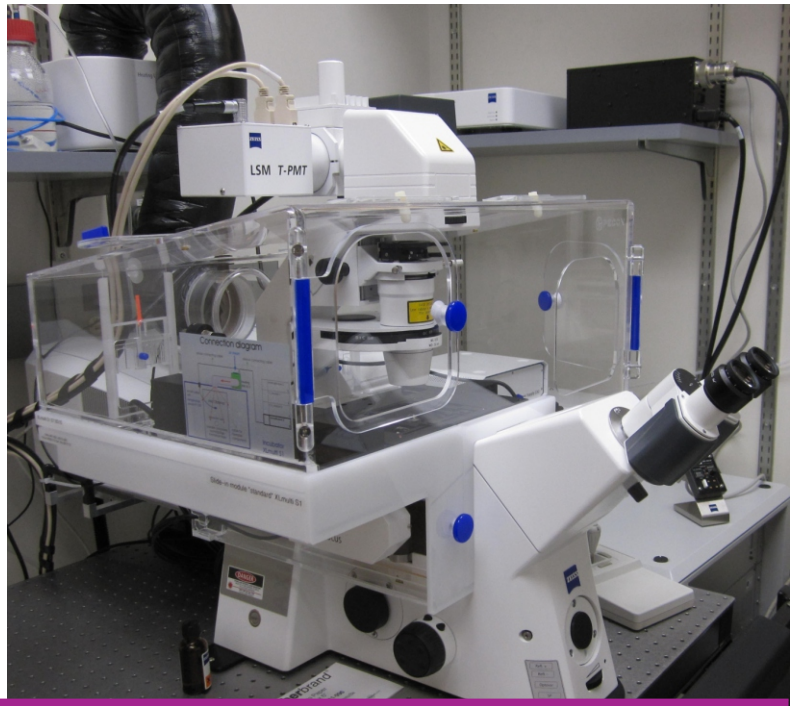
Zeiss LSM 710 Quick Guide

BEFORE YOU START

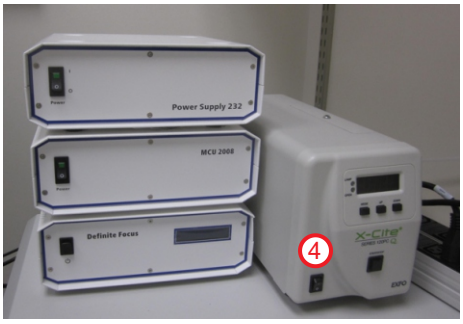
- **RECORD YOUR USE IN THE LOG BOOK.**
- **FOLLOW THIS MANUAL STEP-BY-STEP UNTIL YOU ARE FAMILIAR WITH THE SYSTEM OPERATION.**



A. STARTING THE LSM710

If the system is OFF, start here:

1. Turn on the main power switch ①, located to the left of the monitor, followed by the systems/PC switch ②, and the components switch ③ in numbered order. The "laser" key inside of the plastic cover should always be left on.



2. If you need to see a fluorescent signal through the oculars, turn on the Xcite lamp ④. If you will be using the Argon laser, turn on the key for the laser power supply (clockwise) located on the shelf to the left of the monitor ⑤ and flip the laser power toggle switch on the argon laser control module, located on the black box to the left of the monitor, to the UP (run) position. ⑥ If not on already, turn on the computer to the right of the monitor (switch marked #7).

If the system is ON, start here:

3. Find the pGina log-in window and when it says "connected", enter your VUnetID and e-password.

4. A black DOS window may open indicating registry items are loading: it will close automatically.

5. Double click the ZEN icon ⑦.

⑦

6. In the center of the first Zen window, click "start system".

7. Return to the laser control module ⑥ and note that after several minutes of warm up, the green light comes on to indicate it has stabilized and is ready to use. The output power of this laser is adjustable with the "light control" knob. For typical use, turn the knob clockwise until the red light comes on, then turn it back down until the light just goes out.

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B. SETTING UP YOUR SPECIMEN

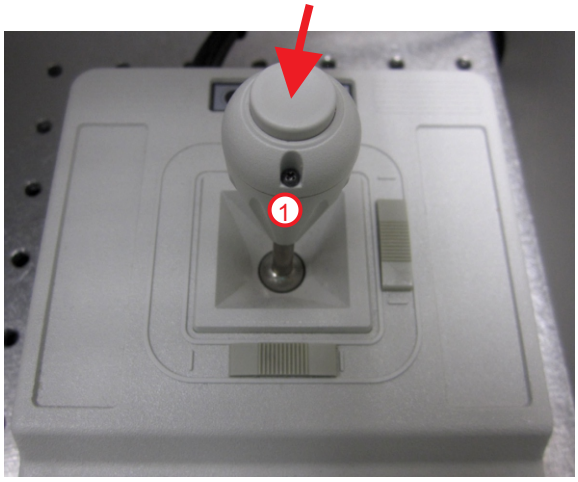
1. Check the objectives before proceeding.

A. Remove the stage insert and clean off any oil with a Kimwipe.

B. Clean the oil and/or water objectives with lens paper.

C. If you find a significant amount of oil or any other substance on any lens or the stage or the objective turret, please inform a CISR staff member. Replace the stage insert.

Do not force the stage insert into place. The insert and stage are precision aligned and must be handled carefully to avoid damage.



5. Move the stage with the joystick(1). The button on the top of the joystick (arrow) changes the speed of movement from fast to slow and back to fast with each press.

2 .**Make sure the sample cover glass is clean, dry and sealed.** If you are unsure which kind of lens to use, start with a dry lens (5X, 10X or 20X). Find details about each lens in the software (see section D, ZEN screen layout). If using a lens marked "oil", apply a small amount of immersion oil to the lens surface. Do not allow oil from other microscopes to mix with the oil on this system. If using a "water" lens, apply a small amount of filtered water from the syringe kept near the microscope.

3. Mount the specimen on the stage. Remember that for *inverted* microscopes - those with the objectives *below* the stage - microscope slides must be placed upside-down.

4. Viewing your specimen through the oculars (eye pieces) requires the ZEN software to control the light path light sources. Please refer next to the section on using this software.

Caution: Do not switch objectives if a specimen has already been placed on the stage with an immersion lens. When switching between oil- or water- immersion and dry, first remove and clean the slide surface prior to switching lenses!

In the event of a spill, quickly wipe up all liquid. Notify a Cell Imaging Core staff member immediately. Timing is critical because expensive optics and electronics inside the scope can be damaged if liquid drips through the top of the microscope.

