

Zeiss 980 User Guide

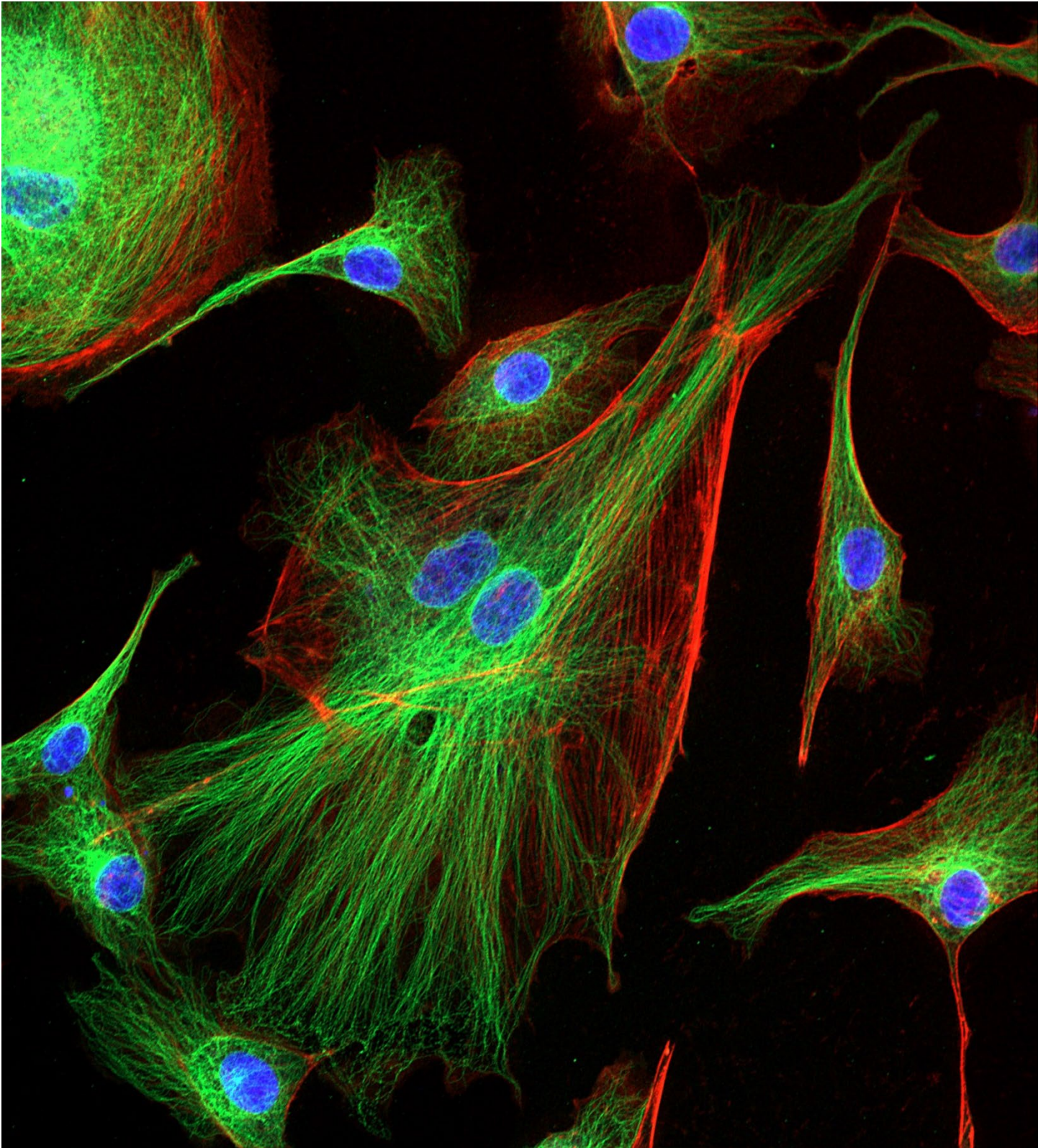


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Starting Up the System

Turn on the **MAIN SWITCH** on the laser rack to the left side of the air table (1).

Turn on the **COMPONENTS** (2) switch on the small remote box.

Turn on the **PC power button** (3).

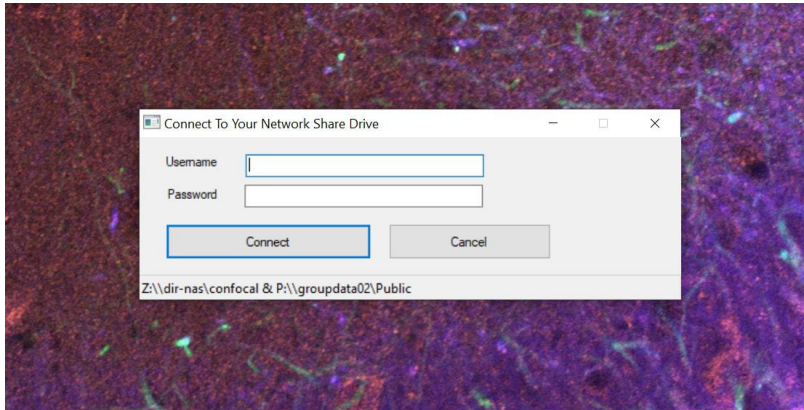


If incubation is desired turn on the two incubator boxes, there are two pieces of tape, one green and the other red, to guide you in which direction to turn on and off the switches.



Connecting to the Confocal Server

Connect to the Network Share Drive with your NIEHS username and password



Launching ZEN Blue Software

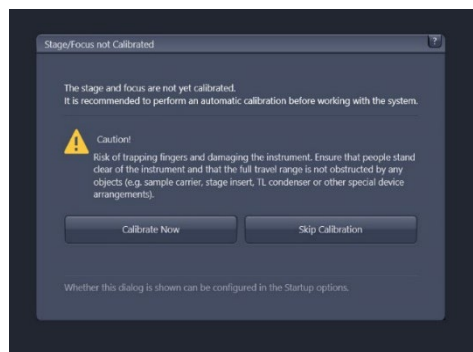
Double click on ZEN 3.6 to launch software.



Move the objective down to its lowest position by turning the focus knob toward you.

Check the area around the stage visually to confirm there are no obstructions.

Calibrate the stage

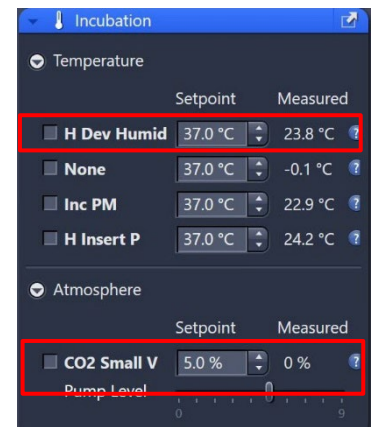


For Live Cell Imaging (Temp, CO₂)

Check that the humidity bottle has a suitable amount of demineralized water. If low, carefully unscrew the bottom glass bottle and fill midway. (Avoid tap water, which leaves heavy deposits.)



Once ZEN launches (see below), activate the required incubation controls in the right-side tool area (in the "Incubation" window). This includes options for heating, CO₂, and humidity control. If using stage-surround heating, activate the "Heating XL" checkbox and wait ~20 minutes for temperature to equilibrate.

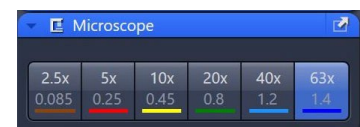
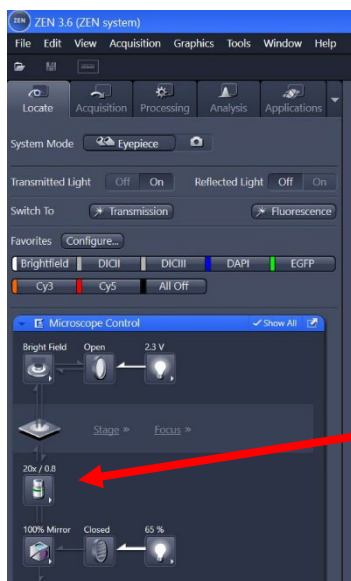


Locating Your Sample

Using the touchpad, select the objective you wish to use. Clean the objective(s) to be used.



Alternatively, under the Locate Tab, select the objective from the 6x motorized nosepiece dropdown menu.



Click the “Locate” tab to permit access to the binoculars.

Select the appropriate objective for examining the slide/dish.

If using water/oil immersion (40x, 63x), add a drop of water/oil directly to the objective front lens. *Never use water/oil on air objectives (e.g. – 10x, 20x), and clean residual water/condensation/oil on slides as necessary.*

Place samples on the stage with coverslip facing downwards.

Use the stage XY joystick to center the objective front lens over an area of interest.

To view the specimen with brightfield contrast (transmitted light), click the “Brightfield” preset button found in the “Locate” tab.

To view the specimen with fluorescence contrast, click on the suitable “DAPI,” “GFP,” or “DsRed” preset buttons.

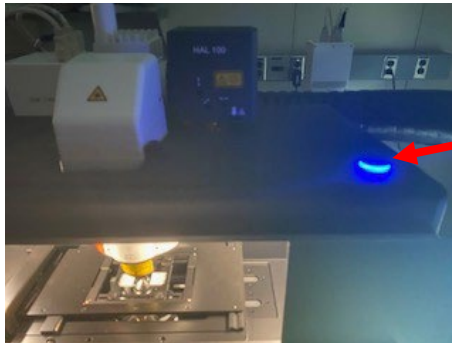


Something New on the Zeiss 980: The AI Sample Finder!

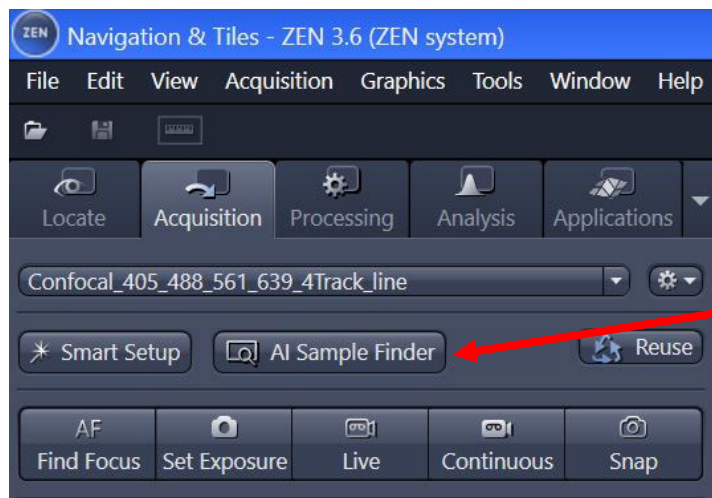
Some may find this new feature very useful. It allows you to capture an overview image of your sample or carrier, which then acts as a navigational guide. This is particularly useful when you have multiple samples, such as brain slices, on a single slide. The AI sample finder will take an overview darkfield image, automatically identify the coverslip, identify the samples, mark the perimeter of the samples (given sufficient thickness), and place grids over the samples for tiling.

