

SOP: Zeiss Live Imaging (Axio Observer)

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Equipment Overview

The Zeiss Axio Observer is an inverted microscope that is fully motorized with an enclosed incubator which is ideal for live cell imaging experiments. It has high resolution imaging, incubation control (CO₂, temperature, heating), a large field of view and fast frame rates (22-67 fps with binning). The system uses an LED light source and various filter cubes for fluorescent imaging. The default settings are indicated below:

Filter Cube	Excitation Range (nm)	Emission Range (nm)
DAPI	335-383	420-470
FITC	455-495	505-555
Rhodamine	540-580	575-640
Texas Red	540-552	593-668
Cy5	625-655	665-715

Notes before use:

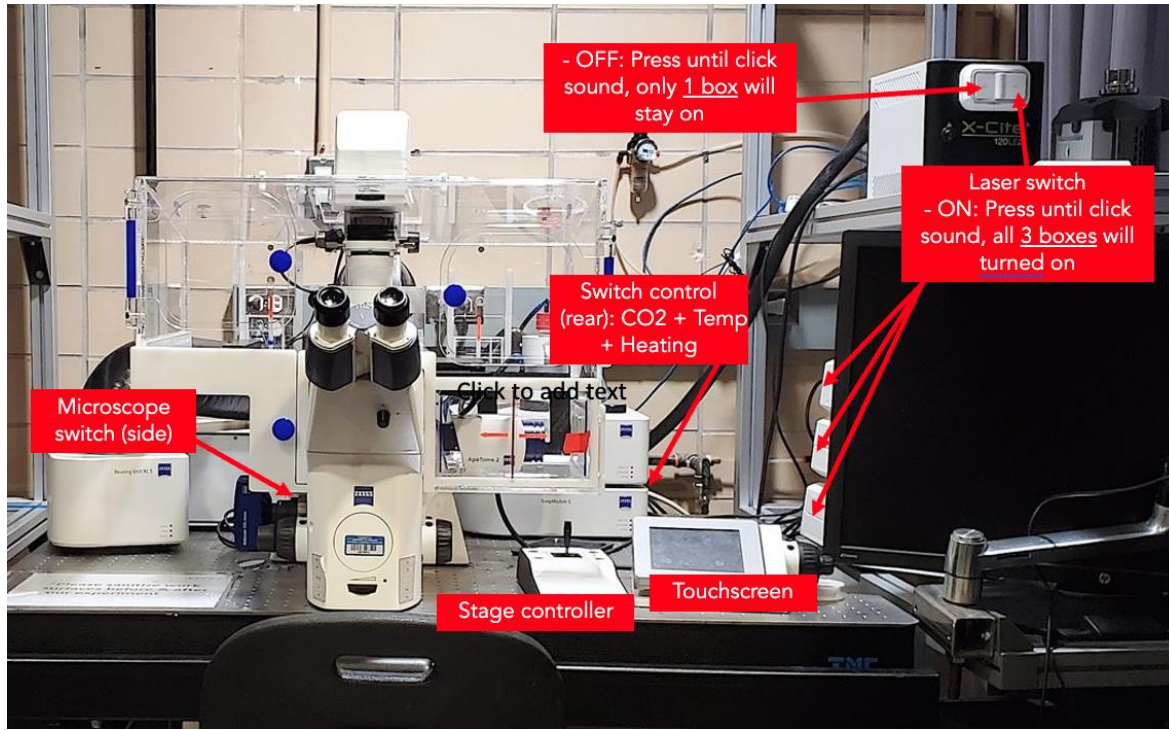
- Log into LMACs to start using the instrument, and sign-out before leaving.
- Ensure incubation chamber is closed before starting the instrument.
- During system start-up, wait 30 seconds between instrument units to prevent communication issues.

Supplies and Reagents

Provided by CRAFT:

- RTU disinfectant or 70% Ethanol
- Kimwipes
- PPE (lab coat and safety glasses)
- Stage insert for holding samples (dish, microplates, slides, etc)

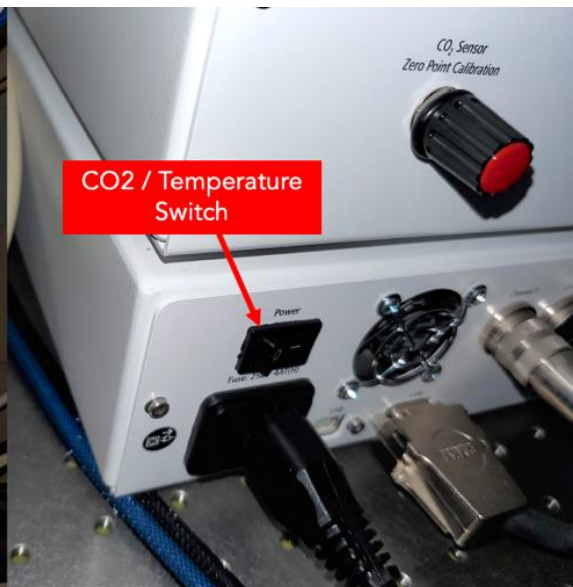
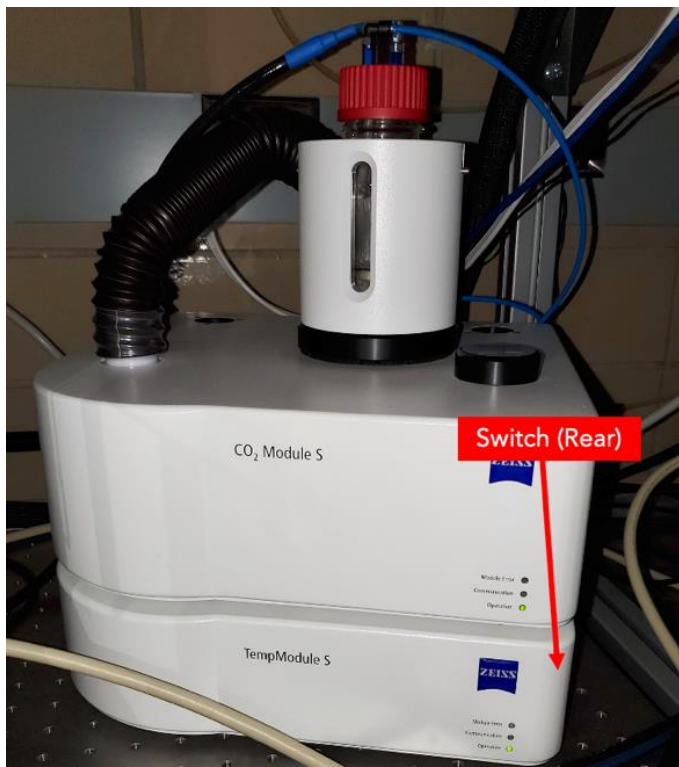
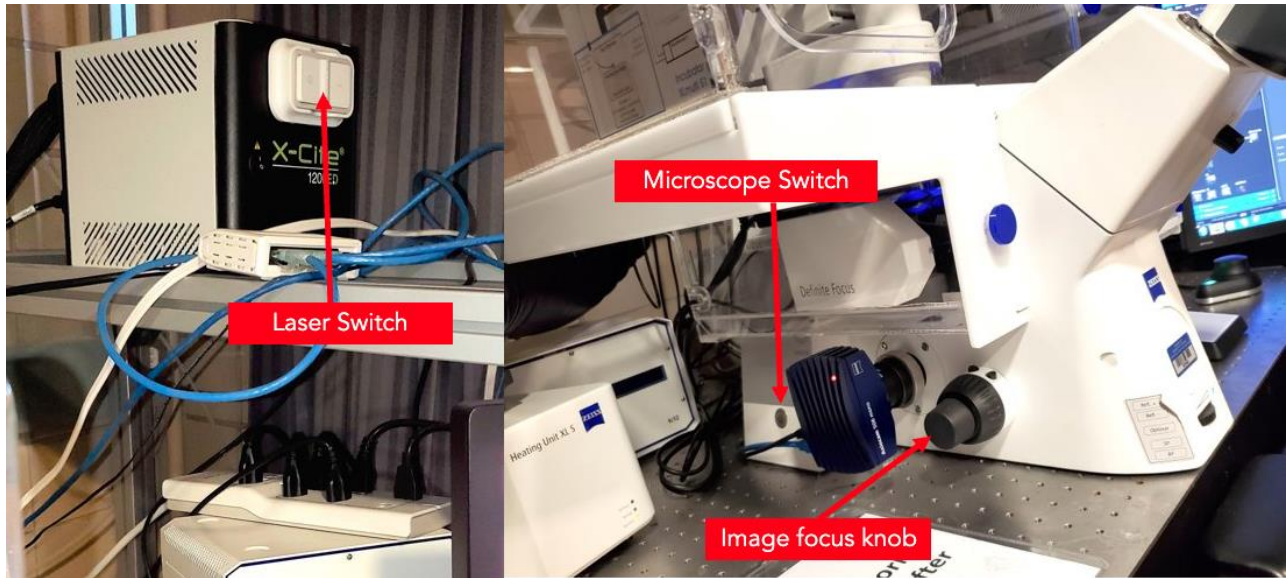
Method