Acknowledgment:

Our funding depends upon your citing the Cell Imaging Shared Resource when you publish data obtained with equipment or services from CISR. This includes images from the microscopes, training in the use of software, consulting on data analysis, etc. The minimum acceptable acknowledgment should read:

"Experiments/data analysis/presentation [include what you use]" were performed in part through the use of the VUMC Cell Imaging Shared Resource, (supported by NIH grants CA68485, DK20593, DK58404, DK59637 and Ey08126).

Please let us know when you publish and, if possible, send us a reprint of the paper. This is how we justify our existence.

C. USING THE TOUCH PAD

1.The touch pad is a convenient way to control the motorized mechanics of the microscope and the settings for the live cell chamber components. These controls are duplicated in the on-screen ZEN software. You also can focus the microscope with the knobs on the right side.

2. The “Home” screen will open on the touch pad as the default ①. The home screen shows details about the state of the microscope and allows turning the white light on for a bright field view of your sample (TL illumination “on” or “off”).

3. The “microscope” screen ② can be used to change objectives, choose fluorescent filter cubes (”reflectors”) for viewing fluorescence through the oculars, and controlling other aspects of the light path, **with the exception of the Xcite light source which can only be turned on through the software.**

4. Click the “control” screen ③ and then the “objectives” tab④. The available objectives (5x, 10x, 20x, 40xW [water emersion], 63xO [oil emersion]) can be moved under the sample by clicking the appropriate button. Information about the chosen objective is displayed below the buttons.

Changing between air and immersion lenses or between oil and water immersion lenses may require cleaning the immersion fluid off of the sample and the lens. The touch pad display will prompt you to do so and will not bring the new lens up to focal position until you close the prompt.

5. Click the “reflector” tab to see the available reflectors⑤. Four colors are available along the top row, with DIC white light (Pol TL) in the second row.

D. ZEN SCREEN LAYOUT

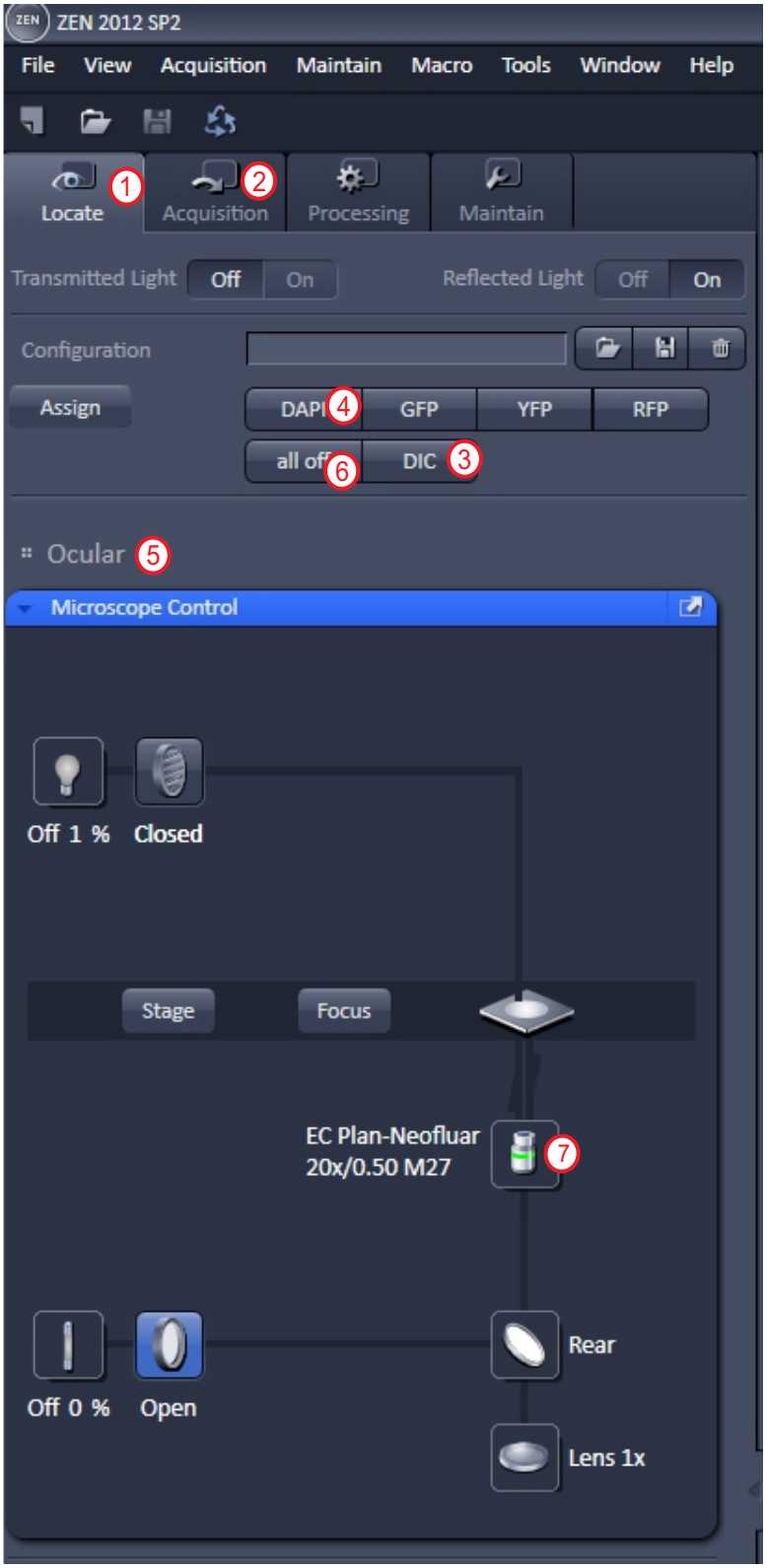
1. The left tool area of the opening screen gives you the choice of examining your sample through the oculars (locate ①) or scanning the image to the monitor (acquisition ②).

2. To look at your sample through the oculars, choose "DIC" for a white light view ③ or one of the fluorophore buttons, for example the blue nucleic acid marker, DAPI ④. These buttons need to be configured and assigned for each user. Please get help from one of the CISR staff to do this.

3. In the "Ocular" portion of the screen, ⑤ changes can be made in light source intensity, open or closed state of the white light or fluorescent excitation light shutters, etc., by clicking on the appropriate icon. Do not use higher than 12% power on the Xcite light (fluorescence) unless you want to bleach your sample on purpose.