Using the AI Sample Finder

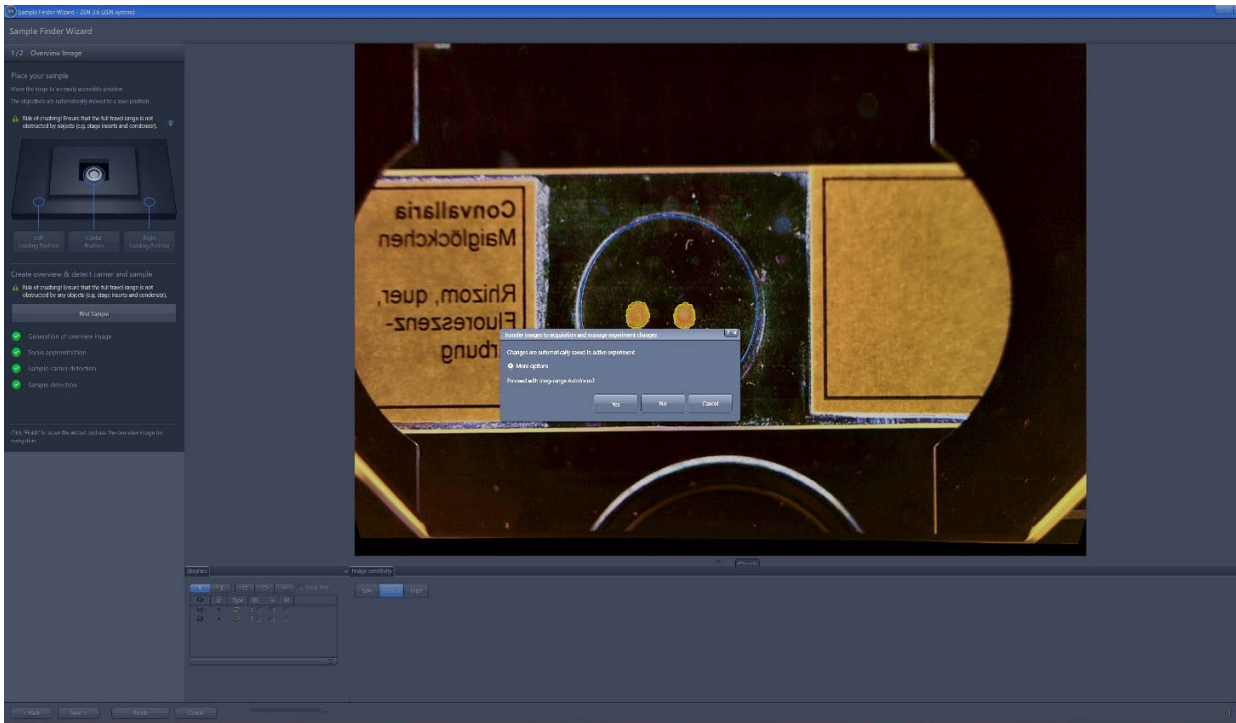
Turn the incubation cabinet light on and to its brightest setting.



Select the Acquisition tab, double click the AI Sample Finder

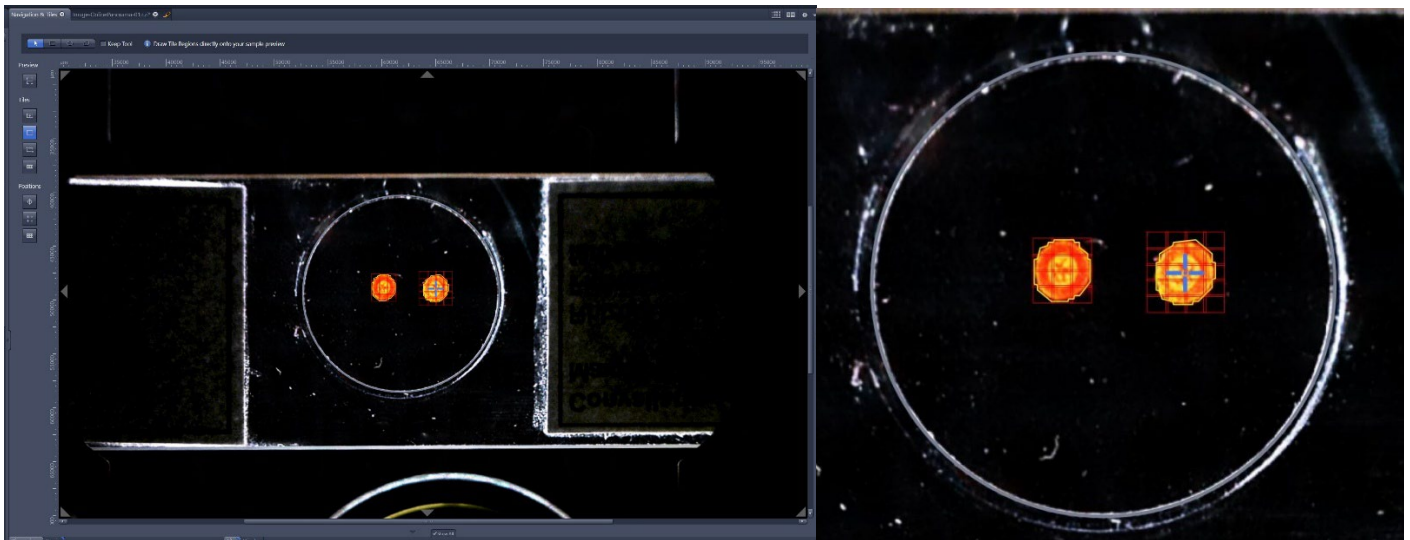


Click Find Sample. This will generate an overview image and approximate the focus.

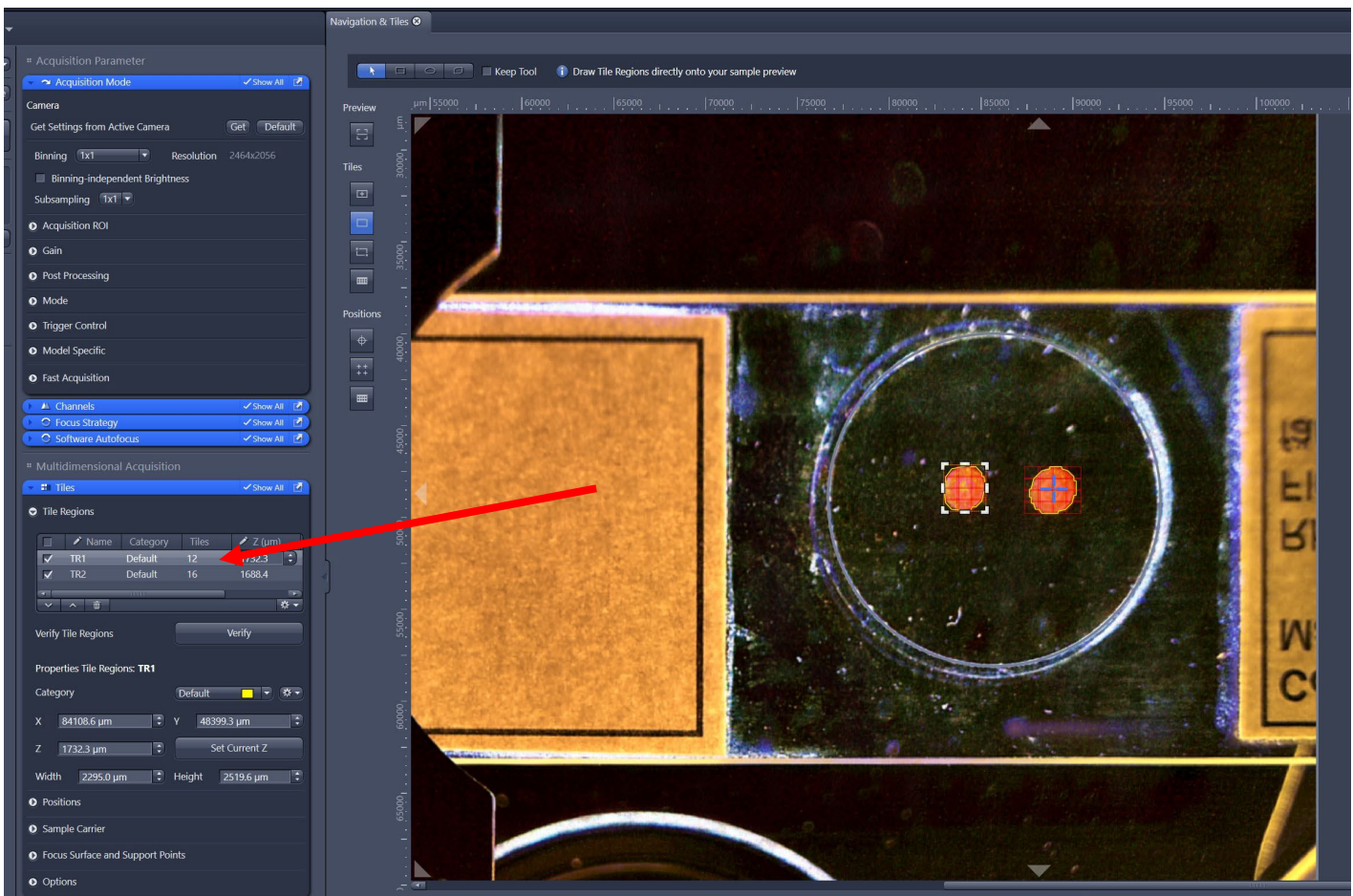


If the sample(s) is sufficiently thick, the AI sample finder will automatically define a ROI. Click finish and proceed with the long range autofocus step.

Turn off the cabinet light and answer Yes to long range autofocus. This will draw a grid of tiles over your sample(s).



If you are not satisfied with the regions and tiling defined by the AI sample finder, under the Tiling panel you can highlight the row corresponding to the region you want to delete, click the trash can, and that ROI and tiling will be removed from your Navigation and Tiles image.