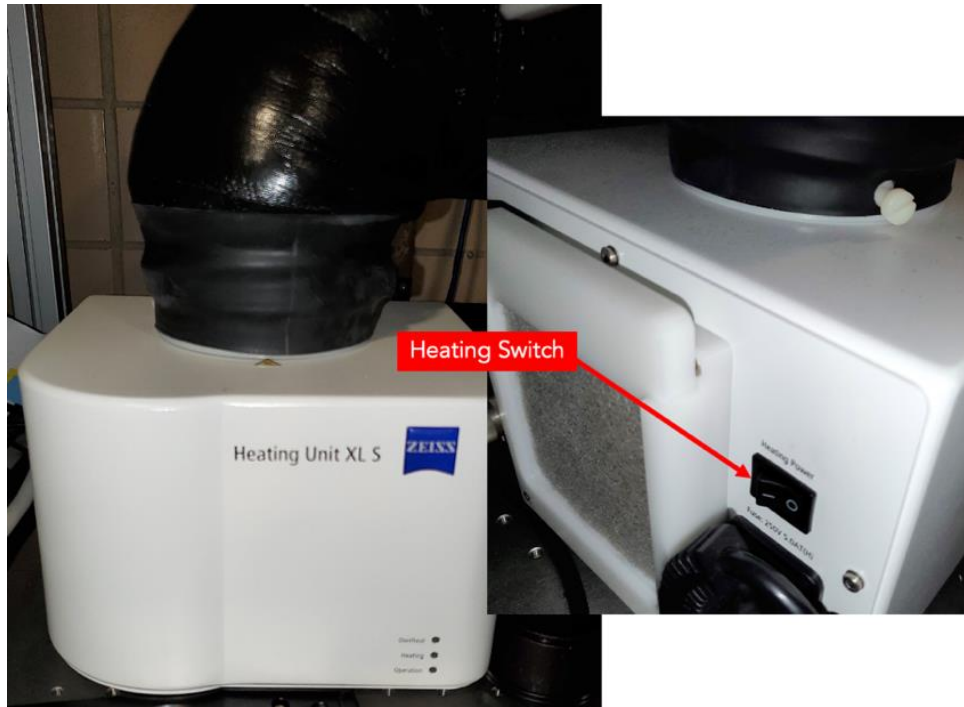


Setup:

To start the system, follow these steps:

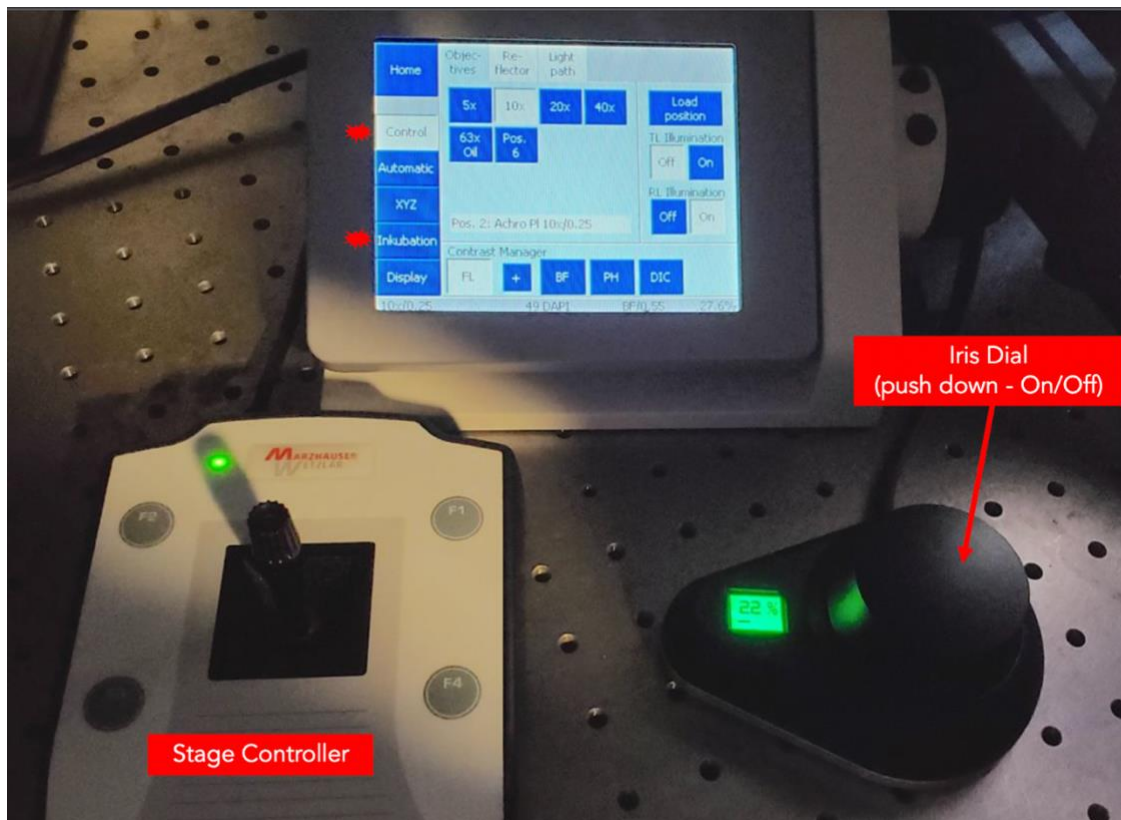
1. Turn on the X-Cite unit by pressing the white switch right, a click sound should be heard. This turns on the control boxes. Wait 30 seconds.
2. If incubation is needed, turn on the rear switch of the TempModule S unit. This controls the CO₂, temperature, and heating all together. Wait 30 seconds.
3. Turn on the Zeiss microscope by pressing the button on the left. This also turns on the touchscreen.





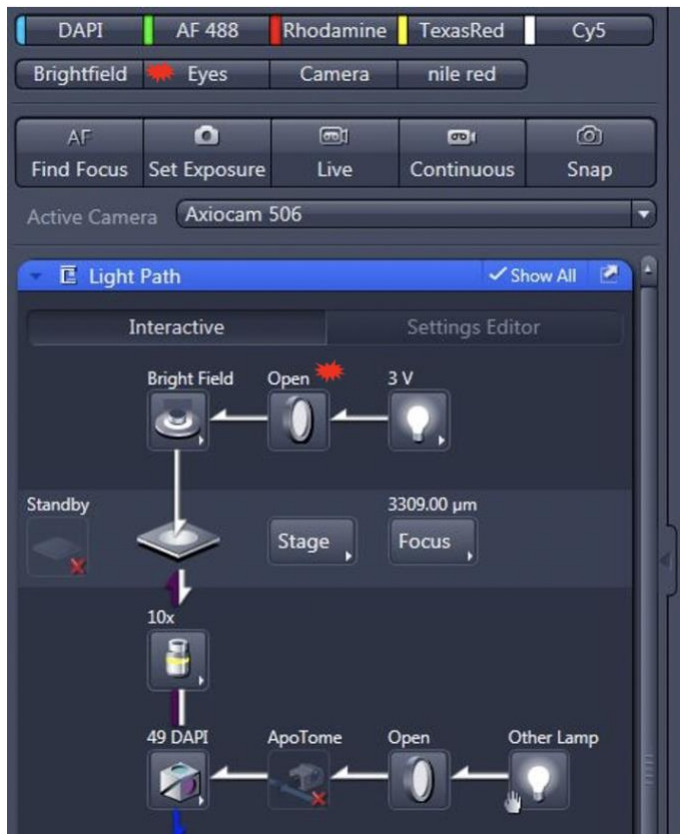
- Specify the incubation settings on the touchscreen by clicking **Home** → **Microscope** → **Inkubation**. Turn on the computer if not on already on.

Note: Wait a minimum of 15 minutes for the incubation system to warm up and stabilize.



Procedure:

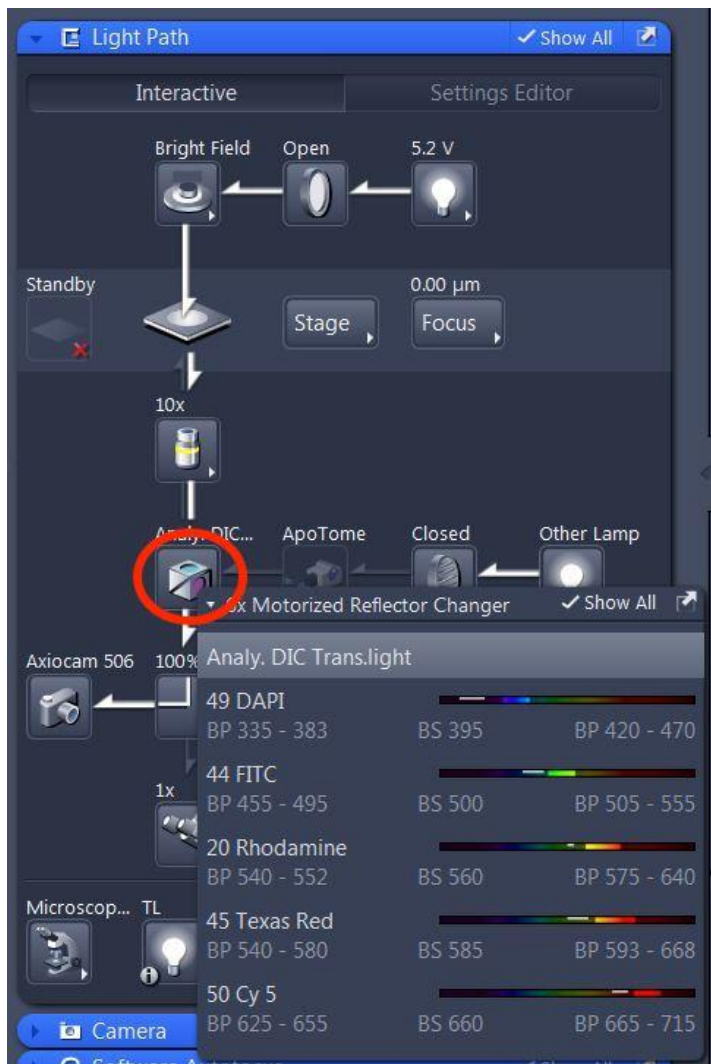
1. Open the ZEN 2 Pro software on the computer desktop.
2. Choose an objective (5x, 10x, 20x) either on the touchscreen (under **Control**) or from the **Light Path** settings in the software.
3. Place the sample into the incubation chamber with the appropriate stage insert. Use the stage controller to position the sample.
4. Locate the sample using the microscope eyepiece by clicking the **Locate** tab → **Eyes**. If no light is seen on the sample, ensure the light path is open and click either **Brightfield** or one of the fluorescent filter cubes:



For brightfield mode: Select the **Brightfield** option and adjust the light intensity using the dial on the microscope.