4. After focusing and visually setting up your sample for image capture, immediately turn off the Xcite light,"all off" ⑥ to prevent bleaching.

5. Click the lens icon ⑦ to get more detailed information about each lens. If you want to capture images with a different lens than the one you used to get your sample in focus, use this drop down list to change lenses Or use the touch pad as described on a previous page. Re-focus your sample using either DIC or fluorescence mode.



E. SETTING UP A CONFIGURATION

1. Click the “Acquisition” button① in order to open the menus shown in the picture to the right. A series of **setup menus** appear, each headed by a blue bar with white lettering.

2. The upper left quadrant (outlined **here** in **green**) contains the main control buttons for opening scanning configurations, starting and stopping scans, and opening advanced setup windows for z-stacks, time series and others.

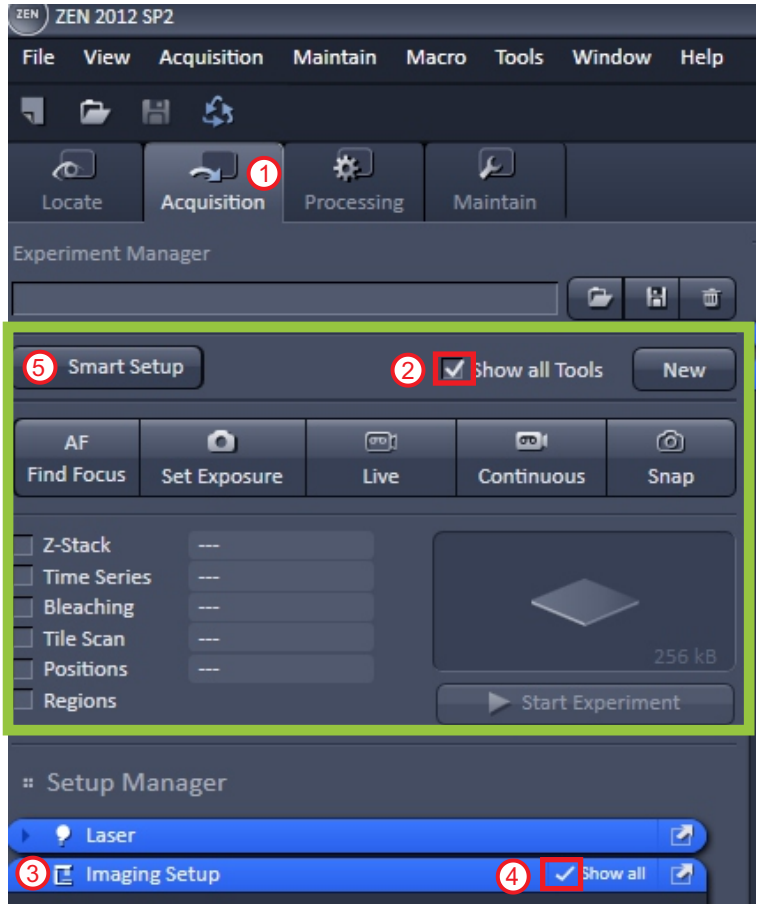
3. If not already done, click the small box to the left of “**Show Manual Tools**”②.

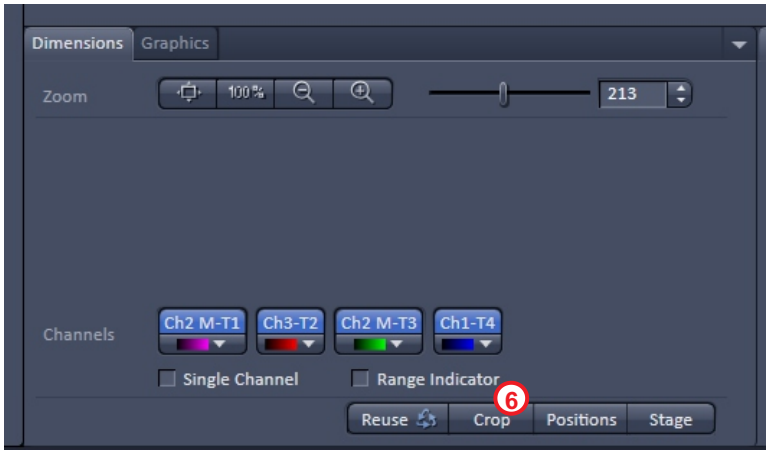
4.To show the items inside of a minimized setup menu, click the small arrowhead at the left end of the blue bar,

With the menu maximized, for example the “imaging setup”,③ click the “show all” box④ in order to see all of the menu functions.

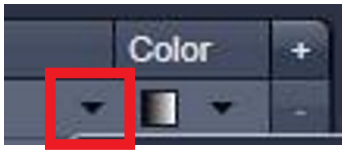
5. To create a new track configuration that will acquire images of your fluorophores, click the “Smart Setup” button⑤.

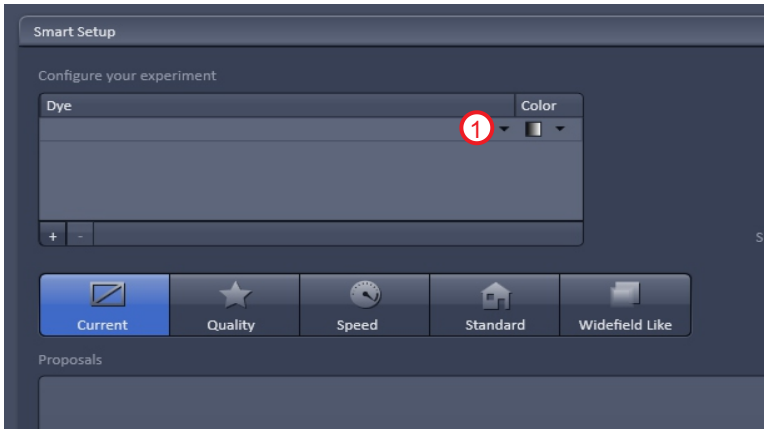
To load the same configuration used in a previously saved image, open the image and click the “reuse” button near the bottom of the screen below the opened image.⑥