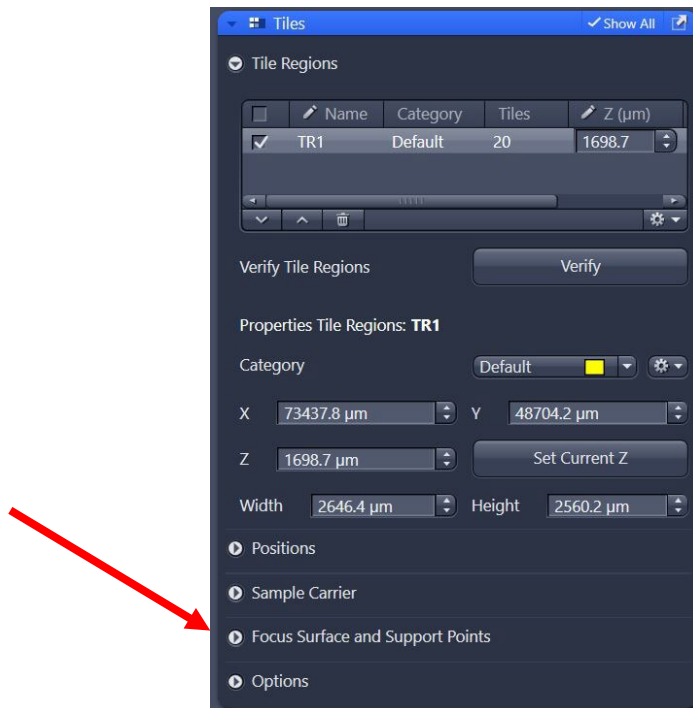
Once you have removed the unsatisfactory tile region, select a tool from the choices in the top left corner (ellipse, freehand, etc.), and draw a region around your sample. The new tiled area will be created automatically.

Note that you can left click and reposition the tiling area as needed.



Creating Support Points

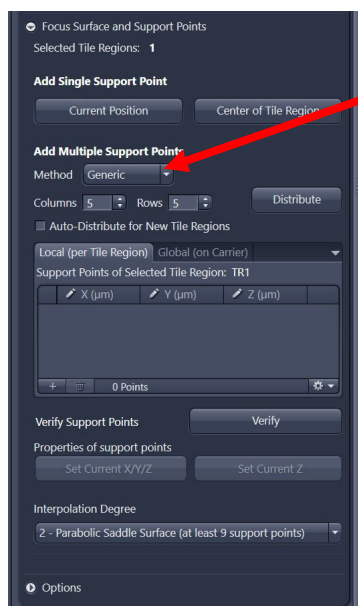
Under the Tiles tab open the *Focus Surface and Support Points* panel by clicking on the caret.



Under this tab you can add either single or multiple support points. In this example we will add multiple support points. Under this option there are two choices, *Generic* and *Onion Skin*.

Generic is a distribution method with a simple column and row approach. This is recommended for most tiling applications, less than 200 tiles, and either rectangular or circular in shape.

Onion skin is used for larger tile applications (>200 tiles) with an irregular shape, for example, whole mouse brain slices.



Click on the tile region (e.g., TR1) to highlight it.

Multidimensional Acquisition

Tiles Show All

Tile Regions

<input type="checkbox"/>	Name	Category	Tiles	Z (μm)
<input checked="" type="checkbox"/>	TR1	Default	20	1698.7

Verify Tile Regions Verify

Properties Tile Regions: **TR1**

Category Default Color

X 73437.8 μm Y 48704.2 μm

Z 1698.7 μm Set Current Z

Width 2646.4 μm Height 2560.2 μm

Positions

Sample Carrier

Focus Surface and Support Points

Selected Tile Regions: 1

Add Single Support Point

Current Position Center of Tile Region

Add Multiple Support Points

Method Generic

Columns 5 Rows 5 Distribute

Auto-Distribute for New Tile Regions

Local (per Tile Region) Global (on Carrier)

Support Points of Selected Tile Region: TR1

X (μm)	Y (μm)	Z (μm)
0 Points		

Verify Support Points Verify

Properties of support points

Set Current X/Y/Z Set Current Z

Interpolation Degree

2 - Parabolic Saddle Surface (at least 9 support points)

Options

Under add multiple support points select Generic Method.

Set the desired number of columns and rows (in our example the tiling region is 4 x 5) then click *Distribute*.

You will now see the support points (dots with a yellow circle around it) distributed over your sample and a list of positions of the support points.

The image shows a software interface for microscopy acquisition. On the left is a settings panel, and on the right is a preview window showing a sample with a tiled region.

Fast Acquisition

- Channels Show All
- Focus Strategy Show All
- Software Autofocus Show All

Multidimensional Acquisition

- Tiles Show All

Tile Regions

Name	Category	Tiles	Z (µm)
TR1	Default	20	1698.7

Verify Tile Regions

Properties Tile Regions: TR1

Category: Default

X: 73437.8 µm Y: 48704.2 µm

Z: 1698.7 µm

Width: 2646.4 µm Height: 2560.2 µm

Positions

- Sample Carrier
- Focus Surface and Support Points

Selected Tile Regions: 1

Add Single Support Point

Add Multiple Support Points

Method: Generic

Columns: 4 Rows: 5

Auto-Distribute for New Tile Regions

Local (per Tile Region) Global (on Carrier)

Support Points of Selected Tile Region: TR1

X (µm)	Y (µm)	Z (µm)
73085.0	47637.4	1698.7
73790.7	47637.4	1698.7
72379.3	48170.8	1698.7
73085.0	48170.8	1698.7
73790.7	48170.8	1698.7
74496.4	48170.8	1698.7
72379.3	48704.2	1698.7
73085.0	48704.2	1698.7

Verify Support Points

Properties of support points

Interpolation Degree: 2 - Parabolic Saddle Surface (at least 9 support points)

Preview

µm | 69000 | 70000 | 71000 | 72000 | 73000 | 74000 | 75000 | 76000

µm | 46000 | 47000 | 48000 | 49000 | 50000 | 51000 | 52000 | 53000 | 54000

Dimensions Carrier

Zoom: 10% Auto Fit

Tools:

Channels: Preview Single Channel Range Indicator

Display

Show Live/Continuous in:

Two red arrows point to the 'Support Points of Selected Tile Region' table and the tiled region in the preview window.

Verifying Support Points

Next, you want to verify the support points. Click on the *Verify* button and a new panel opens, *Verify the Regions/Positions*.

Highlight the first position by clicking on it, click *Move to Current Point*, and click *Live* to begin a live image.

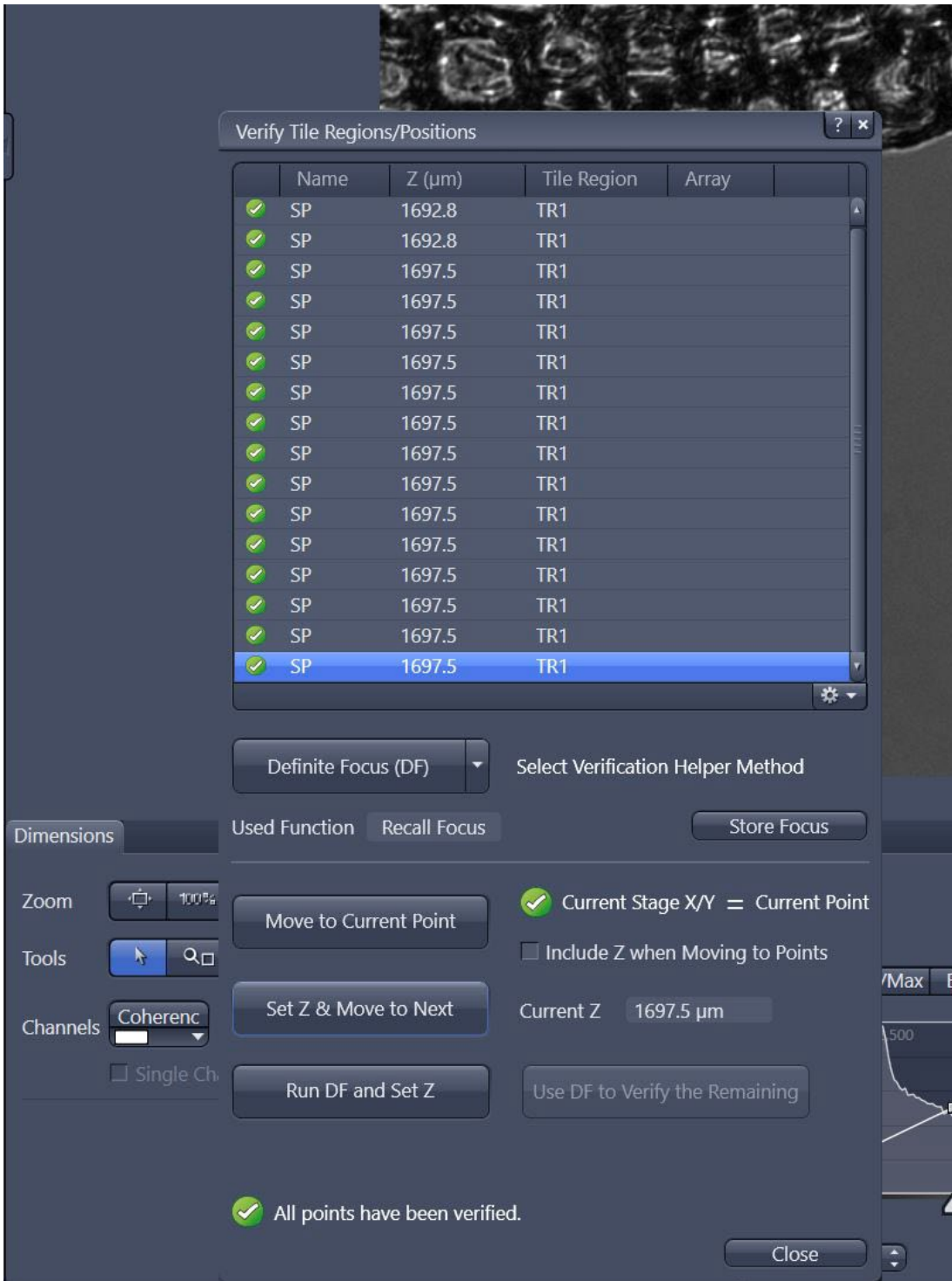
The screenshot displays the 'Verify Tile Regions/Positions' dialog box. The table below shows the list of support points:

Name	Z (μm)	Tile Region	Array
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	
SP	1698.7	TR1	

Below the table, the 'Move to Current Point' button is highlighted with a red arrow. The 'Verify Support Points' button in the left sidebar is also highlighted with a red arrow. A warning icon at the bottom of the dialog indicates 'Not all points have been verified.'

Adjust the focus then click *Set Z & Move to Next*.

Repeat this process of focus and Set Z & Move to Next until all the points have been verified. Once all the support points have been verified you will see a message at the bottom (in green) stating that all points have been verified, and there will be a green check mark next to each position.