For fluorescence mode: Select a filter channel (ie. **DAPI**) and push down the **iris dial** to turn on/off the illumination. As seen in the photo above, a snowflake icon indicates the illumination is ON. Turn the dial to adjust the intensity, 30% is a good starting point. For live samples where photobleaching is an issue, a lower power is recommended.

*Note: Dye emission and excitation info can be found by selecting the filter cube under the **Light Path** tab.*



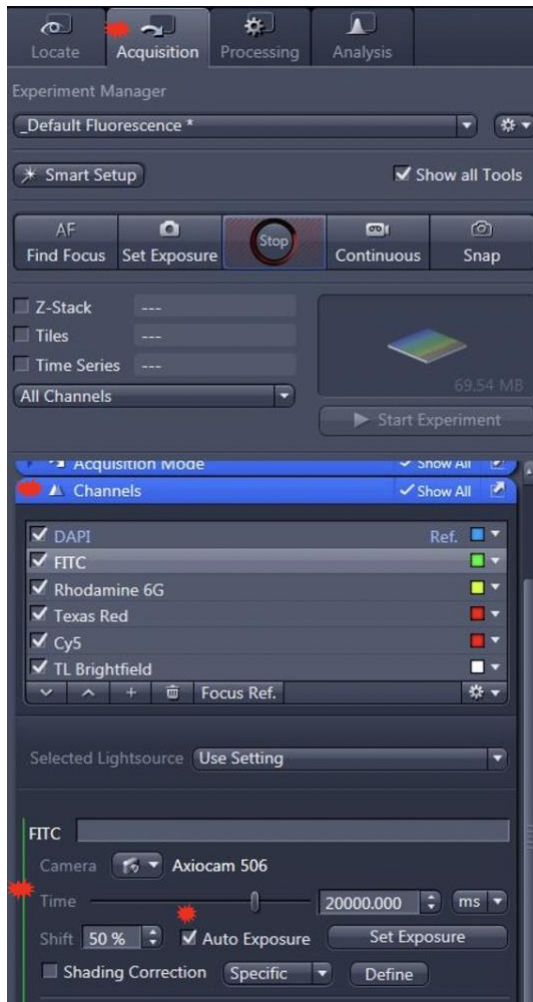
5. Adjust the position using the focus knob on the microscope for Z movement, and stage controller for the X/Y movement.

Note: position the objective ~1 cm away from the sample and slowly get closer to it. Unless using a water/oil immersion objective the objectives do not need to touch the sample directly. The working distance of the 10x for example is 0.5 cm.

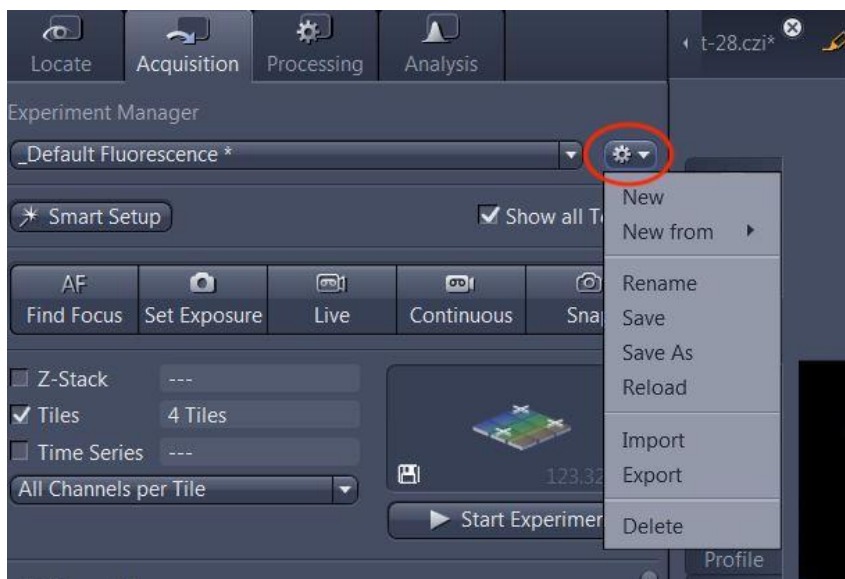
6. Press **Live** to view the image on the computer screen.

Image Acquisition:

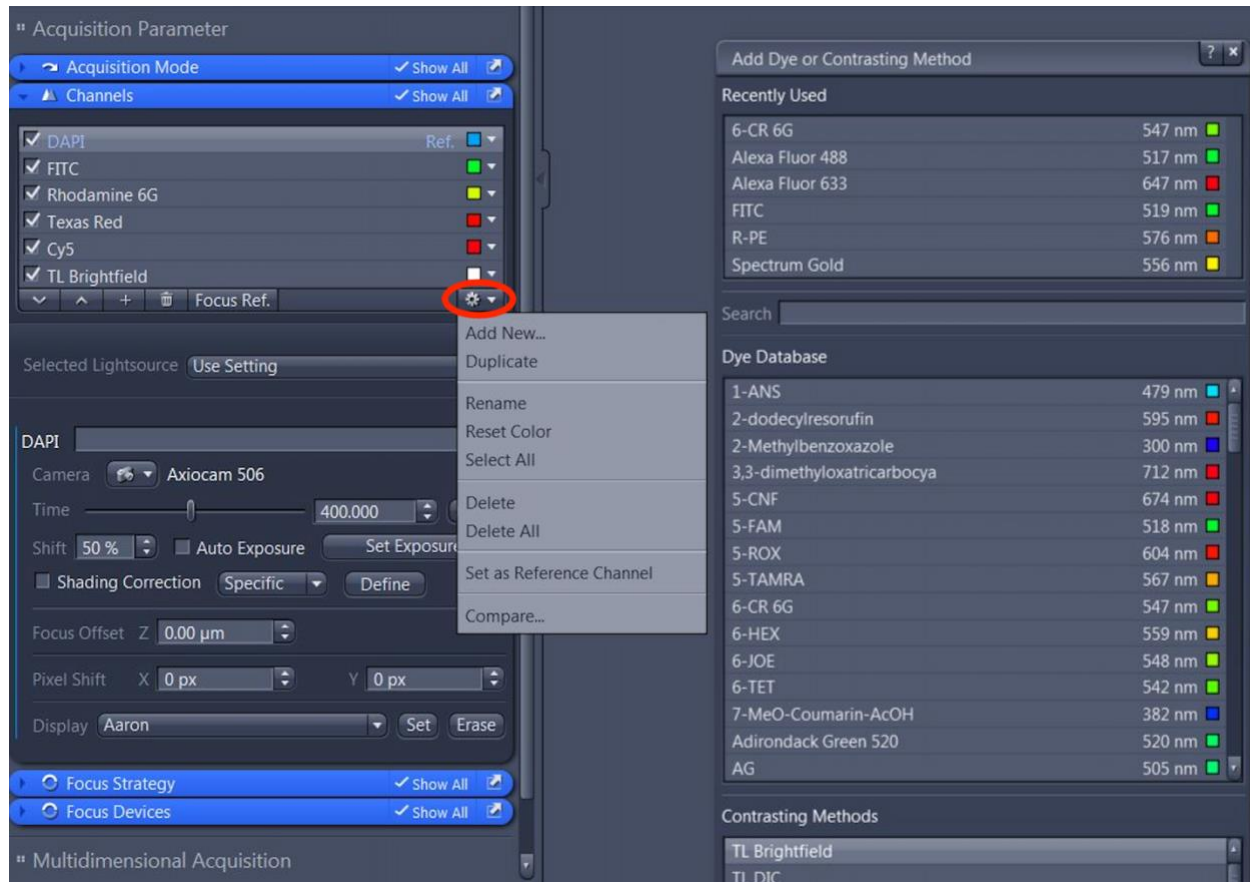
1. Click the **Acquisition** tab, ensure **Default_Fluorescence** is selected under the **Experiment Manager** and select the channels of interest. There are 5 filter cubes and one brightfield channel. The filter cubes cover a wide range of dyes.



Note: Users can create their own experiment manager and save all their settings. However, do not save these modified settings to **Default Fluorescence**.



Note: Dye selections can be added or deleted using the gear icon:

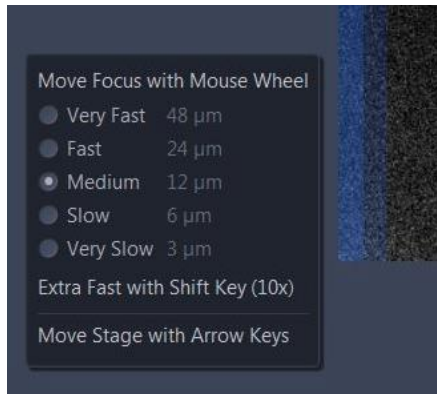


2. In the **Channels** section, choose a channel to preview. Click **Live** and select **Auto Exposure** to see the live image on the monitor. There are 2 exposure settings to choose from and each exposure only applies to the selected channel:

- a. **Auto Exposure** is used to dynamically adjust the exposure for every image/location.
- b. **Set Exposure** is used to ensure exposure settings are fixed after being set. It copies the settings to all files that follow (ie. 2nd, 3rd, etc). This feature is often used for multiple location or large image acquisition where artifacts may negatively impact auto exposure and cause the image to be too dark; otherwise, **Auto Exposure** is preferred.