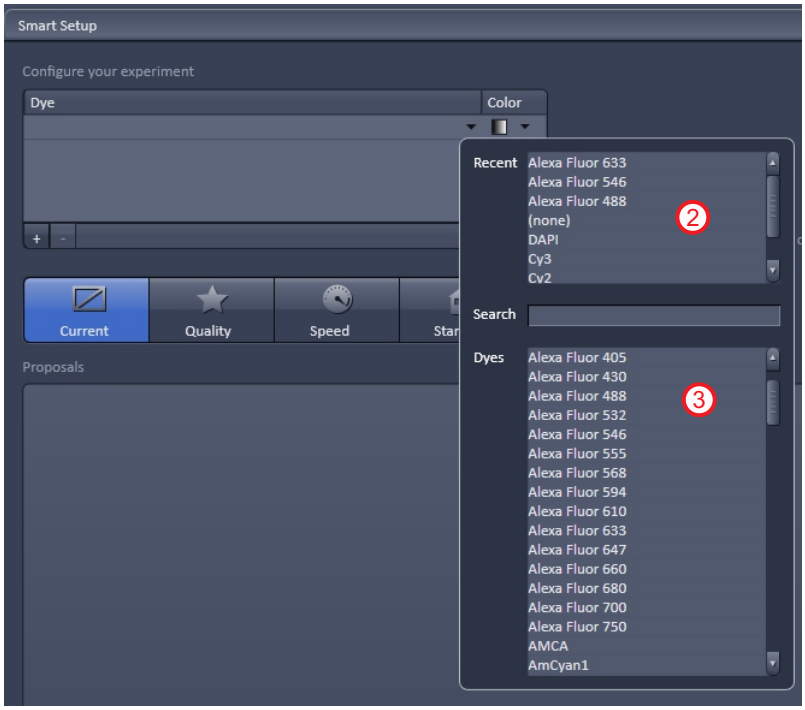


7.The smart setup has a drop-down list of dyes/ fluorophores to choose from. Click the small arrowhead to the left of the first symbol in the “color” column①.





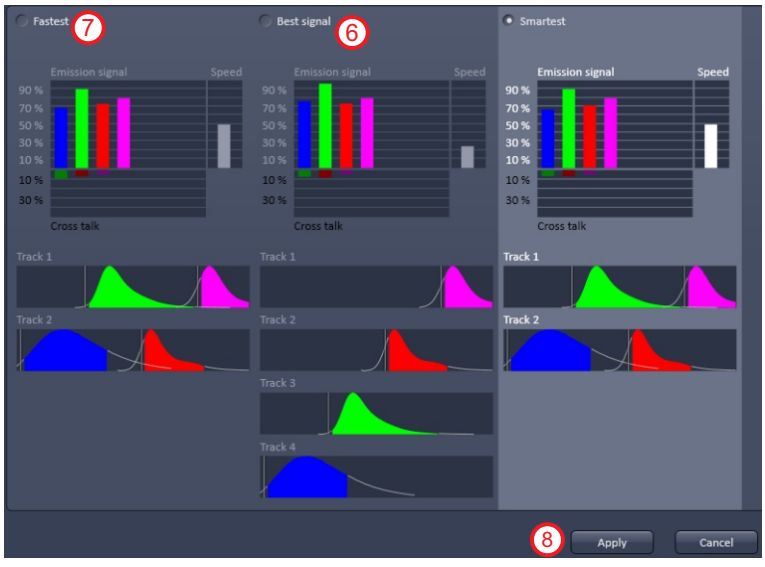
Previously used dyes are listed in the “recent” box② and a more extensive list in the “dyes” box③ . Click on the first dye that you want to include in your image.

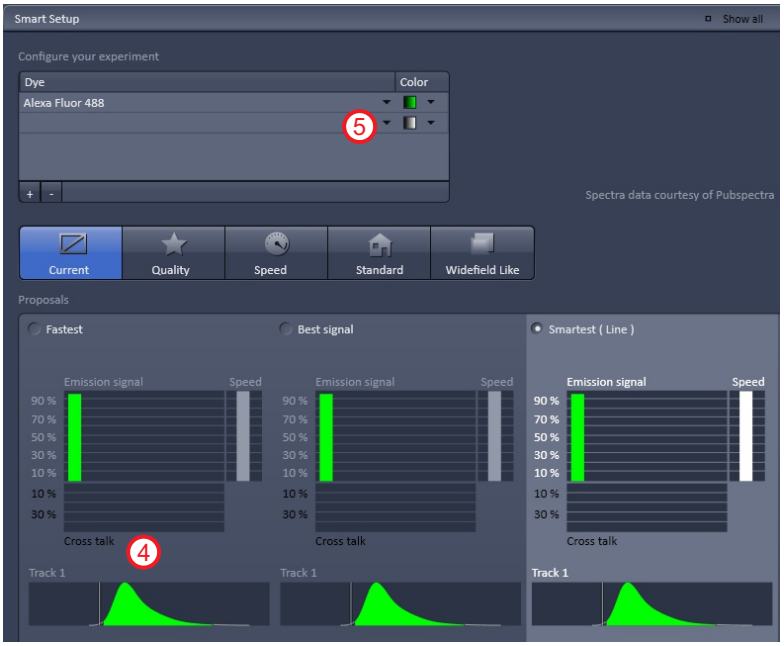


8. A new window will open that shows the emission bandwidth that will be collected④, A new blank track appears in the color column so that a second dye configuration can be added⑤ .

9. To add more dye colors, repeat the above process.

10. Next a choice of fastest scanning, best quality, or their suggestion for “smartest” scanning must be made. The example shown below is for four dyes that have some theoretical bleed thru in all configurations but in this case the “best signal” choice has the least and would also be the brightest⑥ . “Fastest” scan is best for live cell imaging when all colors should be captured simultaneously.⑦