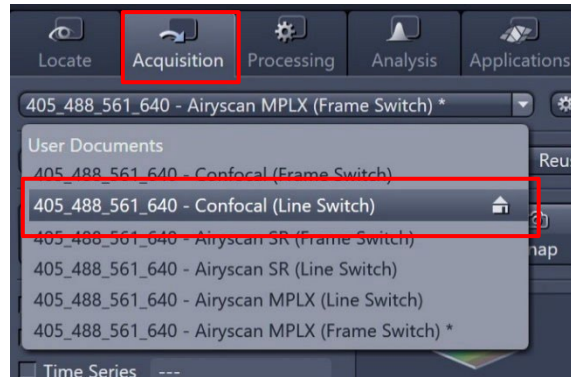
Now you are ready to acquire an image!

Acquiring an Image

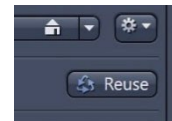
For routine confocal scanning there are three potential methods to load an experiment:

Method 1: In the “Acquisition” tab, use the drop-down box to select a pre-defined configuration with an appropriate combination of laser lines required.

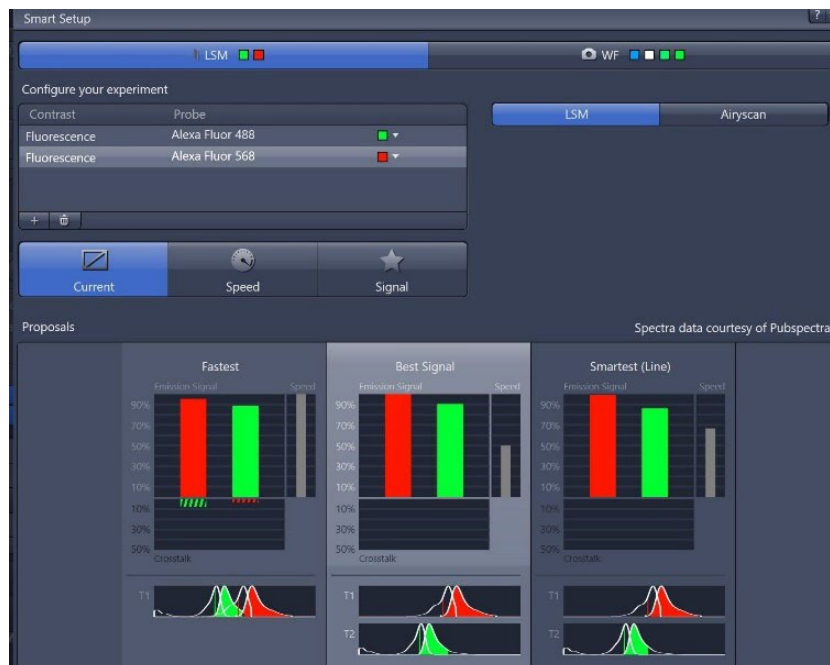
The “**405_488_561_640 – Confocal (Line Switch)**” experiment is an excellent default starting point for up to 4 colors that require very little adjustment of filter stringency.



Method 2: Open any previous image (saved with the .CZI file extension) with similar channels; click the “Reuse” button to repopulate all settings from the image.

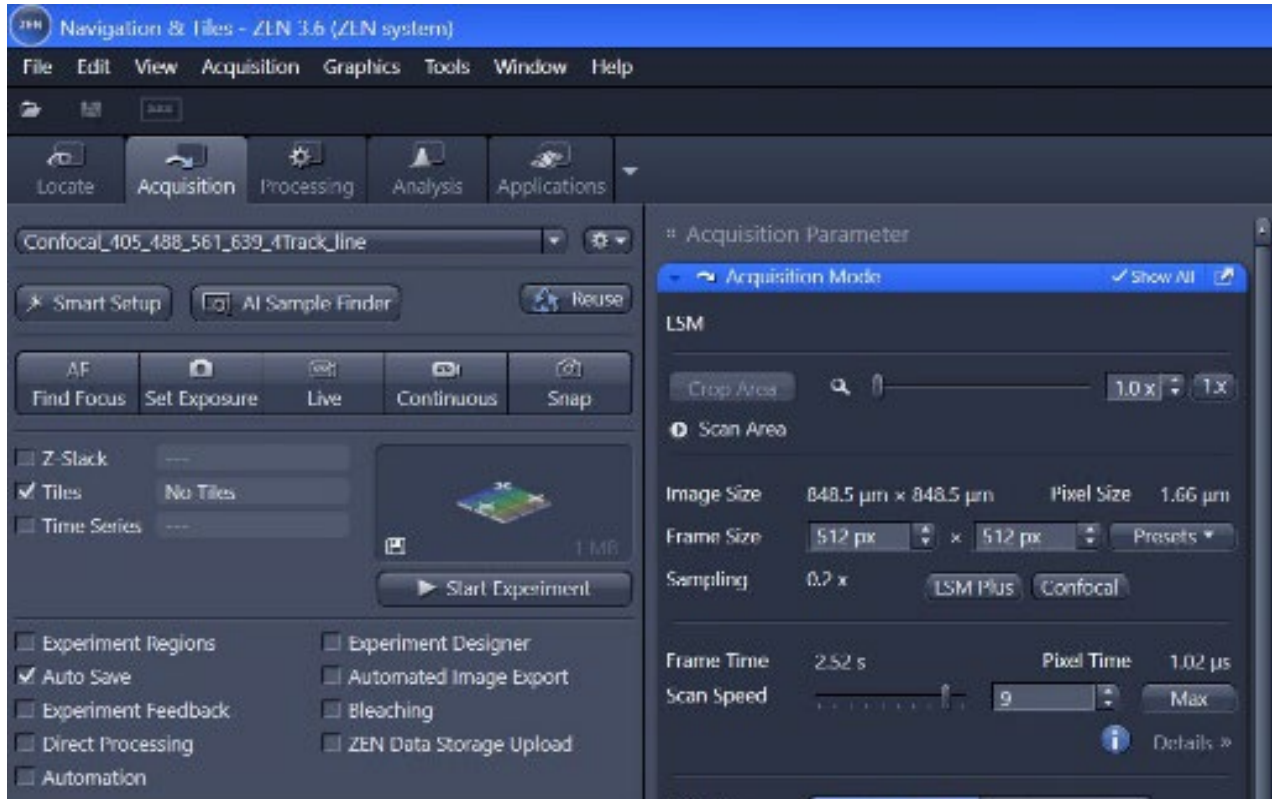


Method 3: Use the “Smart Setup” feature to initialize a stepwise wizard for adding specific fluorophores to the current experiment. The light path will be adjusted based on the given combination, with multiple proposals given to optimize speed, filter flexibility, or a compromise of both.



Under the predefined experiments select from Airyscan Multiplex (MPLX), Airyscan Super Resolution (SR), Confocal, or Widefield experiments.

The example below is loading the **Confocal_405_488_561_639_4track_line** as the experimental setup.



Since the AI Sample Finder was used the *Tiles* box is automatically checked.

Note that the *Auto Save* box is also checked. This is only recommended for a larger experiment like multi-tile Z-stacks, time series, etc., rather than routine imaging.

Adjusting Channel Sensitivity

Highlight a single track in the “Channels” window to view the available parameters associated with that wavelength.

Select the “Live” button to start a fast XY scan.

Activate the Range Indicator checkbox to toggle the scanned image into a display mode where **red** pixels indicate saturation (maximum intensity reached) and **blue** pixels represent a zero value (minimum intensity).

Select the longest wavelength and set the pinhole slider to 1 AU (Airy Units); this will maximize the optical sectioning capability for the given objective lens.

To fine-tune the image sensitivity parameters:

Increase the Master Gain until a relatively small number of red pixels (indicating saturation of the detector) appear in the image. Try to avoid values above 800 V.

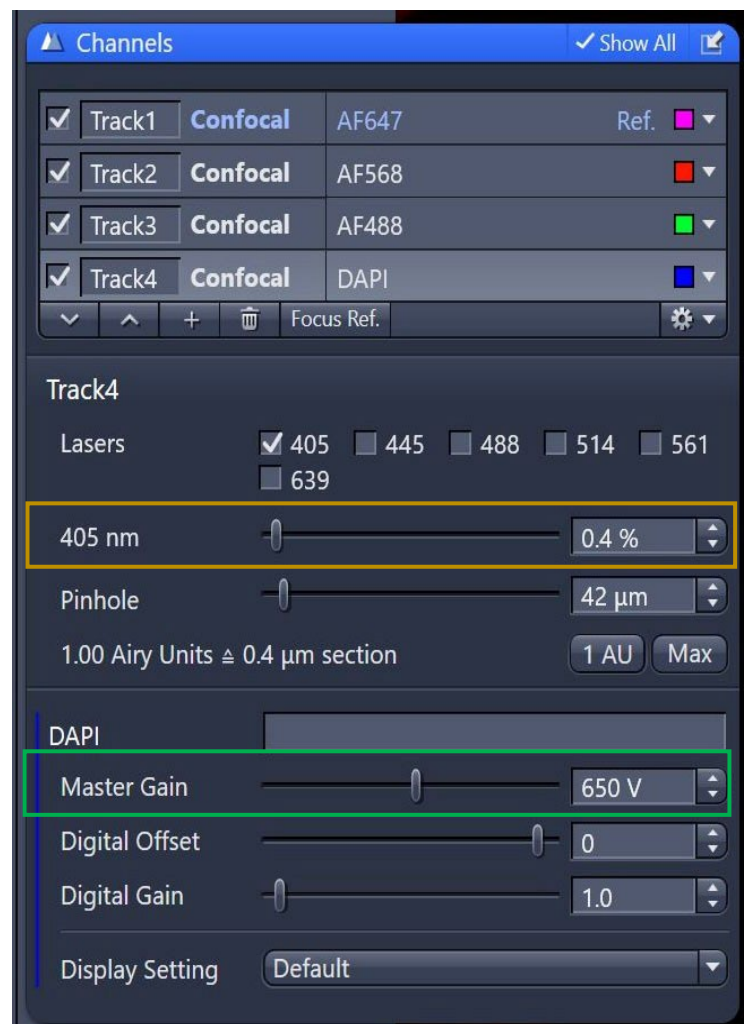
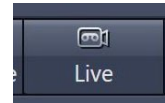
Increase the Laser Power if the detector gain cannot achieve the desired intensity. Values between 1 – 3% are relatively safe starting points.

Adjust the focus with the fine adjustment knob to identify the brightest or preferred Z-position; re-adjust the gain and laser power again if necessary.

Optional – decrease the Digital Offset to reduce background signal. Caution should be used here, as true signal may be eliminated and sensitivity is not altered.

Optional – increase the Digital Gain to amplify all signal and background, thereby stretching the histogram. Again, sensitivity is not directly altered with this function.

Repeat step #5 for additional highlighted tracks/channels as needed



Adjusting Scanning Parameters

In the “Acquisition Mode” window, select a suitable Area and Zoom setting to capture an area of interest. (Alternatively, use the Crop button after a “Live” image has been stopped to adjust the desired field of view.)