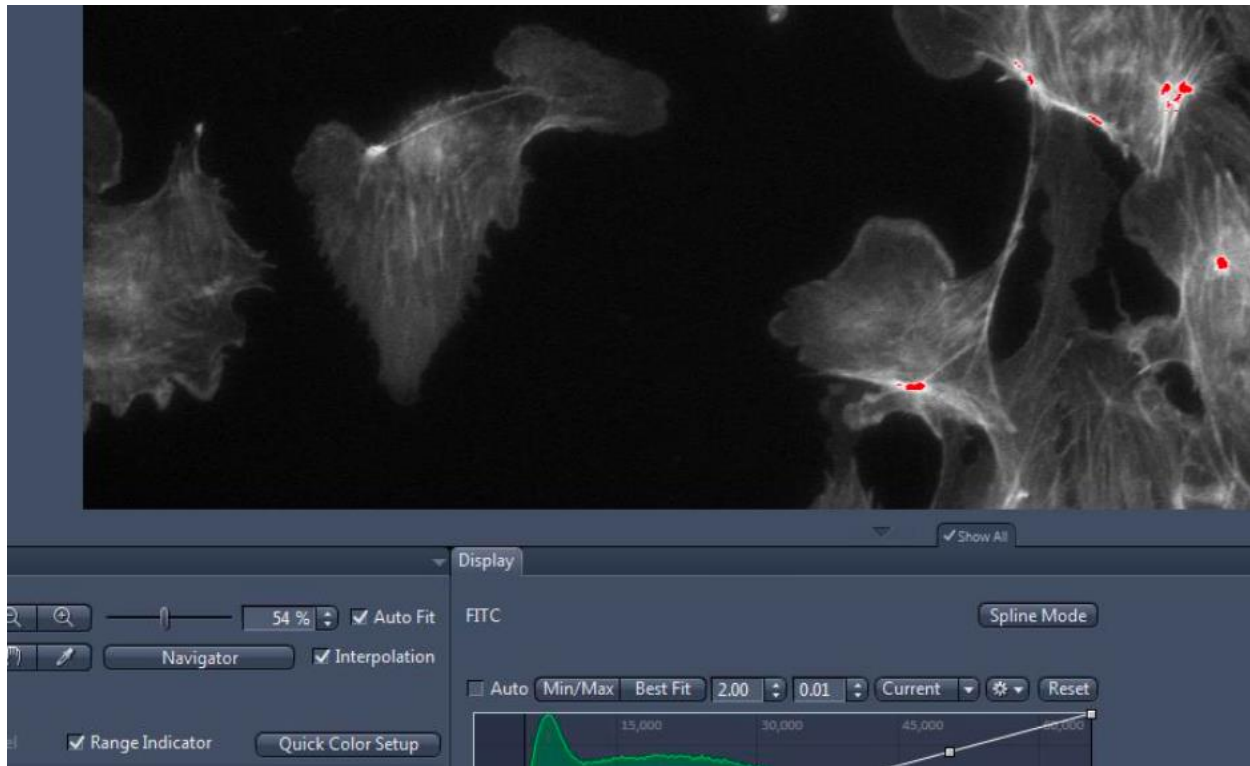
3. Set the exposure time.

4. Use the mouse scroll wheel to zoom in/out of the image prior to focusing. The focusing on the scroll wheel can be adjusted by holding down the CTRL key:



Note: double-click to move a location to the centre.

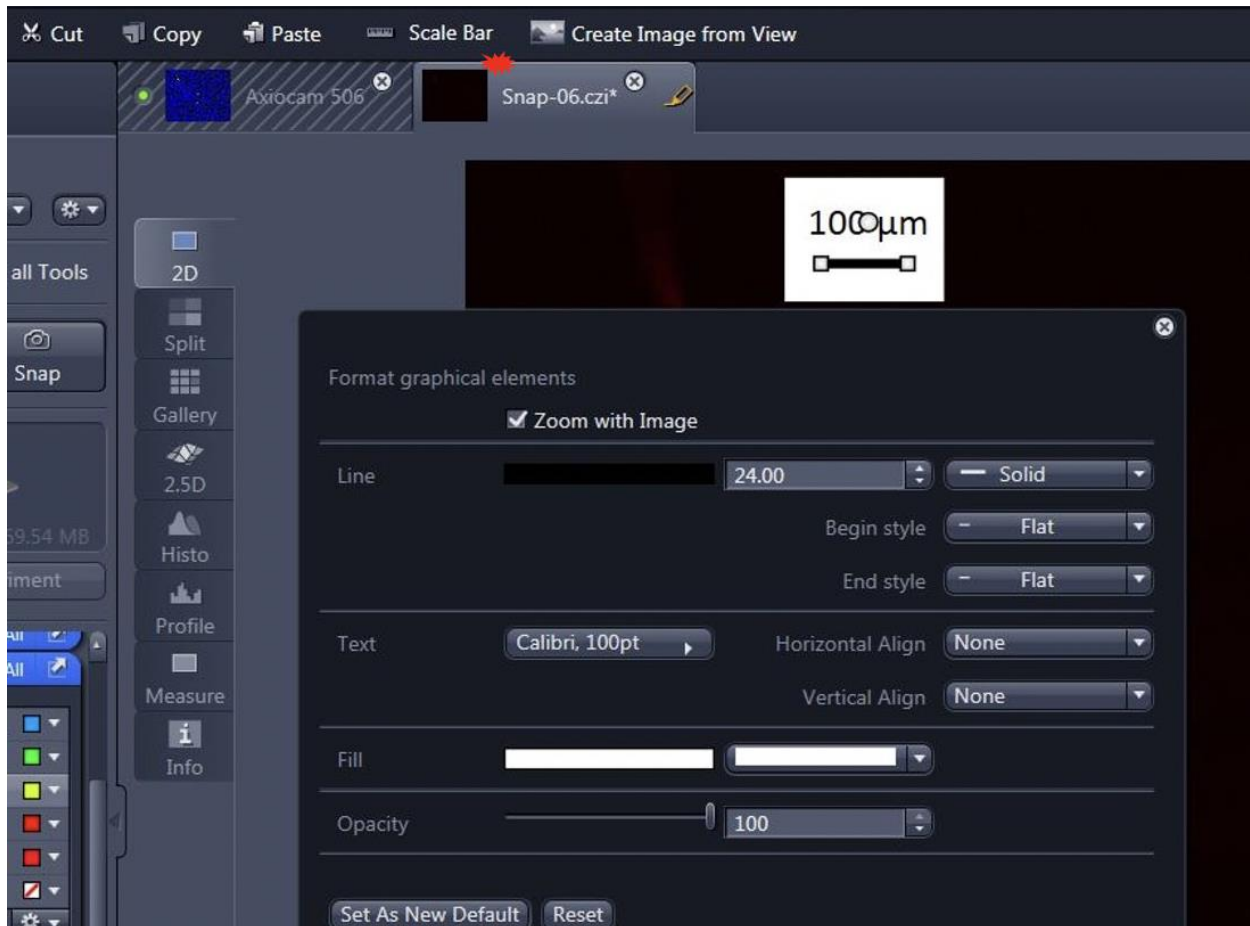
5. In the **Display** tab (bottom right), click **Auto**. The histogram shows the intensities and population of pixels in the image (look up tables). Unclicking **Auto** and selecting **Min/Max** will allow the user to MANUALLY set the maximum and minimum intensity values. Selecting **Best Fit** will permit the software to find the best intensity setting that will capture all the channels.



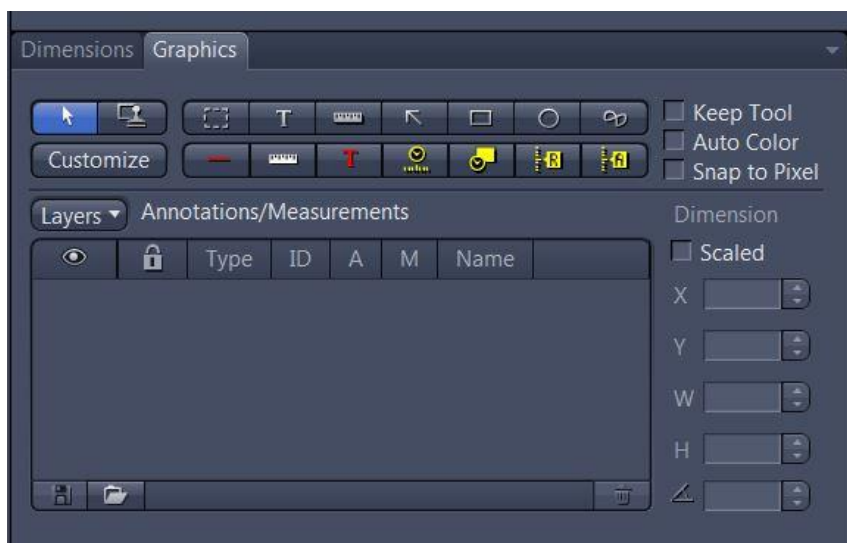
Range indicator: selecting this option will show signal saturation in the image (red). Adjust the exposure of each channel as needed to remove over saturation.

Note: the camera previews the sample in black/white, but colour can be applied to the image after acquisition.