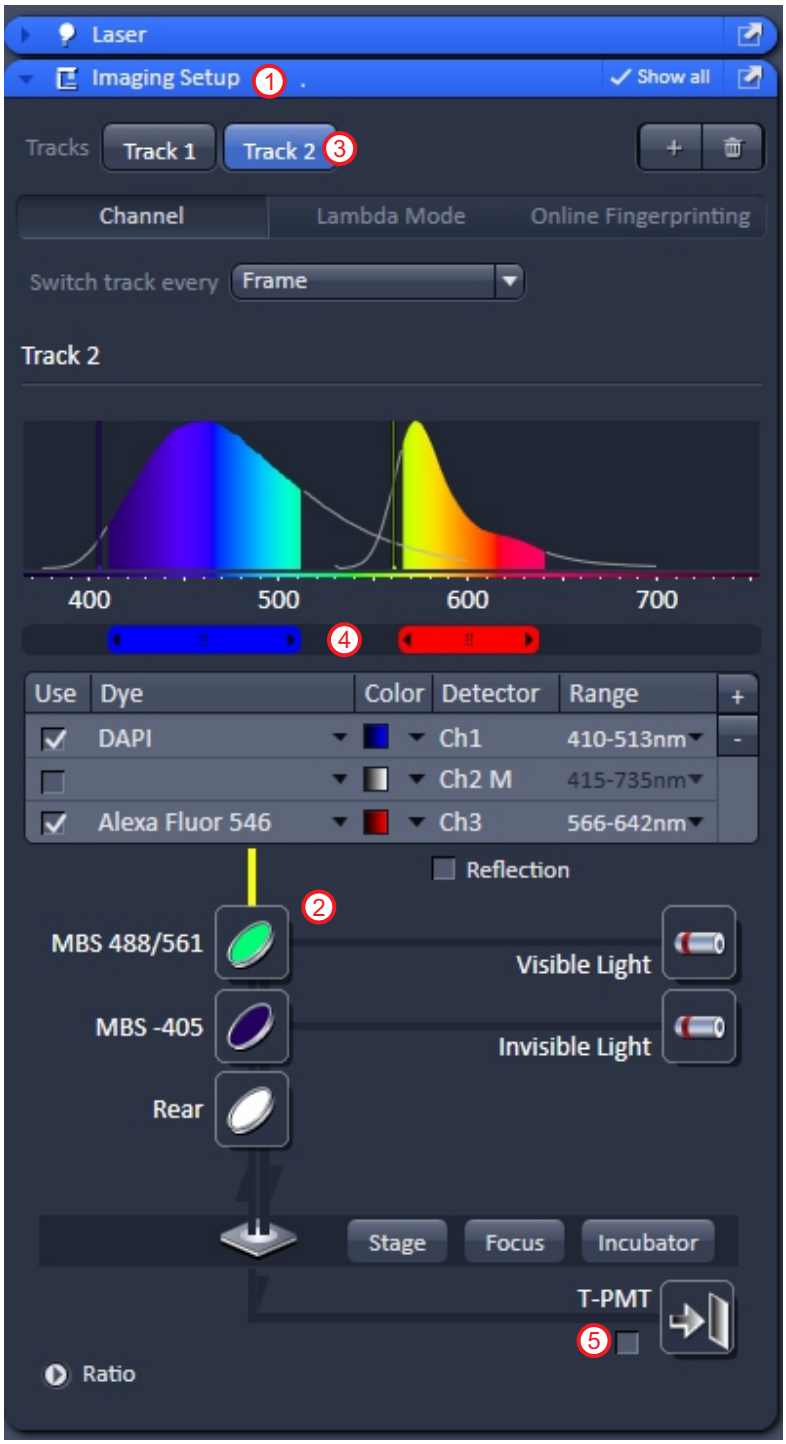
11. When satisfied with the configuration, click the “apply” button⑧.

12. After the configuration is loaded, the menus within the setup manager are updated to show which track has which color(s) in "imaging setup"① as well as the pre-configured light path for each track②. The highlighted (blue bar) track is the one shown in the light path③. In the example shown here, "smartest" configuration was chosen in smart setup so there are two tracks with two colors in each track.

13 There are many options within the Imaging Setup menu for changing the optical configuration, including advanced features such as Lambda Mode. For routine imaging, only two of the options will be discussed here.

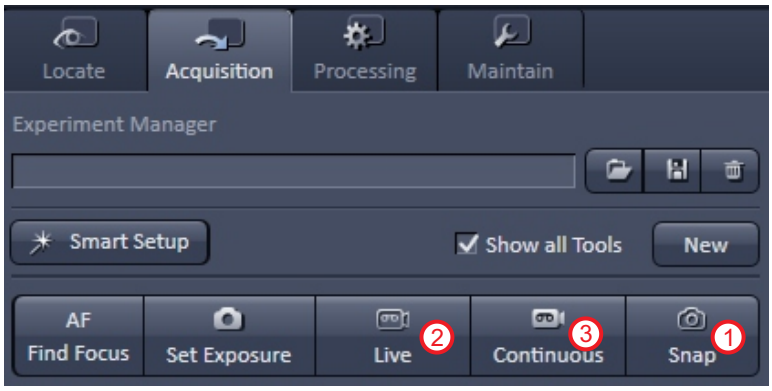
14. First, the emission frequencies collected for the image, both the bandwidth and the wavelengths of the beginning and end of the band can be controlled by dragging the ends of the colored bar located below the spectrum④. Care should be taken when changing the preset bandwidth so as not to create crosstalk between colors or reduce signal intensity unnecessarily. Ask a staff member for help if needed.

15. Second, a grey scale, DIC image can be added as an additional channel by clicking the box marked "T-PMT" at the bottom of the menu⑤. This can be done on any of the tracks (only need one), the choice based upon which laser you prefer to use to collect the DIC image.

