apotome 2

### Objectives:

Magnification	Type	N.A.	WD (mm)	Immersion
5x	EC Plan NeoFluar	0.16	18.5	Air
10x	Plan Aplanachromat	0.3	2	Air
20x	Plan Aplanachromat	0.8	0.55	Air
40x	Plan NeoFluar	1.3	0.21	Oil
63x	Plan Aplanachromat	1.4	0.14	Oil
100x	Plan Aplanachromat	1.4	0.17	Oil

**Cameras:** (1) AxioCam 506 monochrome, (2) AxioCam 506 colour

**Light Source:** Zeiss Colibri 7, solid state LEDs

### Fluorescent Filters:

Position	Name	Excitation	Dichroic	Emission	Example Fluorophores
1	Penta band	385/469/555/631/735	405/493/575/654/761	425/514/592/681/785	DAPI, GFP, mCherry, Cy5, Cy7
2	DAPI 5060C	377	409	447/60	DAPI, Hoechst
3	FITC 44	475	500	530/50	eGFP, Alexa488, FITC
4	Cy3 63 He	572	590	629/62	Tx Red, mCherry, Alexa568
5	Cy5 50	640	660	690/50	Cy5, Alexa647
6	Blank				Brightfield