6. When satisfied with the settings, click **Snap** to acquire an image. A new image tab will appear:

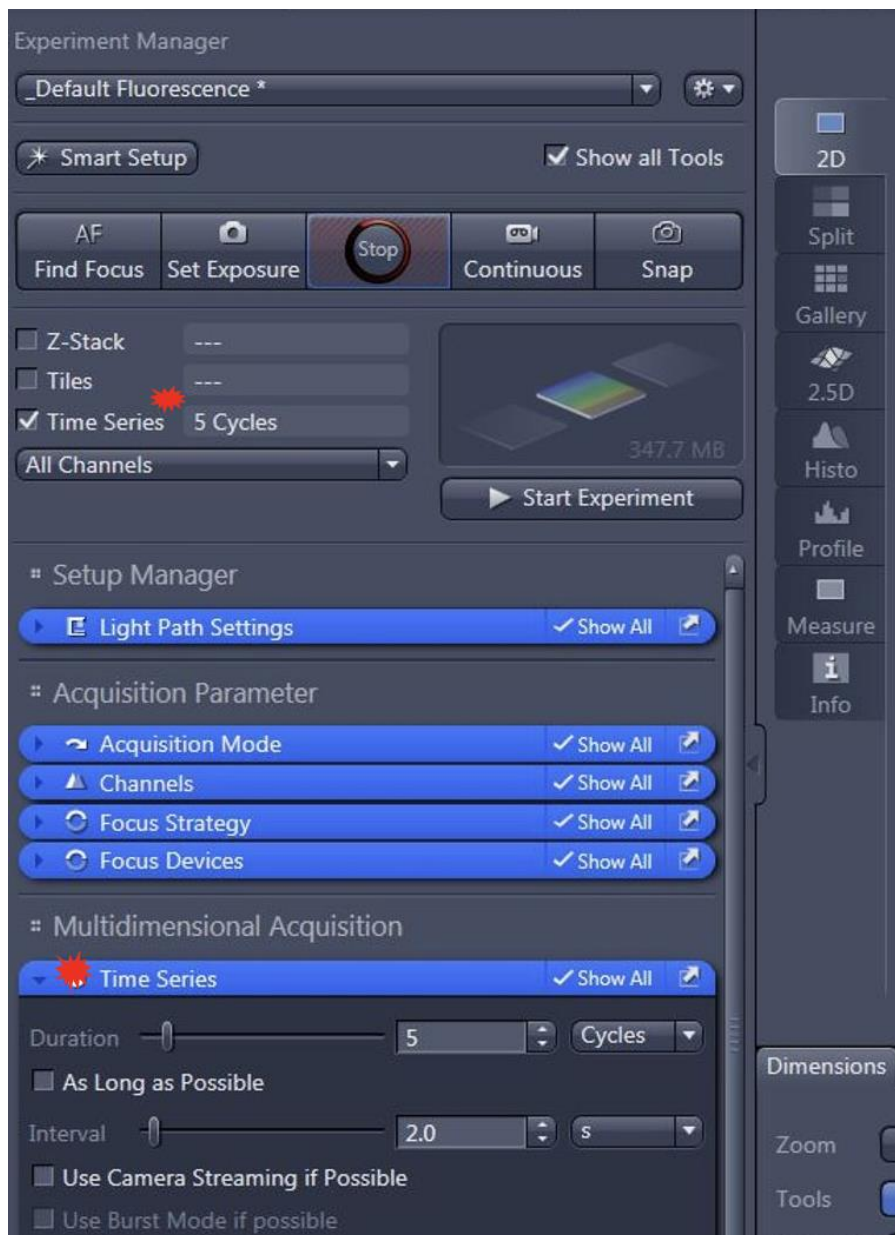


Note: In the **Graphics** tab, various annotations (text, scale bar, shapes, etc) can be added. To modify an annotation, right-click and select **Format Graphical Elements**.



Time Series:

This feature is under the **Acquisition** tab. Images can be captured in intervals. Modify the settings as desired, then press **Start Experiment** to begin.



The **Cycles** define the number of images. In the example above, 5 images would be acquired with one image taken every 2 seconds.

Burst Mode: when activated, the system buffers data in the main memory. This enables recording of the fastest framerates achievable by the camera hardware. For the sake of speed, it supports only single channel time lapse acquisition. Disable this setting if a multi channel image needs to be acquired.

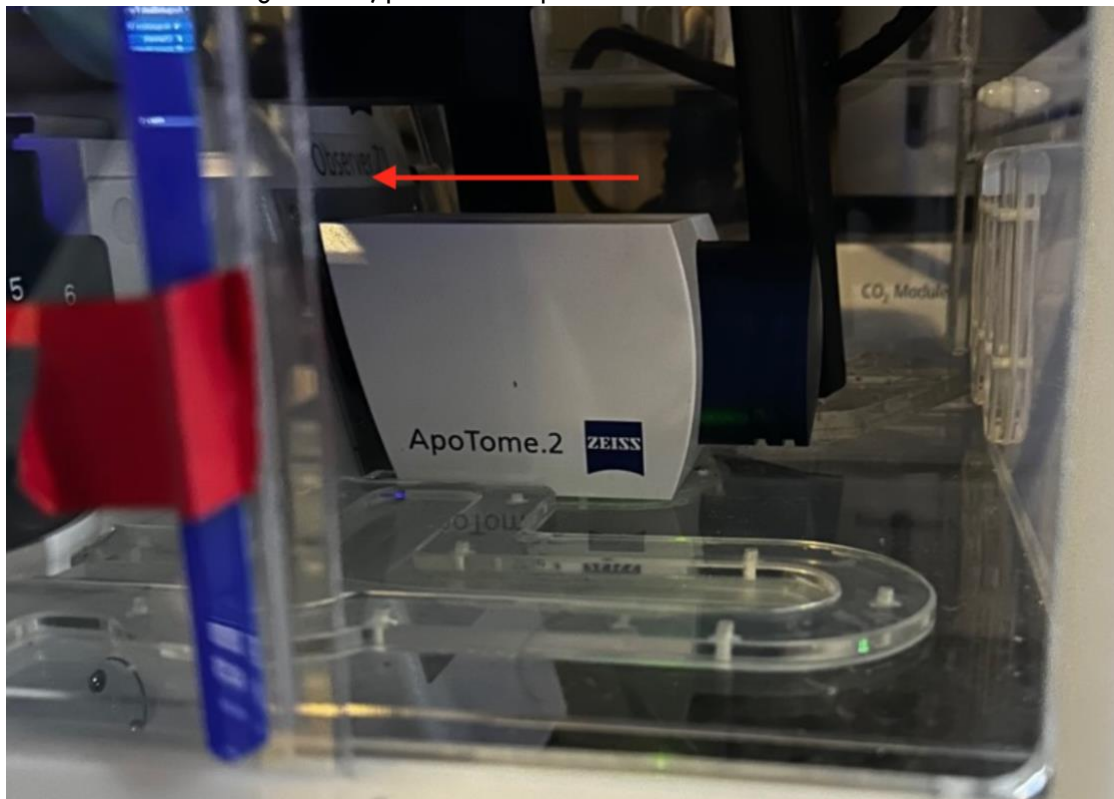
Streaming Mode: when active, the software tries to use free running/streaming mode of the active camera. Use this setting to acquire videos.

*Note: If users want faster acquisition, they can do binning found under the **Acquisition Mode** tab.*

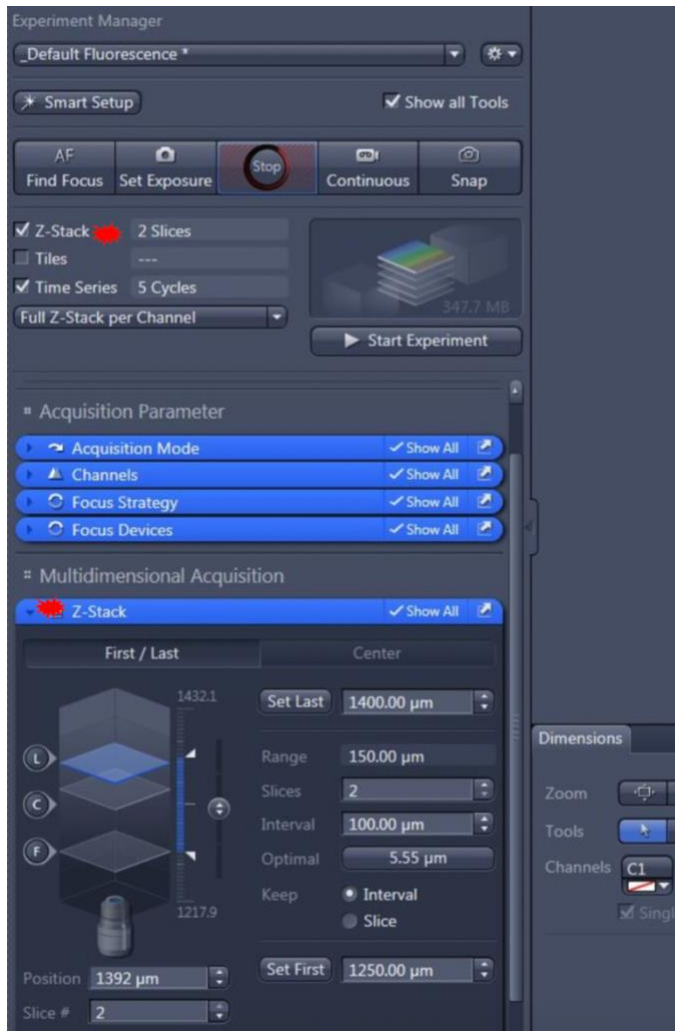
Z-Stacking:

The instrument has the capability of creating Z-stacks using software reconstruction of several images. This is not a true confocal though. We recommend the confocal microscopes at the facility for 3D images.

1. Ensure the Apotome box inside the incubation chamber is pushed in, a beeping noise should be heard. If not doing z-stacks, pull out the Apotome box until a click is felt.



2. Enable **Z-Stack** under the **Acquisition** tab.



There are 2 modes:

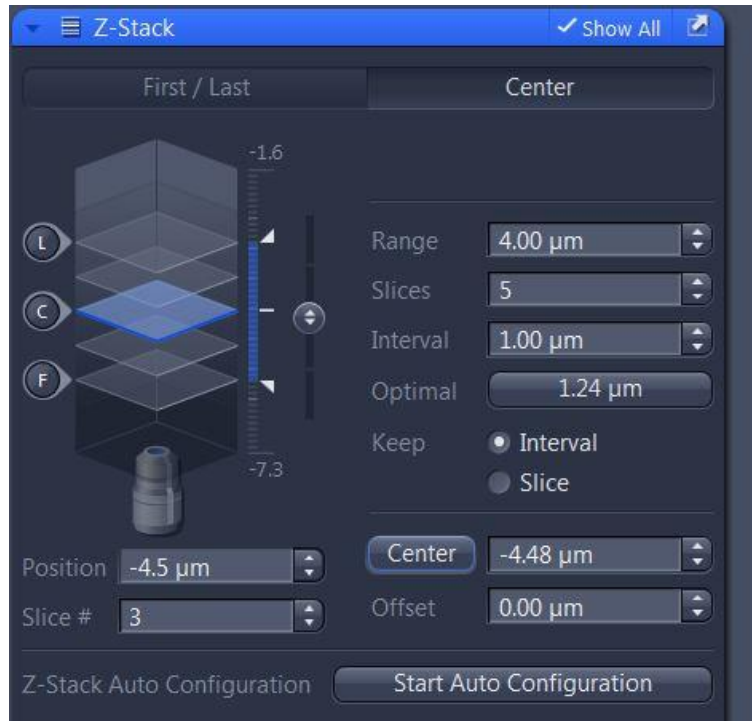
- A) **First/Last:** use this to set the first and the last plane of the Z-stack. This is suitable if thickness of a sample is unknown.
- B) **Centre:** you set the centre plane of the Z-stack. This is suitable the thickness of a sample is known and will be the fastest method to setup a Z-stack.