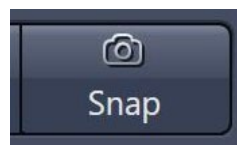
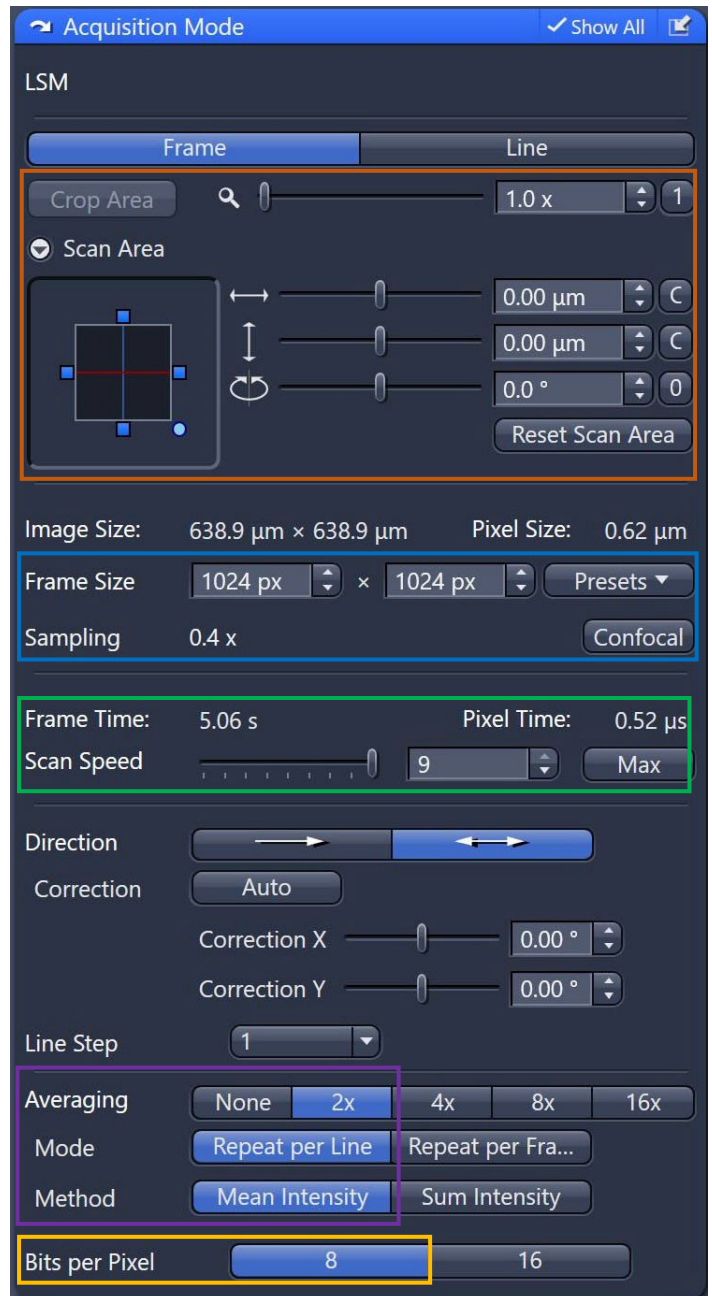
Select a Frame Size to represent the desired area per pixel. While a preset value of 1024 x 1024 is a widely-used starting point, the Confocal button will calculate the best possible diffraction-limited sampling for the given wavelength and objective lens.

Adjust the Scan Speed slider to yield a sensible scan time. A setting of 6 – 8 is usually safe, though noisier samples may benefit from a slower setting.

Select a value for Averaging, which can improve image quality via successive scans with identical settings. A small amount of averaging (2 or 4) is typically more effective at improving signal-to-noise ratios than a slower scan speed.

Select a dynamic range (8, 16 bit) with the Bits per Pixel setting. 8 bits is often sufficient, but more regimented image quantification may benefit from a 16 bit setting. Note that file sizes will jump significantly with higher bit depths.

Click the Snap button to acquire a single (or multi-channel) frame.



Adding a DIC Channel

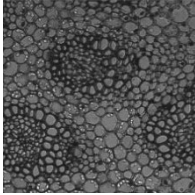
To add a DIC transmitted light image, select either the red or green channel and check the T-PMT box under the Acquisition tab.



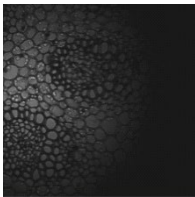
To have the best DIC image check your Kohler illumination, if you need help with this ask Jeff, Erica, or Rob, or check out the Kohler illumination cheat sheet on the next page.

Setting Koehler Illumination

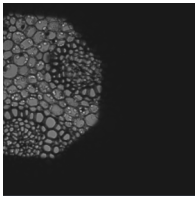
First, bring your sample into focus.



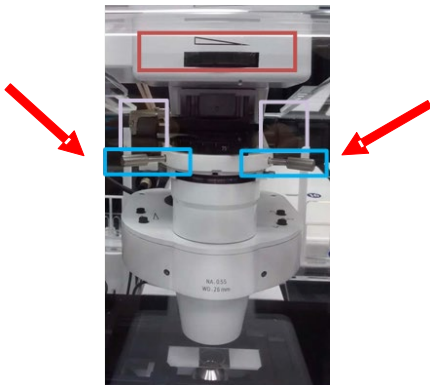
Close the field diaphragm until you can see at least one edge.



Adjust the condenser height until the edges of the field diaphragm are sharp.



Center the diaphragm image using the two centering screws

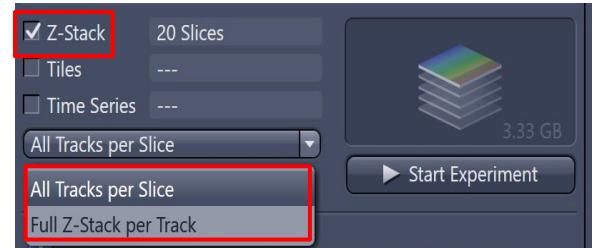


Open the field diaphragm until it just covers the field of view. Koehler illumination is now set.

Acquiring a Z-Stack

Activate the *Z-Stack* checkbox in the main experiment tool area.

Select a preferred scanning mode (e.g. – “All Tracks per Slice,” “Full Z-stack per Track”) in the drop-down box. The “All Tracks per Slice” mode is the default, but may be slower for experiments that use multiple filter changes.



Expand the Z-Stack control window.

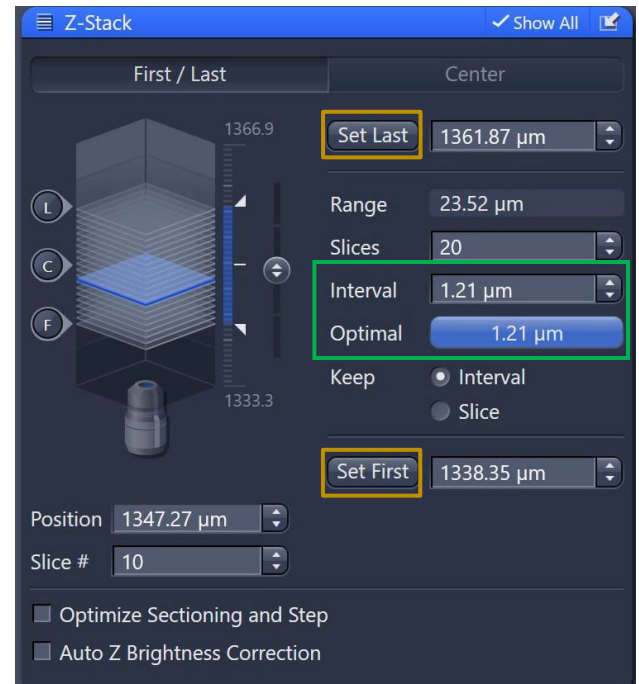
Highlight a channel that exhibits signal throughout the desired sample volume, then click the “Live” button.

Use the focus knob to locate one end of the specimen, then click the Set First button.

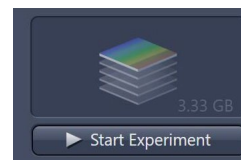
Focus to another end of the specimen, then click the Set Last button. This will mark the total range of the volume. (*The relative orientation of the sample does not matter, as the system will always move the focus drive against gravity during acquisition.*)

Stop the “Live” scan.

Set the Interval to define a step size during the Z-stack. For the best 3D reconstruction, consider using the value of the Optimal button. This will yield a sampling step size that is 50% of the optical section thickness of each plane.



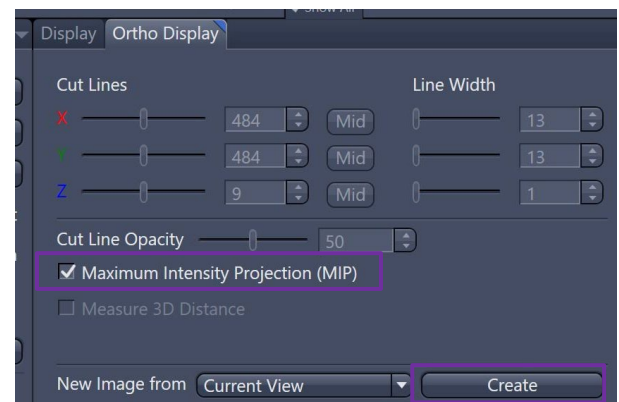
Click on the *Start Experiment* button to begin the recording of the Z- Stack scanning.



Optional – to create a Maximum Intensity Projection image from the stack, navigate to the Ortho tab, which shows a cross-sectional view of the Z-Stack. At the bottom, toggle the

“Maximum Intensity Projection” checkbox and wait for the image to process. If a separate image is needed, click the “Create” button at the bottom and select one or more of the axes shown.

Optional – to view the sample in the rendering/visualization engine, click the 3D tab on the image. Use the mouse to modify the viewing angle as needed. Snapshots of renders can be generated using the “Create Image” button in the bottom tool tray.

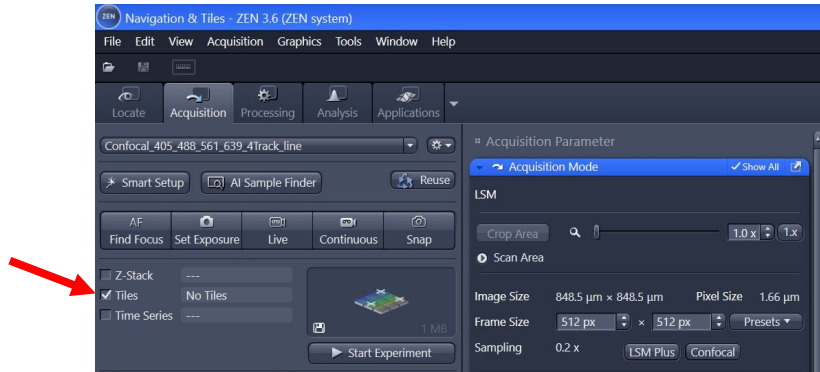


Tile Scan

Tile scanning is a little different on the Zeiss 980. A predefined tile setup may already be in place if the AI sample finder was used.