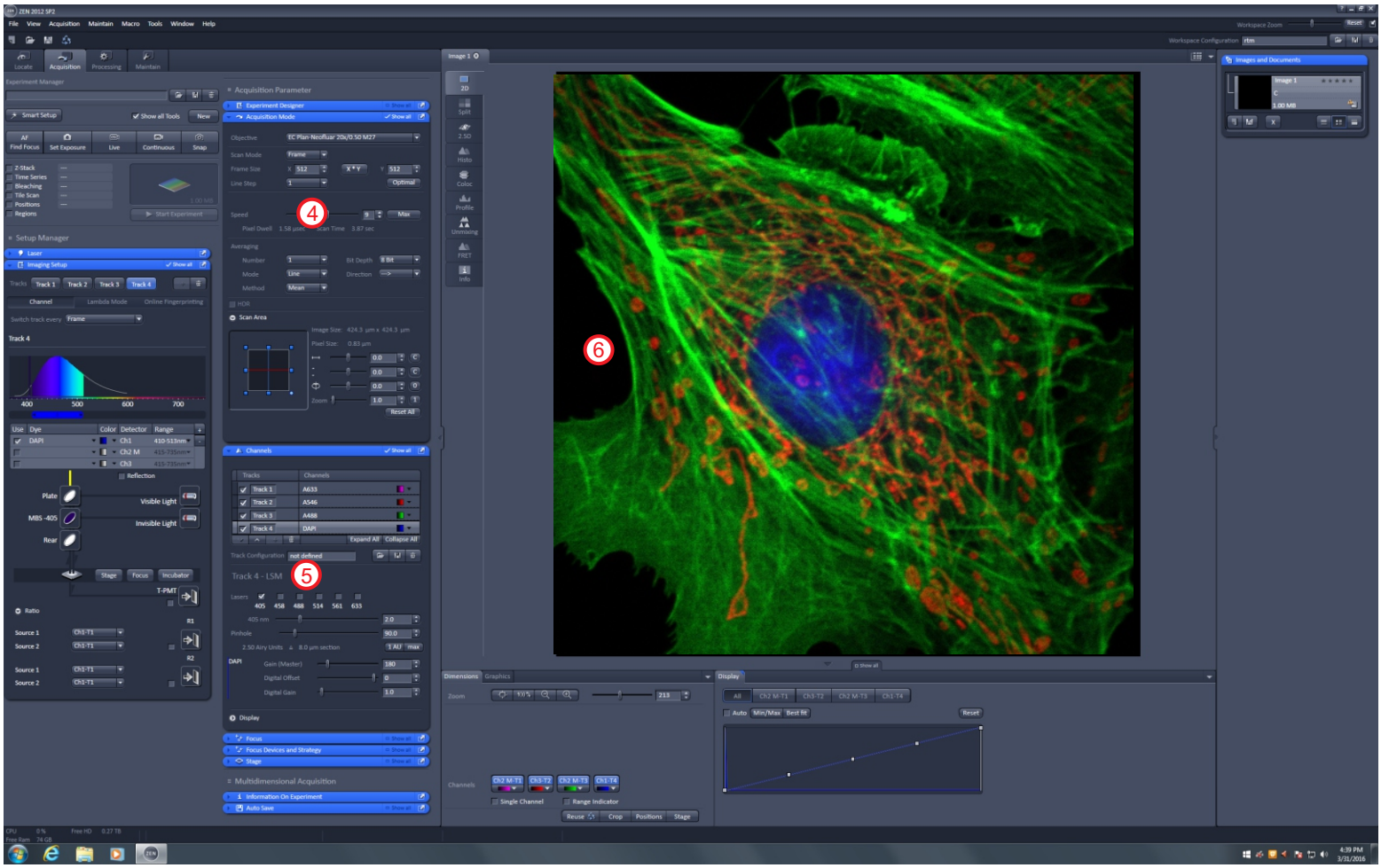


F. LASER SCANNING: OPTIMIZING THE IMAGE

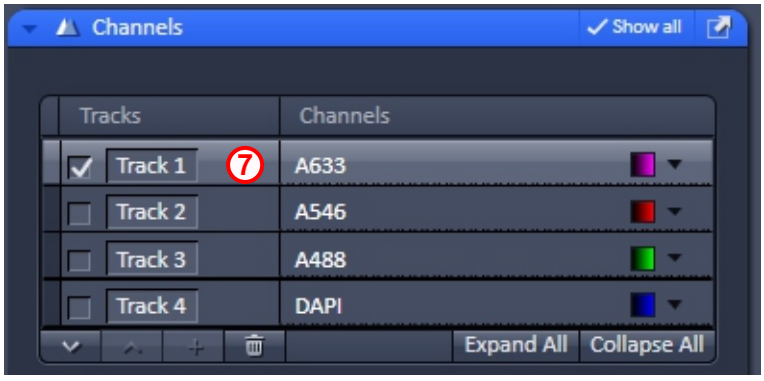
1. Once a configuration is in place, single image scans are initiated with the “snap” button①. To scan repeatedly so that the effects of changing laser power, signal amplification, etc. can be seen quickly, use the “live” button ②. The “continuous” button③ also scans repeatedly but scan rate, frame size and other parameters are controlled by what you set in the “Aquisition Mode” window④ (see later section) and will be the same parameters used for your final image.



2. The settings in the “Acquisition Mode”④ and the “Channels”⑤ menus must be changed in order to achieve the best possible representation of your sample.⑥



3. Within the “channels” menu, un-click all but one track⑦. Choose the track with the dimmest signal, as seen previously through the eye pieces. Or start with the track with the longest wavelength. Click in the blank part of the track title in order to activate its controls (turns it lighter grey ⑦). Now set the pinhole diameter, laser power, master gain and offset for the chosen track, step by step to achieve an optimized image.



4. The first option is laser output ⑧. Keep default output of 2% initially to prevent bleaching.

5. The next option is pinhole ⑨ which determines the optical slice thickness and creates a confocal image. A pinhole setting of 1.00 Airy Units (1 AU) will result in the sharpest image, collecting all in-focus light and rejecting out of focus light. However, a pinhole setting of > 1 AU may be necessary in order to collect enough light to create an optimal image. If unsure what diameter to start with, leave it at the default setting.

6. Increase the Gain (master) ⑩ to 600-700. Set the digital offset to "1" ⑪. Begin scanning by clicking the "live" button. (② on previous page)

If an image appears, skip to step 7

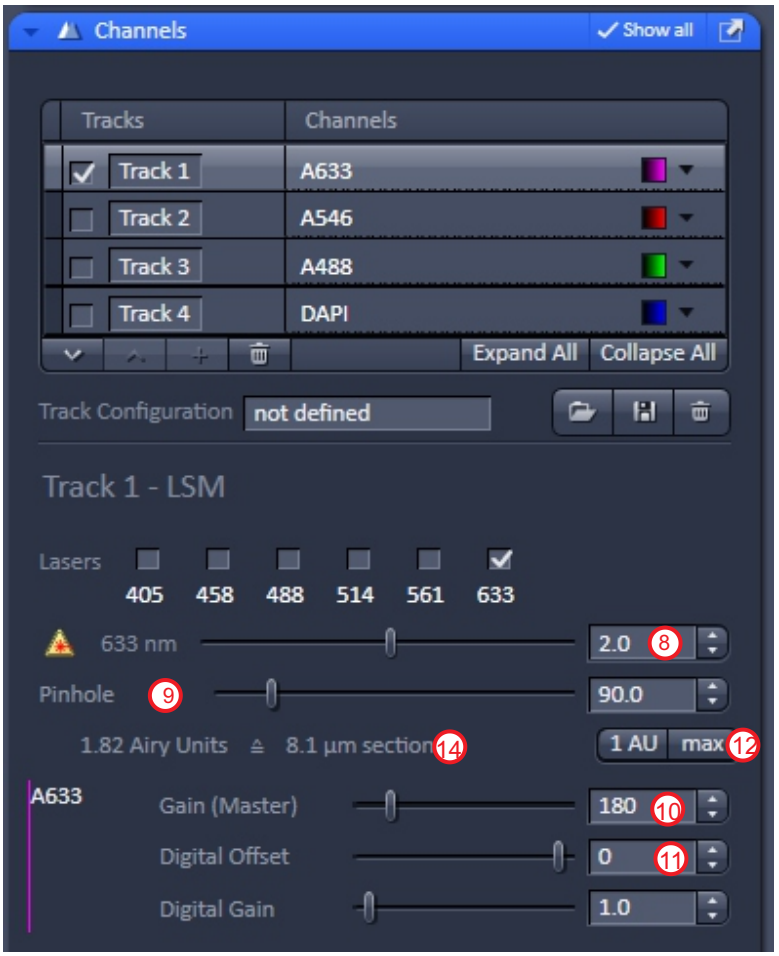
If no image appears, try one or all of the following:

A. click the "max" button ⑫ to fully open the pinhole or use the slider to open it enough to get an image.

B. Check focus while scanning.

C. Increase laser output:

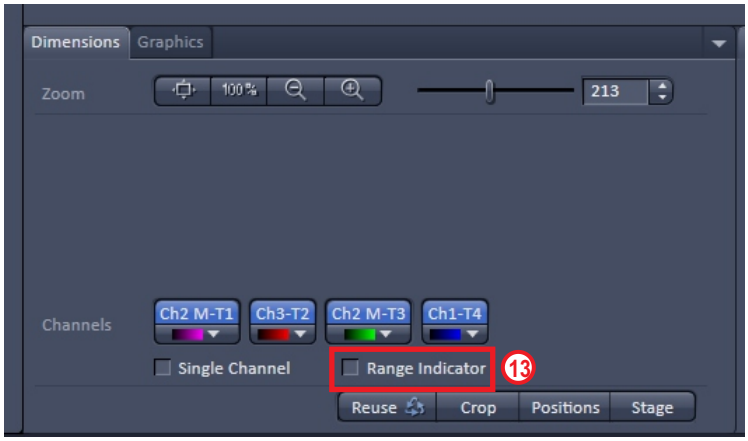
up to 15% for 405, 458, 488 & 514 nm laser lines
up to 30% for 561 & 633 nm laser lines



7. With an image visible, click the box called " range indicator" at the bottom of the center screen ⑬ . In this palette, red pixels in your image mean the PMT is saturated when those locations are scanned (too bright). Blue pixels mean all signal has been removed at those locations (signal lost below threshold). All of pixels with a value of 0 are blue and all the of the pixels with a value of 255 are red. Use a combination of changes in the pinhole size, laser output and Gain (master) to obtain an image with a few red pixels. Digital offset at "1" will result in an optimal number of blue pixels for and 8 bit image. Leave the Digital Gain at "1".

Note: The goal in optimizing is to use the smallest pinhole size (≥1 AU), lowest laser output and a Gain (master) at or below 700 that produces an acceptable image with just a few red pixels visible when using the range indicator palette.

The pinhole size determines the thickness (Z dimension) of the sample from which light is gathered for the image. Larger pinhole size = more light reaching the PMT detector. However, a larger pinhole results is a "fuzzier" image.



8. After the 1st track is optimized, note the final optical section thickness ⑭. T**he pinhole size for all other tracks must be set so that the section thicknesses match across all tracks.** Stop the scan.

9. Uncheck the finished track, check the next track and repeat the above steps for each additional track one at a time, with the limitation about pinhole size mentioned in step 8. To capture a complete image, put check marks in all tracks and click "snap". Save image in .czi format on our cisrstore server (see image saving details on page 12).

G. IMPROVING IMAGE QUALITY

Why it's important to optimize your image using the entire intensity range:

In this image the intensity ranges from 30-174. The picture looks flat and dull, and it's difficult to discern detail.

In this image, the intensity ranges from 0-255, but *many* pixels are saturated, and detail is lost in a wash of white. It's not that it's hard to see detail; it's no longer there.

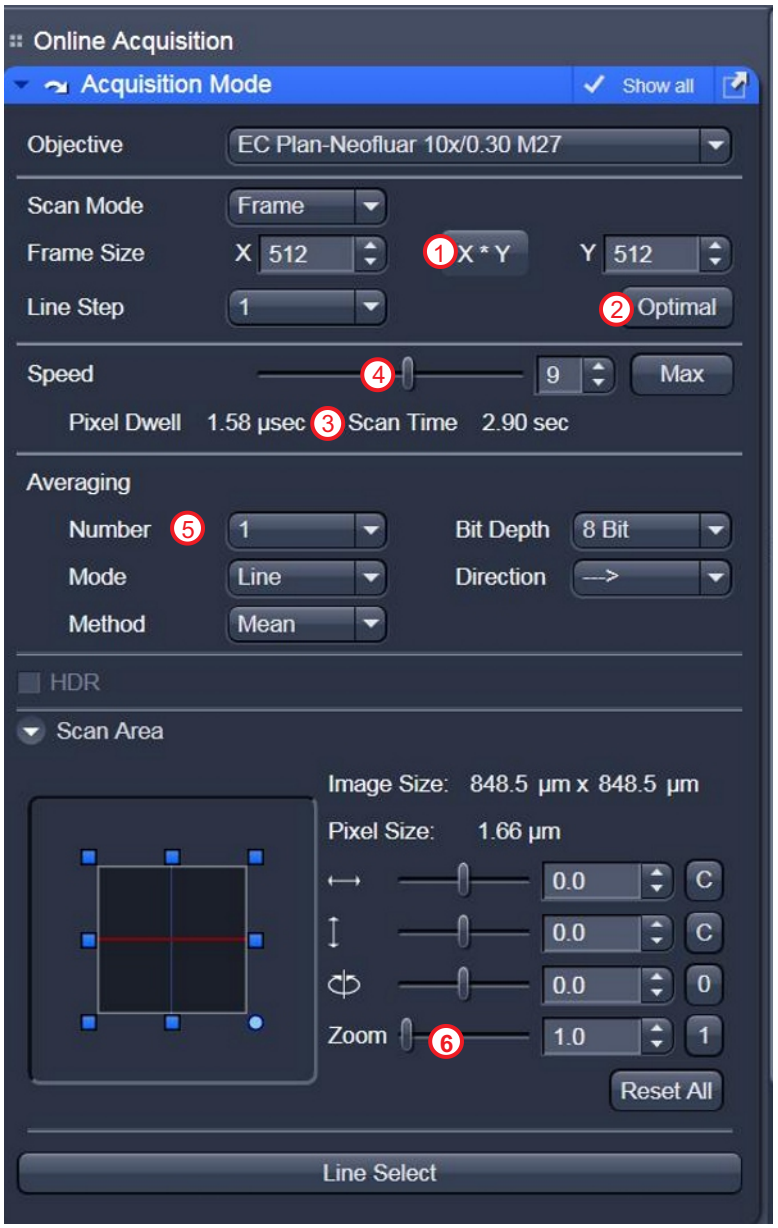
In this image, the intensity also ranges from 0-255, but now very few pixels are saturated. All detail is preserved. This image gives us the most information about the original sample.