First/Last Mode:

1. Under the **Z-Stack** tab, click the **First/Last** Button.
2. While in live mode, adjust the Z-drive knob until you reached the upper plane of the Z-stack. The blue plane is the actual focus plane. Click the **Set First** button to set this as the first position of the Z-stack.
3. Repeat the previous step in the opposite direction and click **Set Last** to set the last position of the Z-stack.
4. Click **Optimal** for the software to adjust the best settings for the number of slices and intervals. Alternatively, users can set a desired interval and number of slices manually.
5. Click **Start Experiment**.

Center Mode:

1. Under the **Z-Stack** tab, click the **Center** Button.
2. While in live mode, adjust the Z-drive until focused to the centre of the sample. Then, click the **Centre** button to set it as the middle of the Z-Stack.

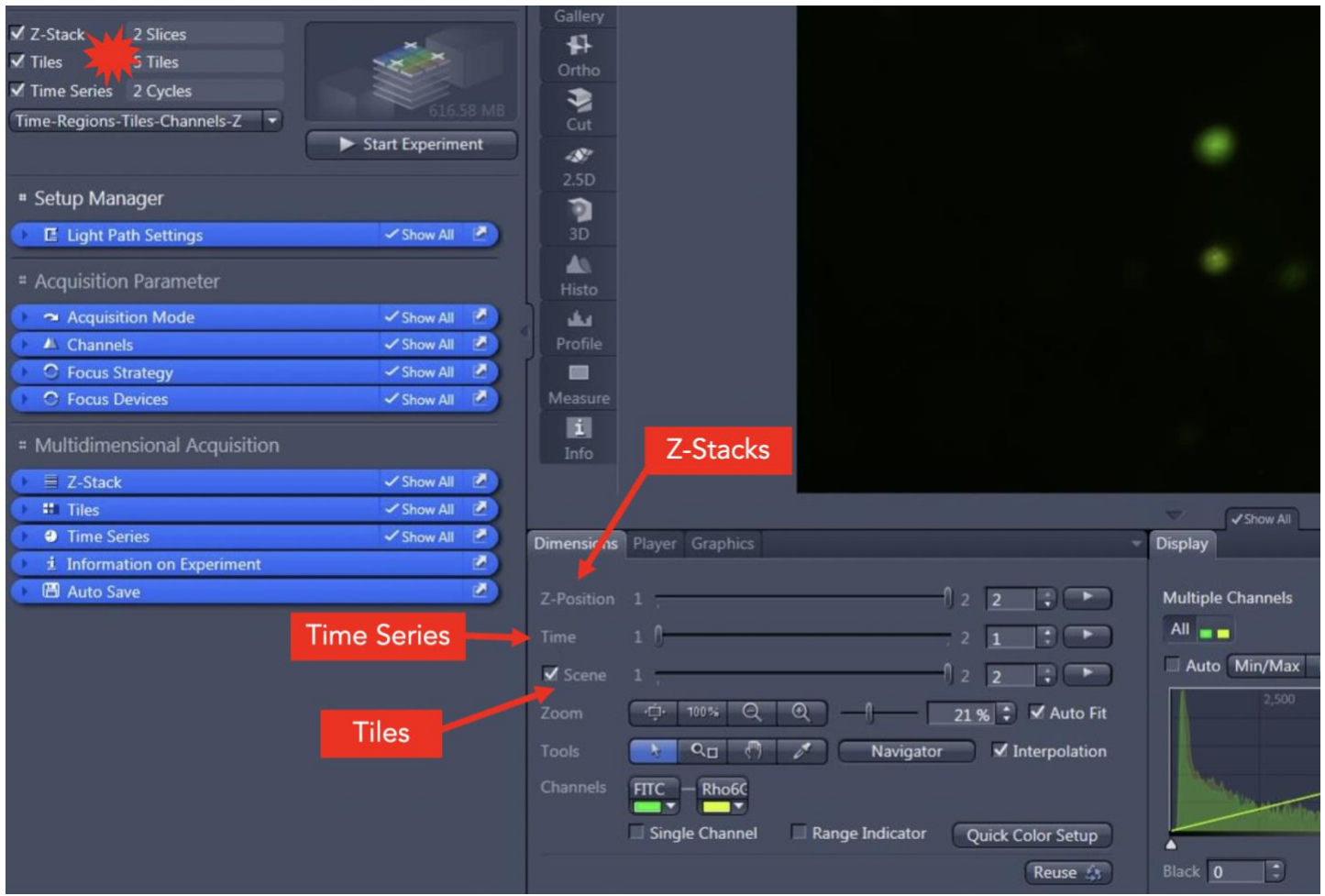


3. Click **Optimal** for the software to adjust the best settings for the number of slices and intervals. Alternatively, users can set a desired interval and number of slices manually.

*Note: depending on what's in the **Keep** section, either the **Interval** or **Slices** will be held constant.*

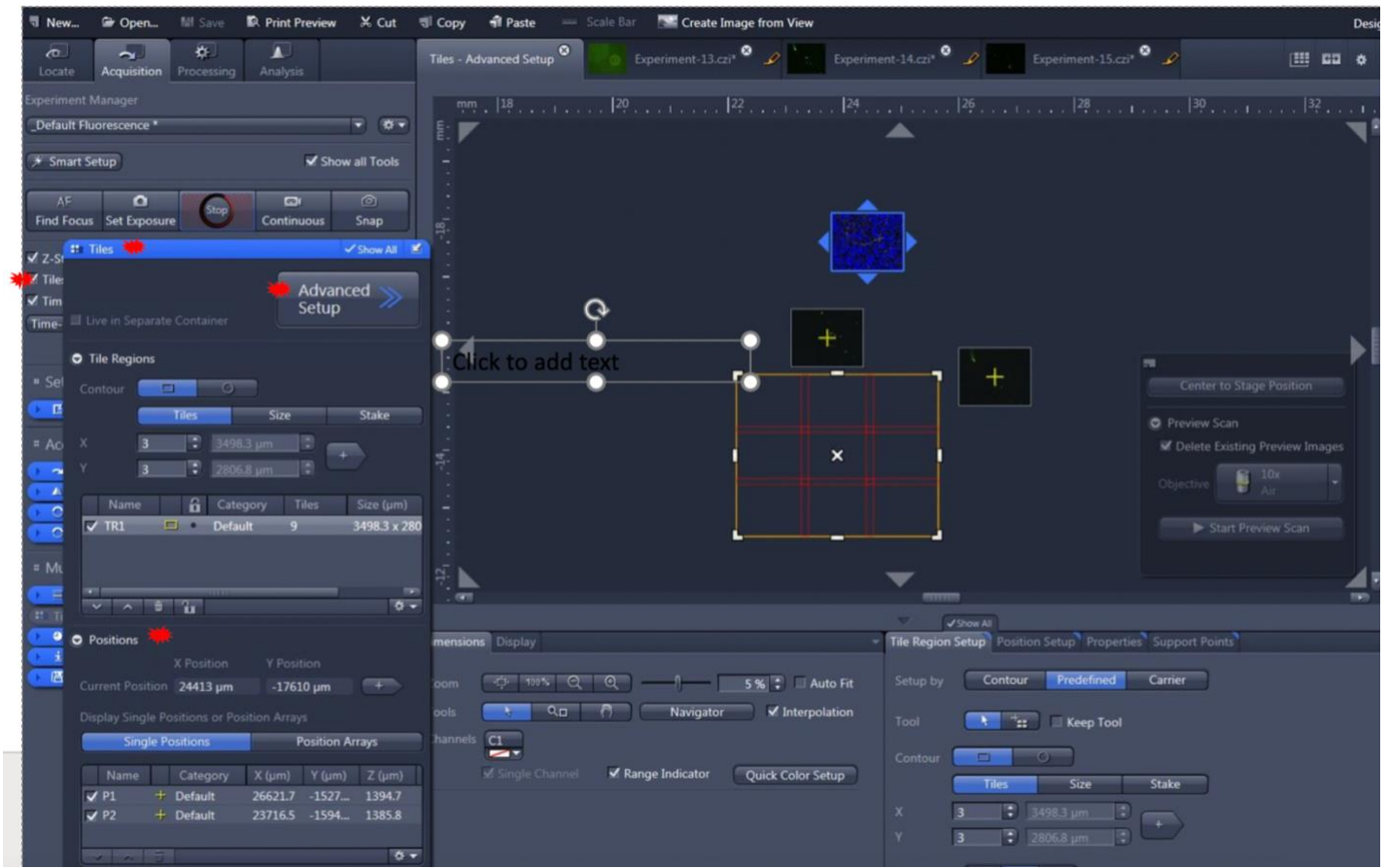
4. Click **Start Experiment**.