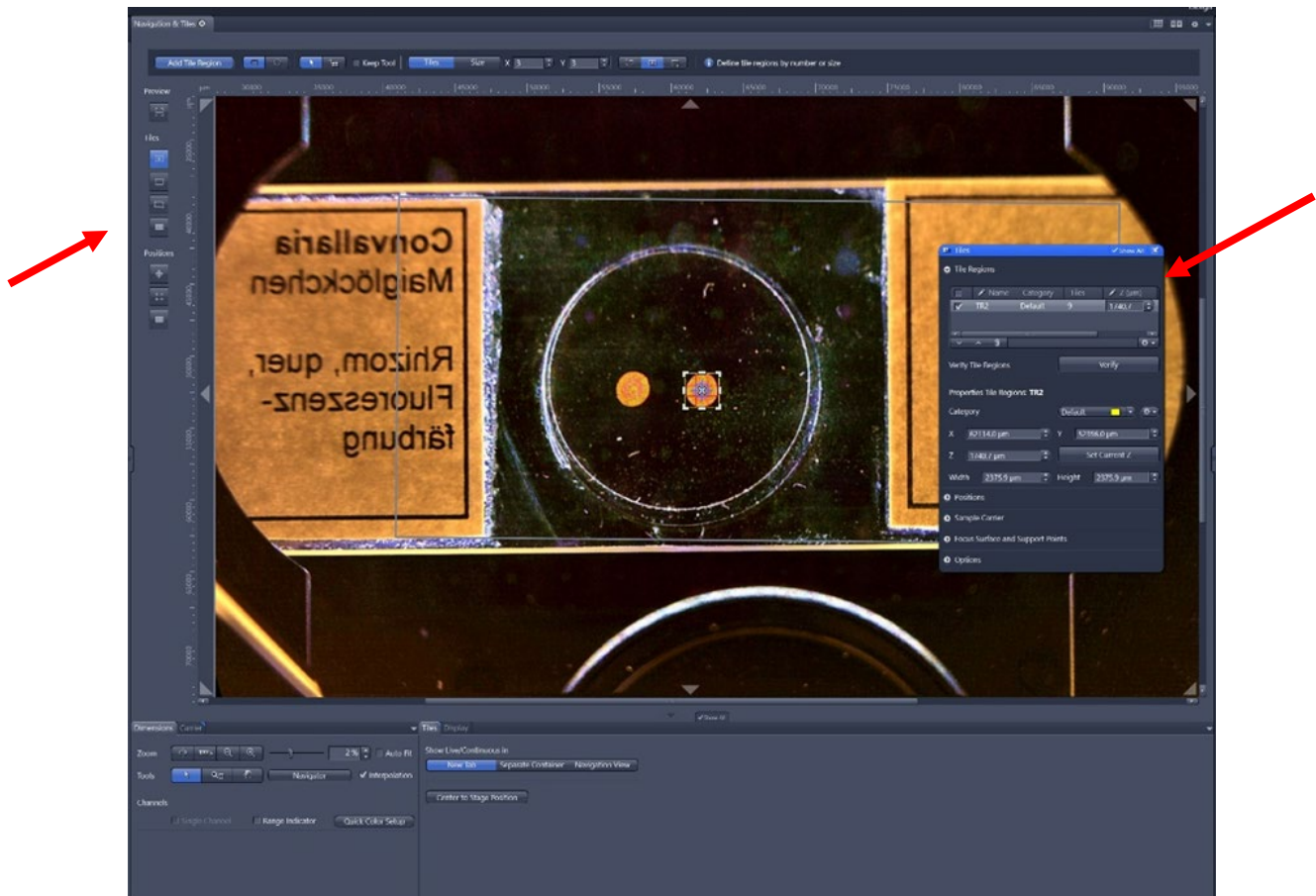
The 980 has a lot of flexibility in tiling, the tiling area defined by the AI sample finder can be changed, and you can define the tiling region by number or size.

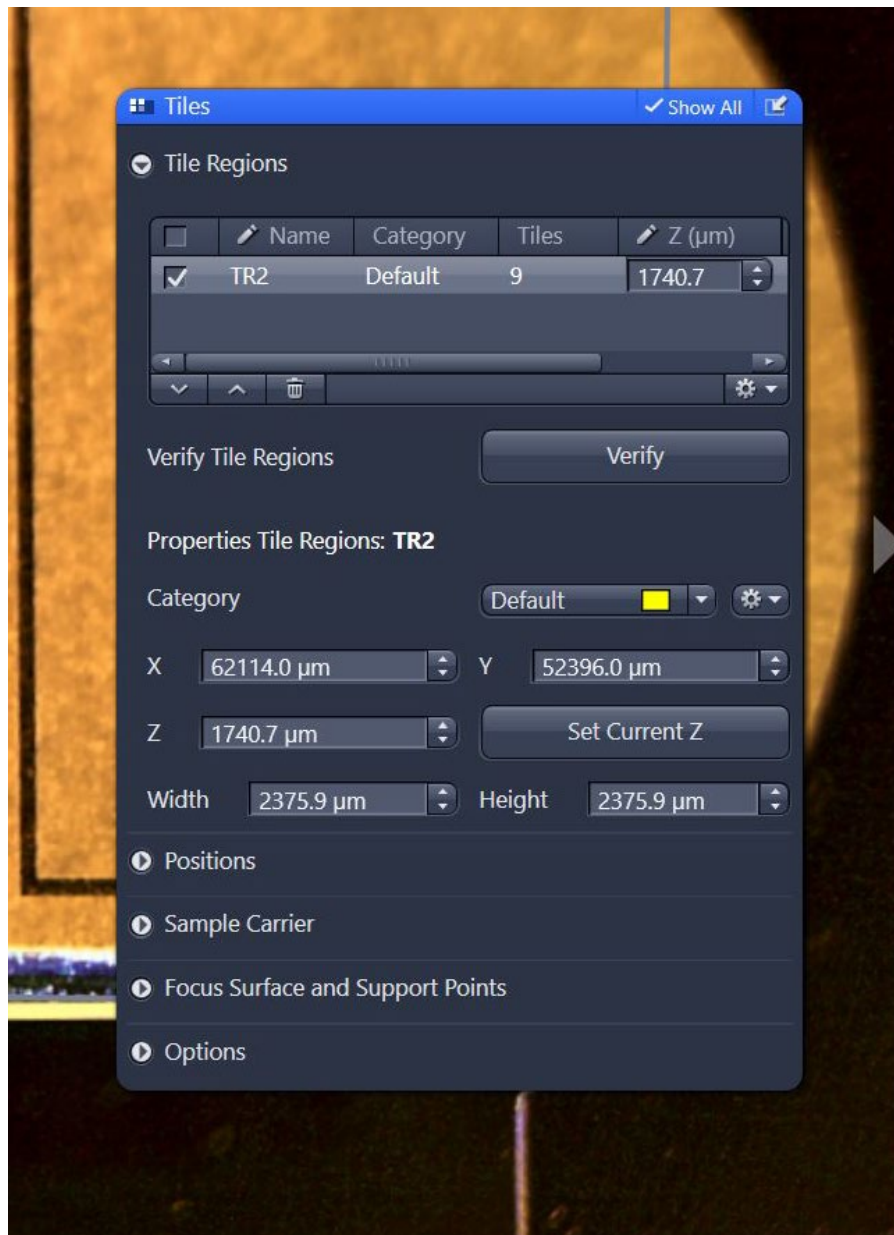
First, check the *Tiles* box.



On the left-hand side of the Navigation and Tiles image there are options for setting tile regions, positions, and acquiring a preview scan.

Undock the Tiles panel for easier navigation.





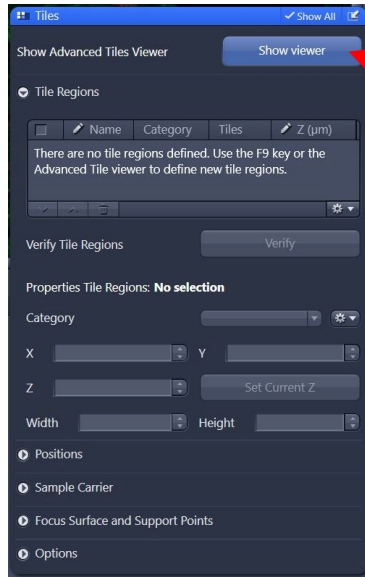
The Tile Regions box has a number of options available:

- Verify Tile Regions
- Positions
- Sample Carrier

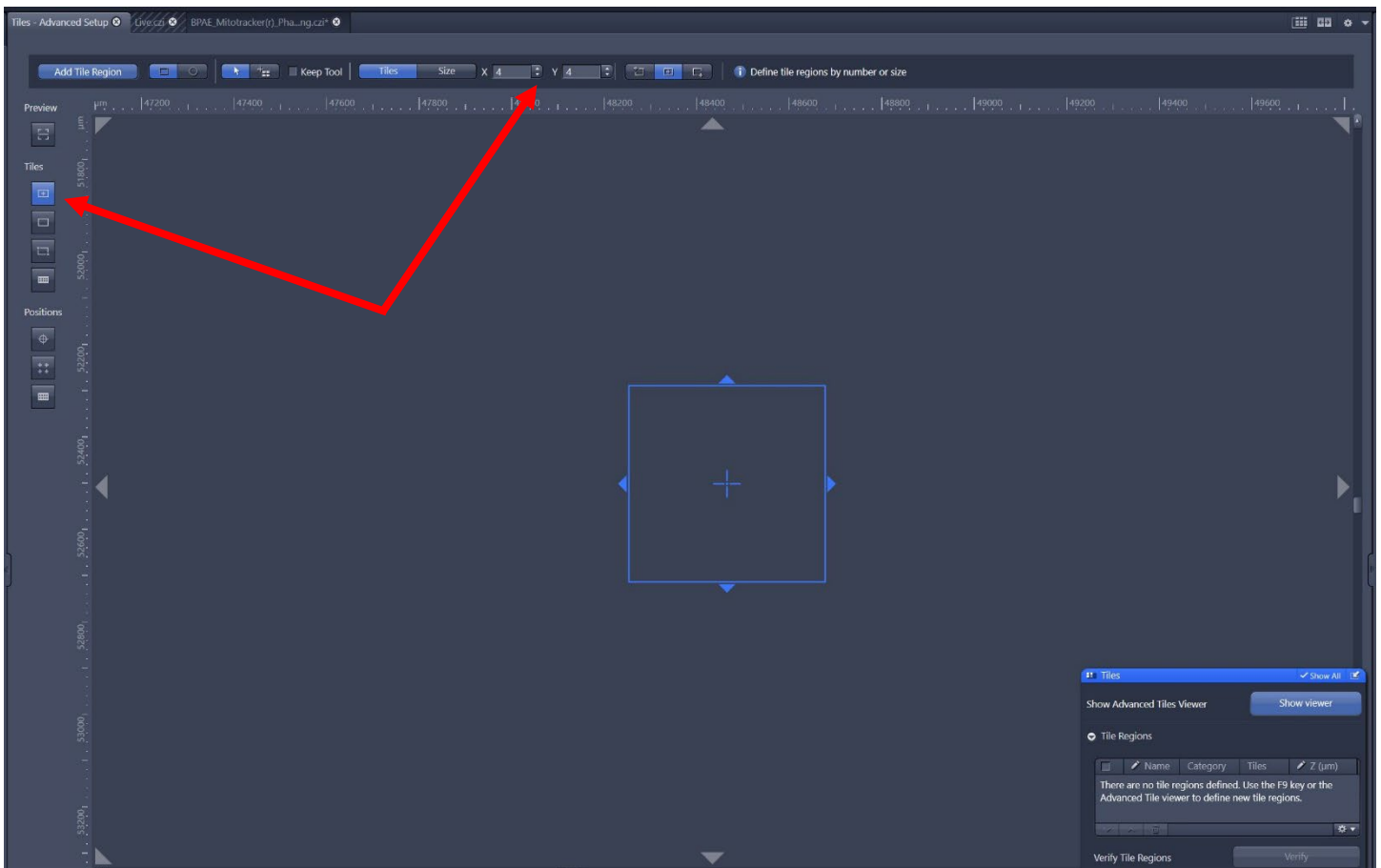
Tile Scan: Predefined Region

To create a tile region of a predefined size, check the *Tiles* box and undock the Tiles panel.

Click on the Show viewer box.



Along the left hand side and top is where you can set up tiles of predefined size.



The example above is for a 4 x 4 tile. Click *Add Tile Region*.

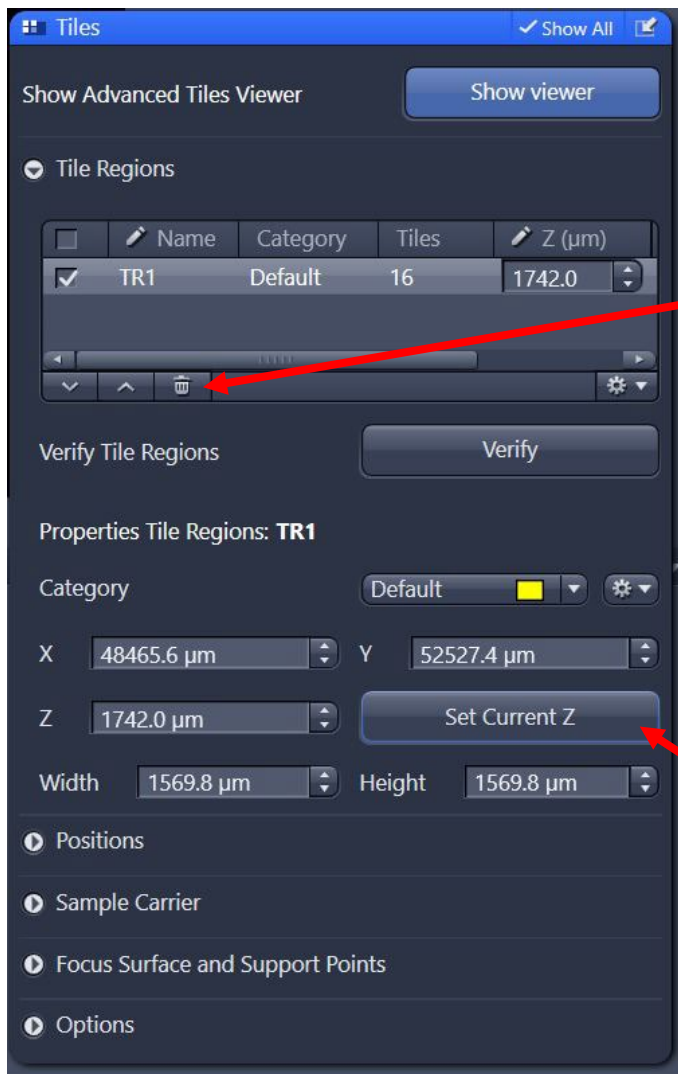
You can use the scroll wheel to scroll out and see your 4 x 4 tile region.

Other options for creating a tiling region are by drawing a contour (second button down), or staking opposite corners to specify two or more marker positions (third button down).

Whichever way you choose to create your tiling region, clicking Add Tile Regions adds the region to the list.

You can delete the region by highlighting the Tile Region (TRx) in the Tiles dialog box and clicking on the trash can icon.

Before starting the experiment adjust the focus and in the Tiles box click *Set Current Z* button.



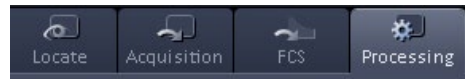
Click Start Experiment to begin data acquisition.

Stitching a Tile Scan

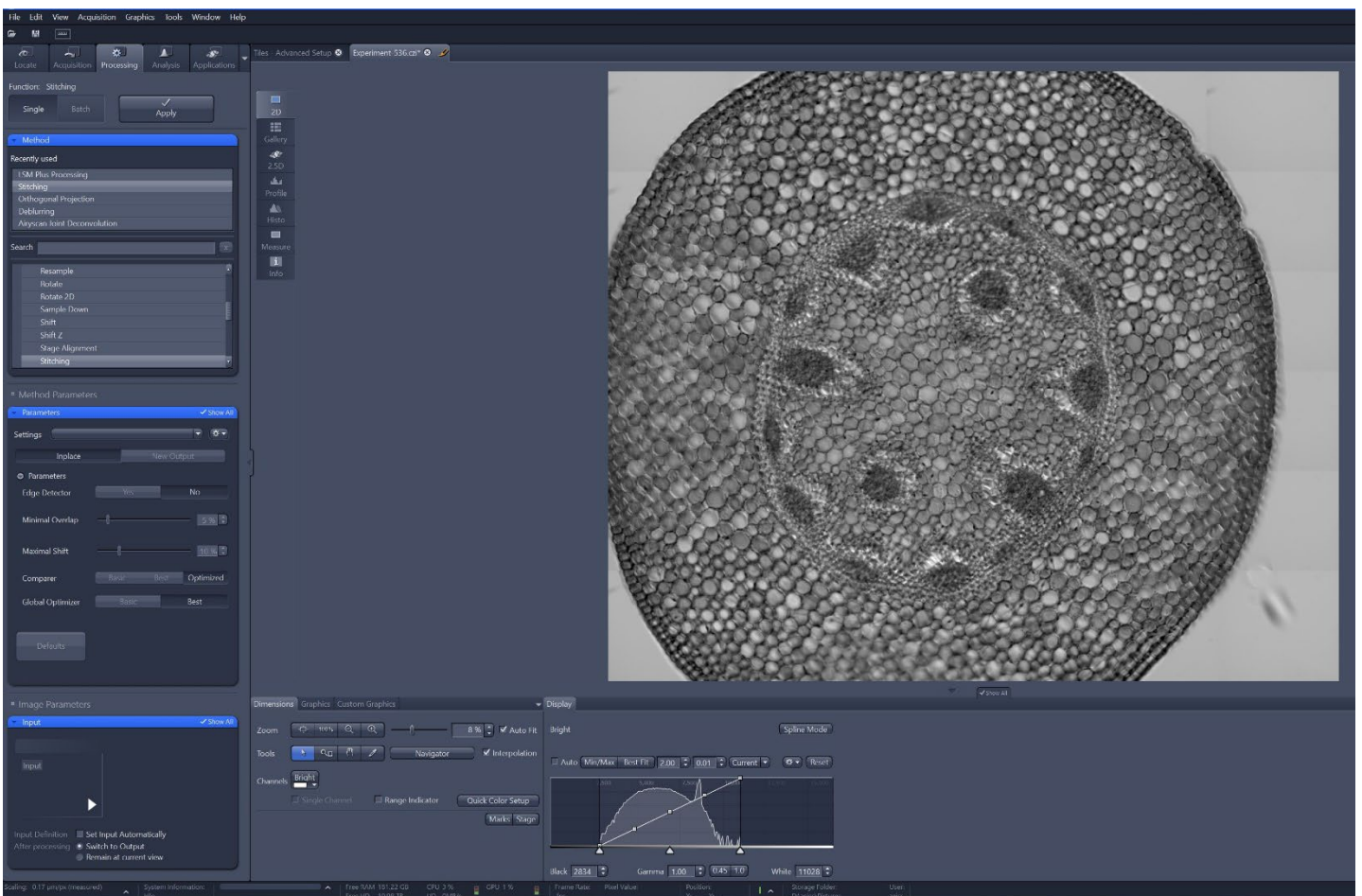
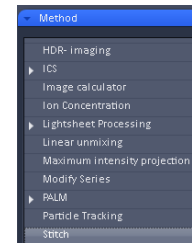
After you take your image save your file. Either click on *File*, then *Save*, from the dropdown, or click on the disk icon on the top left.



Click on the *Processing* tab.



Under Method click on *Stitch*. If you don't see *Stitch* in the Recently Used dropdown you can simply search for it using the Search box.



Open the Input tab and check the *Set Input Automatically* tab. The image you wish to stitch should now be the Input thumbnail.

Open the Parameters tab. Select *New Output* and check the *Fuse Tiles* box.