*Note: **Time Series**, and **Tiles** can be combined with **Z-Stack**. If all 3 acquisition modes are selected, these can be viewed under the **Dimensions** tab after the run is complete.*



Tiles Acquisition:

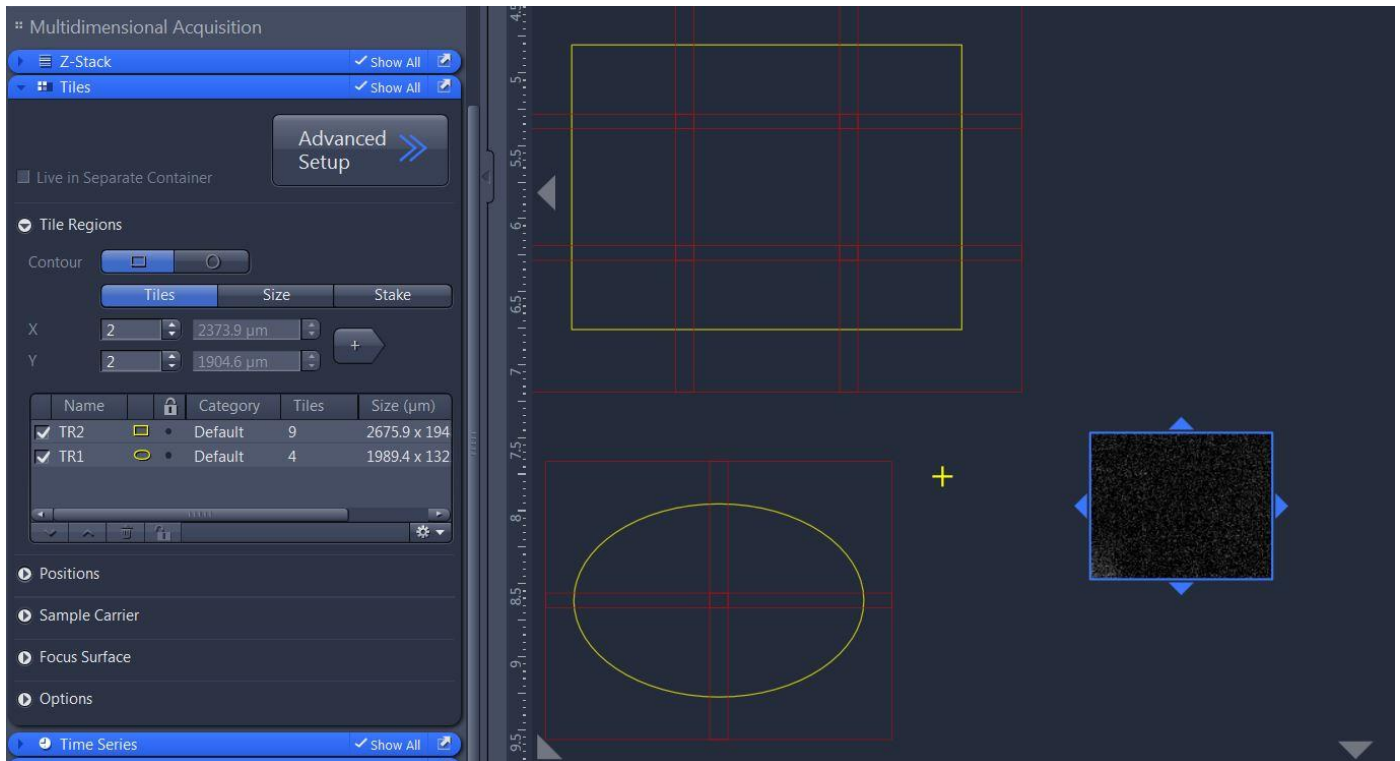
This setting allows images at various locations to be taken at the same time, or a cluster of images stitched together.



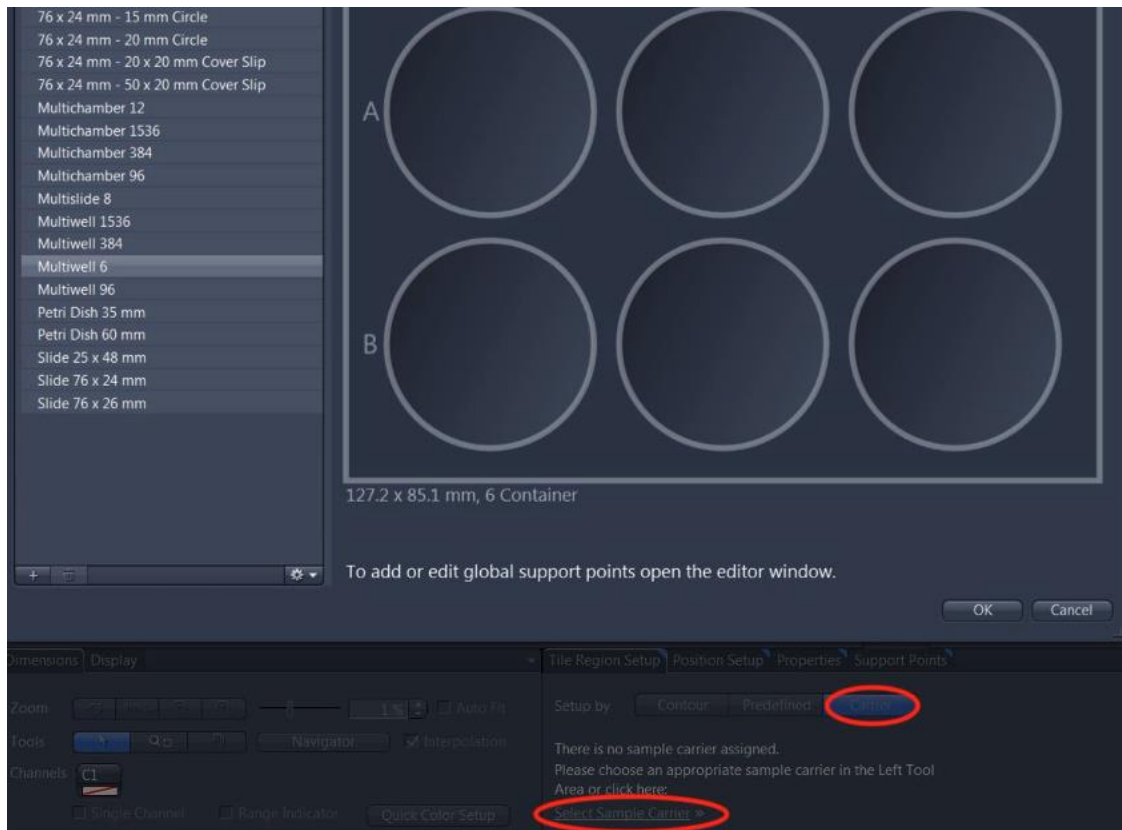
1. Select the **Advanced Setup** of the **Tiles** tab.

2. Specify the tile regions to be captured:

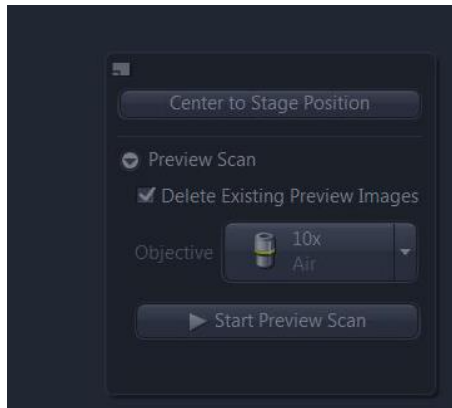
- a. Individual locations can be added under the **Positions** subtab. To cycle through the different positions you can double-click each line.
- b. A field of tiles of either rectangles or circular shapes can be added under **Tile Regions** subtab.



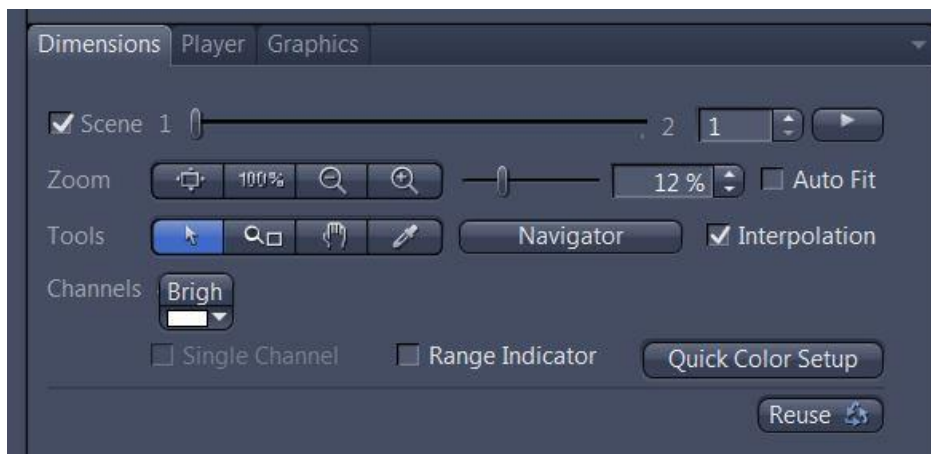
c. Various carriers can be selected, such as plates or petri dishes. Under the **Tile Region Setup** tab, click the **Carrier** button and select the carrier of interest:



Note: you may do a preview scan with a low magnification.



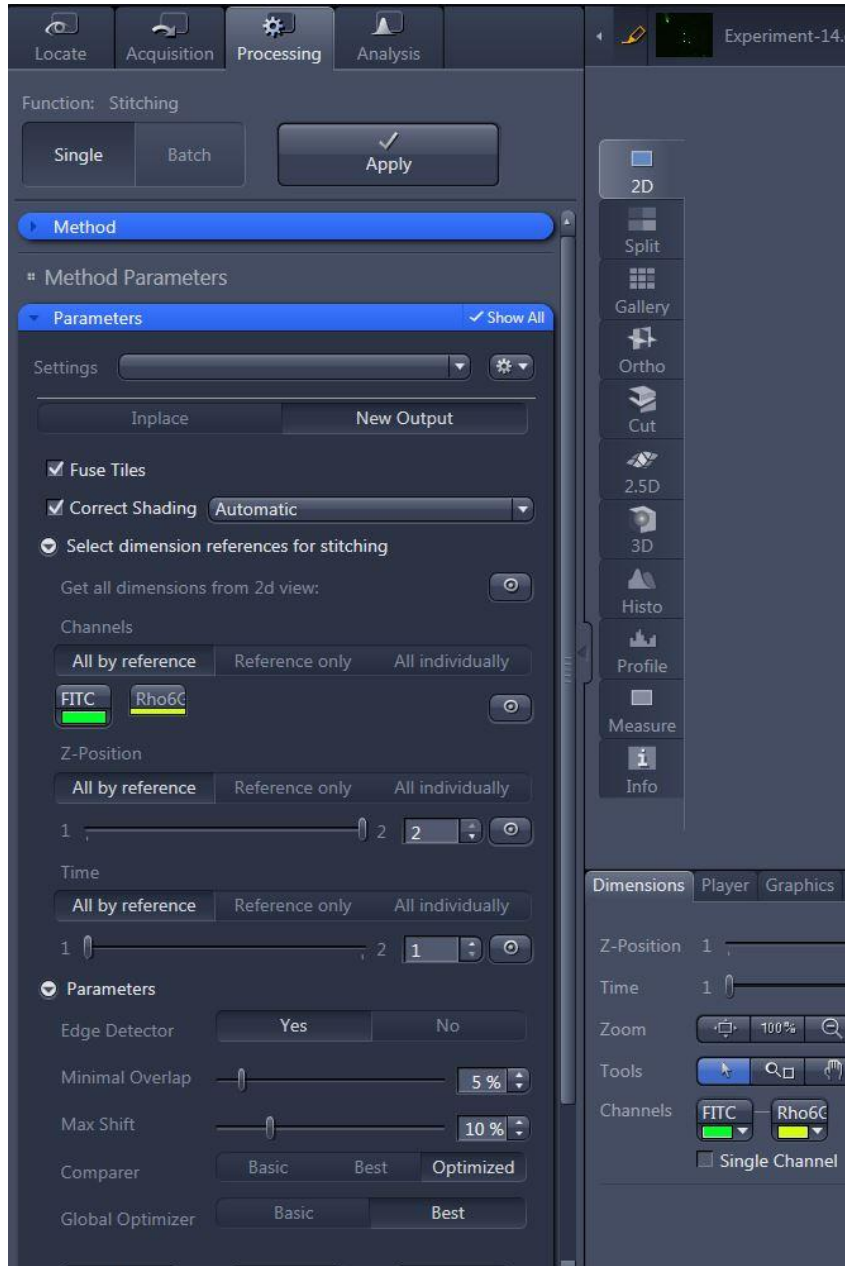
3. Tile overlap settings can be customized from the **Options** tab. A higher % overlap indicates tighter stitching of the images together.
4. Check off **Stage and Focus Backlash Correction**.
5. Optional: check off **Custom Stage Speed** if liquid is involved. Reduce it to 50 or less to prevent sample plate from shifting vigorously when moving to different locations.
6. When ready, click **Start Experiment**.
7. When tiles are acquired, they will appear in the **Dimensions** tab as **Scenes**:



Note: Each **Scene** is an image of a tile region OR a specific location. Each **Scene** can be selected and saved independently (see **Saving/Exporting** section).

5. The acquired tile images can be processed by clicking the **Processing** tab → **Stitching (Method tab)**. A **Parameters** tab will appear. Select **New Output** and set the following:
 - a. Check off **Fuse tiles, Correct Shading (Automatic)**
 - b. Edge Detector: Yes

- c. Minimum Overlap (5%), Max shift (10%),
- d. Comparer = Best,
- e. Global Optimizer = Best
- 7. Click **Apply** to make the changes.



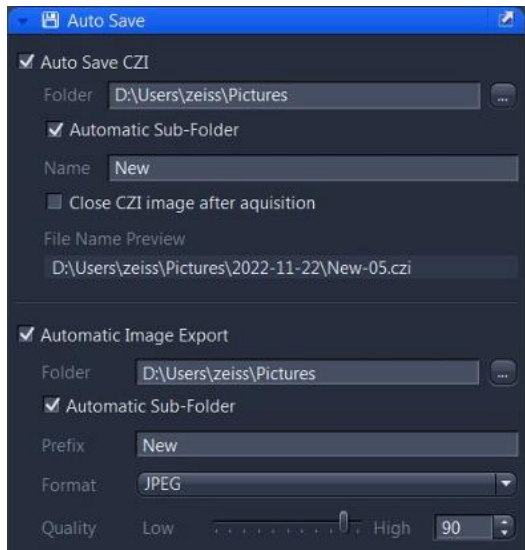
Saving/Exporting:

- A. To save a file with the original data, click **File** → **Save As**, then specify the location of the external USB or hard drive to save the **.czi** file.
- B. To save individual images as they appear with colour and annotations for specific channels, right-click and select **Create Image from View**. Then, export images using these steps:

1. Click **File** → **Export/Import** → **Export**.
2. Choose the file type (ie. JPEG). Select **Burn-in Graphics**.
3. Specify the save location (USB, external drive). Saving as **.czi** file is recommended to keep the original data. This file can be opened on many analysis programs such as IMARIS, ImageJ (open source), or ZEN lite (free version).

C. To autosave immediately after an experiment or scan is complete, follow these steps:

1. Under the **Acquisition** tab, check off **Auto Save CZI**. This will ensure all raw data will be saved.
2. Optional: select **Automatic Image Export** to save files in either JPEG or TIFF.

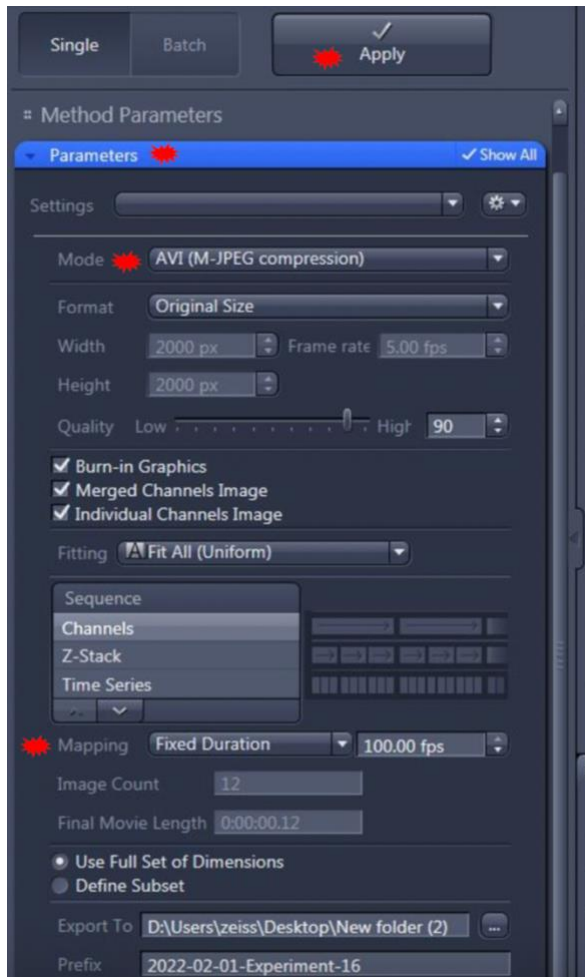


3. Identify the save location and provide it a file name.

Video Rendering:

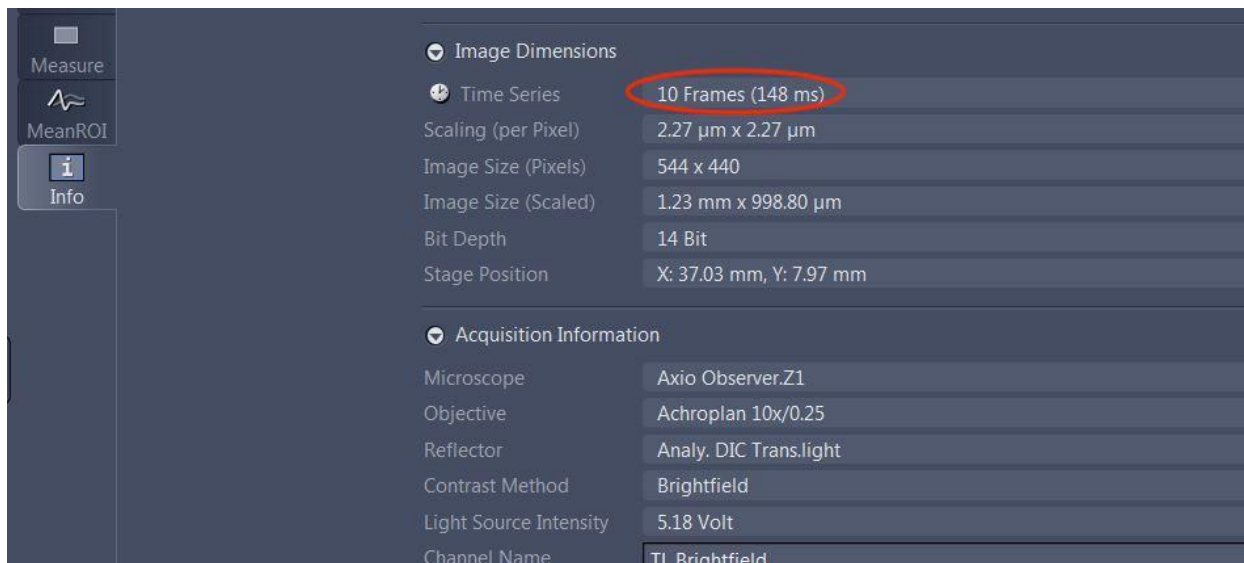
This feature is only available after images are obtained from Time Series. This is useful in applications for looking at wound repair or cell proliferation over an extended period of time.

1. In the **Processing** tab, select **Single** → **Movie Export** (under Method). A **Parameters** tab appears. Select the following:



a. Mode: **AVI (M-JPEG compression)**

b. Mapping: fixed duration → choose the appropriate frame rate. Choose the same fps in the export as the camera actually captures the photos found in the **Info** tab of the time series:



2. Click **Apply**, and the video will be exported.

Cleanup:

1. Exit the software. Ensure data is saved to a USB or external hard drive. Never leave original data on the computer.
2. Shut down the system by turning off the units in the following order: TempModule S unit, microscope, then XCite.

Note: skip this step if there is another user immediately after.

3. Ensure incubation chamber doors are closed to prevent dust from entering inside the unit.
4. Sign out of the instrument in LMACs.
5. Wipe the working area with RTU disinfectant and kimwipe.

Troubleshooting:

Issue	Solution
If no functions work in ZEN software.	Restart the system, and ensure hardware is started before the software.
If no fluorescent light is seen.	Push down the iris dial (snowflake = ON)

Note: if an issue cannot be resolved, contact a CRAFT staff member.