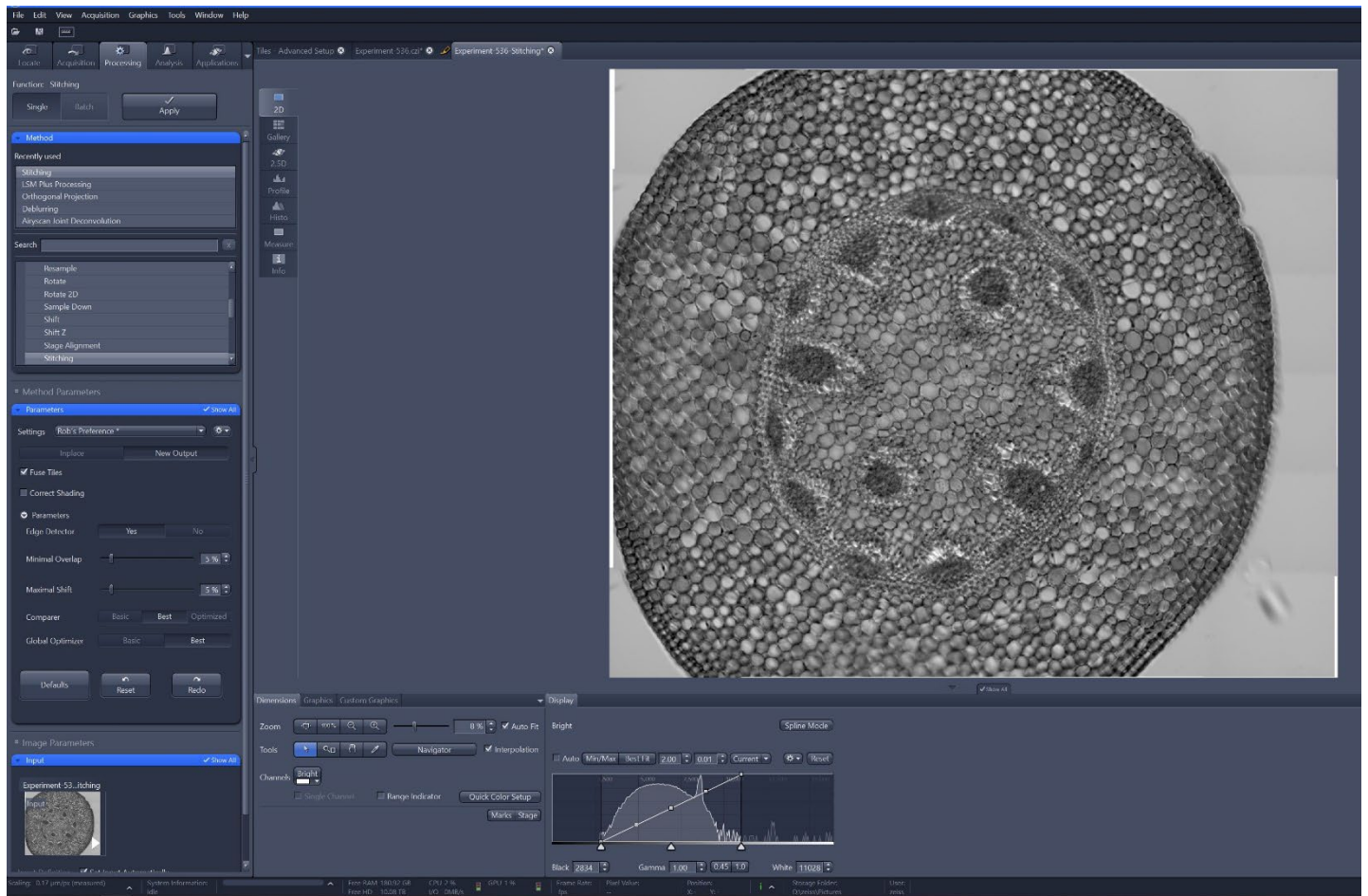
Select Edge Detector = Yes

Minimal Overlap = 5%

Maximal Shift = 5%

Comparer = Best

Global Optimizer = Best

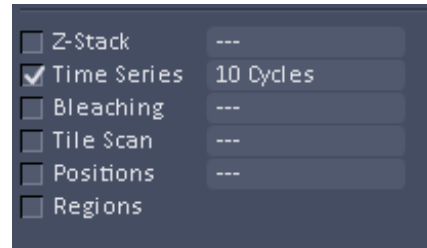


Click *Apply*.

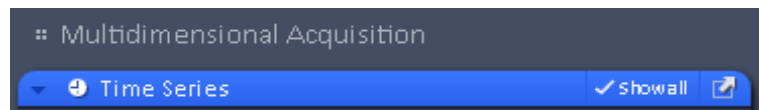
The stitched image will appear as a new output with “stitching” appended to the image name.

Acquiring a Time Series

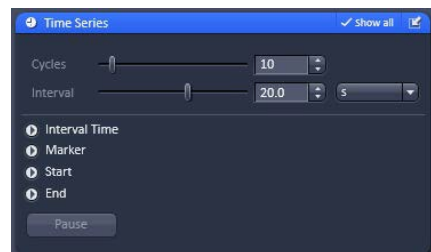
To acquire a Time Series first check the Time Series box.



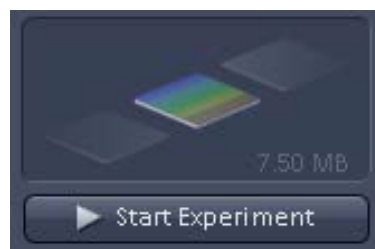
The Time Series tab will appear under the Multidimensional Acquisitions on the left side. To move the Time Series box, click the undock tab.



Set the number of cycles for the number of times you want to acquire the images, as well as the interval between cycles.



Click Start Experiment.

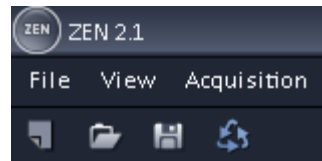


Saving Your Image

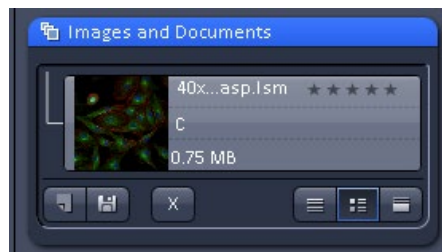
After you have taken your image, you may save your file by using one of the following options:

Click on File, then select Save from the dropdown.

Clicking on the disk icon on the top left.



Clicking on the disk icon on the right-hand side under the Images and Documents panel.



Storing and Exporting Data

To save an acquired or processed image, click on that image in the right tools area to highlight it. Click the Save button (disk icon).

Enter a file name and save as a .CZI format. Data should not be saved onto the C:\ of the system!

After saving a copy of the .CZI data (which retains all hardware information used during the experiment), images can be exported to various formats as needed.

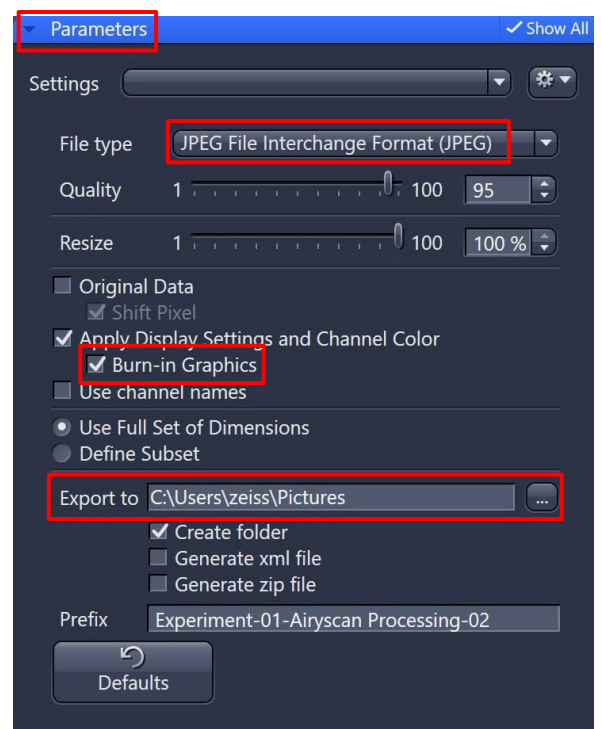
To export, go to the menu entry **File** **Export** **Export**.

In the “Parameters” window, choose the file type and any relevant compression settings.

If scale bars do not need to be exported, the “Burn-in Graphics” checkbox can be deselected.

Double-check a destination path.

Click the “Apply” button at the top.



Setting Up an Airyscan Experiment

Move to an Airyscan-optimized objective (typically 63x/1.4 oil) and ready the specimen.

Load either a preset configuration – such as “**405_488_561_640 – Airyscan (Frame Switch)**” – or use the “Smart Setup” feature, select the Airyscan mode, and add fluorophores.

Consider adjusting the scan area $> 1.7x$, as larger empty areas will yield longer scanning time.

Highlight a track in the “Channel” window, and click the “Live” button.

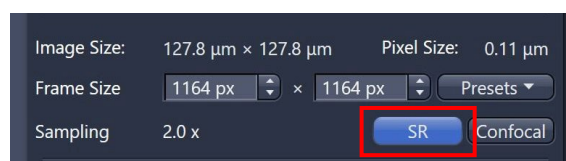
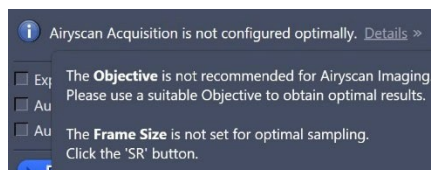
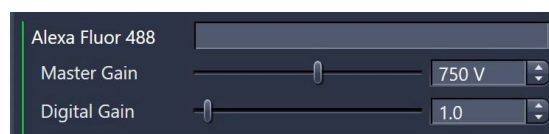
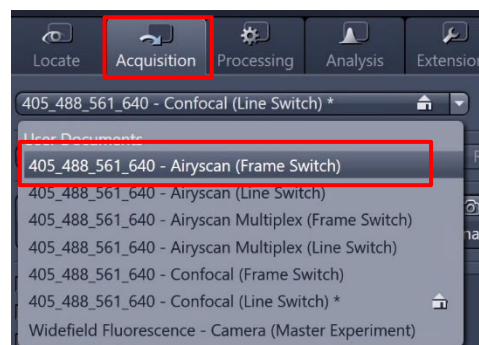
Adjust the Master Gain and Laser Power (with the help of the Range Indicator tool) to minimize saturation. (See *earlier section, ‘Adjusting Channel Sensitivity’*) Extra care should be used in this mode to minimize grossly saturated pixels. As before, avoid use of gains above 800 V. **Note that the Pinhole and Digital Offset are not adjustable in this mode.**

Click the Airyscan detector view button along the bottom toolbar; confirm that the hexagonal detector view is centered and aligned.

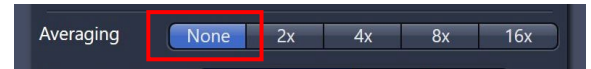
Repeat step #5 for additional tracks/channels as needed.

Check the optimal settings (e.g. – Frame Size, Scan Area, objective lens) as instructed in the experiment warning(s).

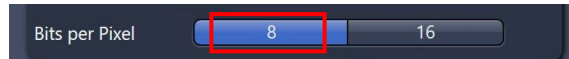
Using a super resolution-limited pixel count (“SR”) is critical for maximizing the capabilities of the Airyscan concept. Note that these values may be significantly higher than standard confocal resolution experiments.



The benefits of Averaging are less critical to routine operation of the Airyscan. To keep scan times feasible, consider starting with 1x Averaging values.



Raw Airyscan data is efficiently collected with 8-bit data depths; Airyscan processing always yields 16-bit image results.



Click the “Snap” button and wait for the scanning to complete.

After the scan, navigate to the “Processing” tab and select Airyscan Processing in the “Methods” window. Apply the setting to generate the pixel- reassigned and deconvolved result.