1. The "Acquisition Mode" window has a number of controls that may further improve the quality of your image. Change the pixel resolution to make each pixel smaller and enhance the ability to zoom in after acquiring the image. Click the "x*y" button to see standard resolution sizes (1) or click "optimal" (2) for the best resolution possible for a particular objective and field size. Higher than optimal resolution will result in oversampling. Higher resolution imaging requires longer scan times (3).

2. Change to a slower scan speed (4) to improve the image, but again at the expense of more time.

3. Increasing the "Averaging" number to >1 (5) may improve image quality, specifically better signal/noise ratio if the noise is random.

4. Change the "Scan Area" settings as needed to reduce or expand the scanned area (zoom). (6) Or use the "crop" button located at the bottom of the central screen (7) for more precise control of the image contents.



H. Z STACK

1. Creating a z-stack of images refers to collecting a series of overlapping images at different focal planes without changing the x:y stage position. 3D reconstructions of your sample can then be created from a z-stack of images

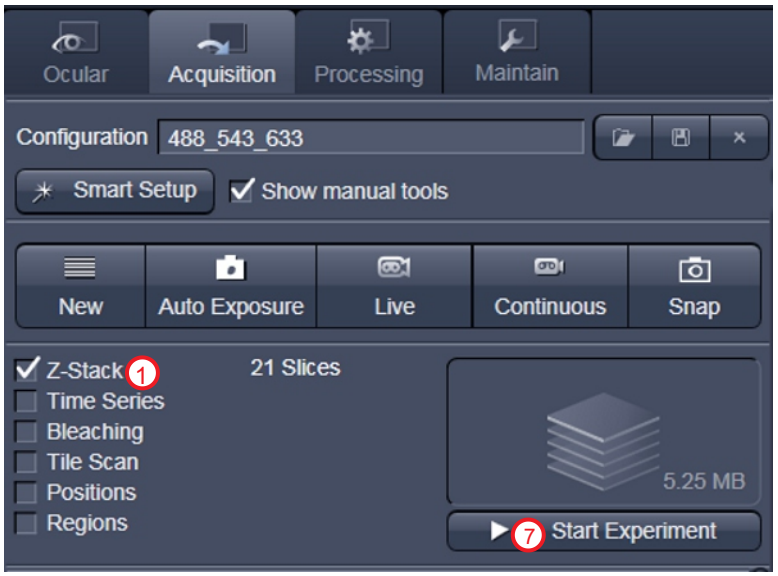
2. Place a check mark in the z-stack box within the menu control list (1). Maximize the z-stack menu and choose the first/last submenu (2). Go to the channels menu and turn off all but one channel; leave on the channel that will most easily show the beginning and ending focal plane. Start scanning with the "live" button and focus the microscope toward you **(counter clockwise on right hand knob)** to lower the objective to the start of your z stack and click "set first" (3). Turn the focus knob in the opposite direction to reach the desired depth and click "set last". (4)

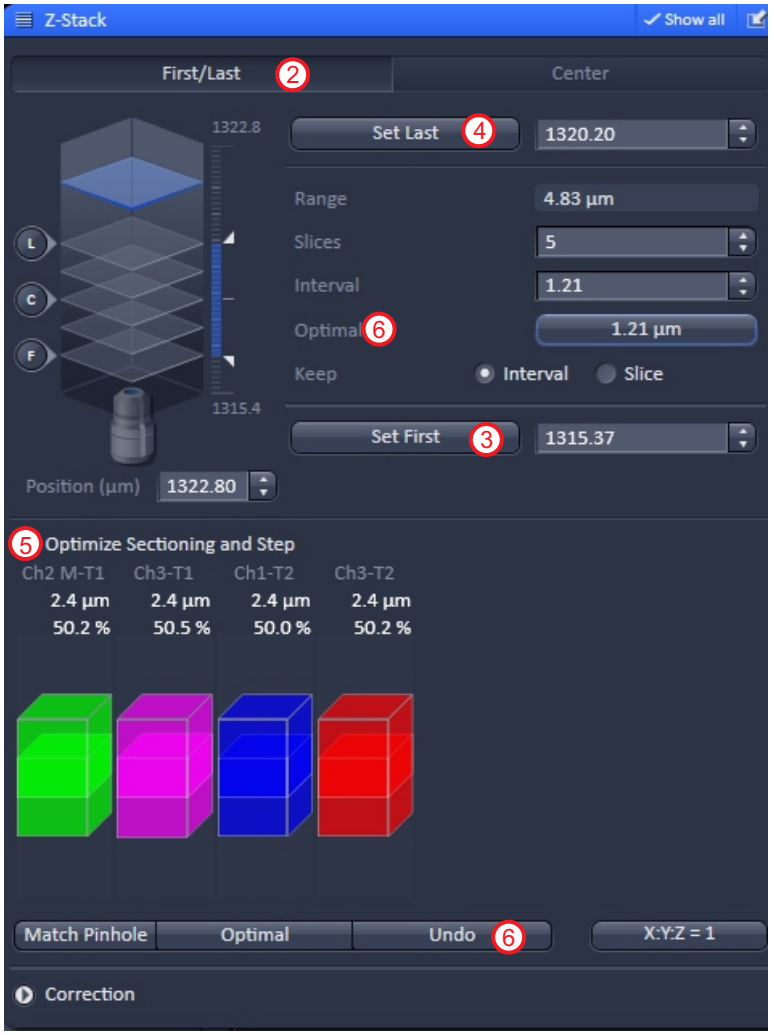
3. Set an optimal overlap between images by clicking the "Optimize Sectioning and Step" submenu (5) to reveal additional buttons at the bottom of the z-stack menu. Clicking the "Optimal" button (6) will synchronize the z-stack step size with your chosen optical slice thickness (pinhole size dependent) and the objective for the best software reconstruction of a 3D image.

4. Change any Mode settings as discussed previously in order to allow completion of your z-stack in a reasonable amount of time. When ready, click the "Start Experiment" button (7) (NOT the snap button).

5. Once started, the approximate time required to finish will appear at the very bottom of the screen. The scan can be stopped at any time and restarted from the beginning.

6. When finished, save the stack as you would a single image.



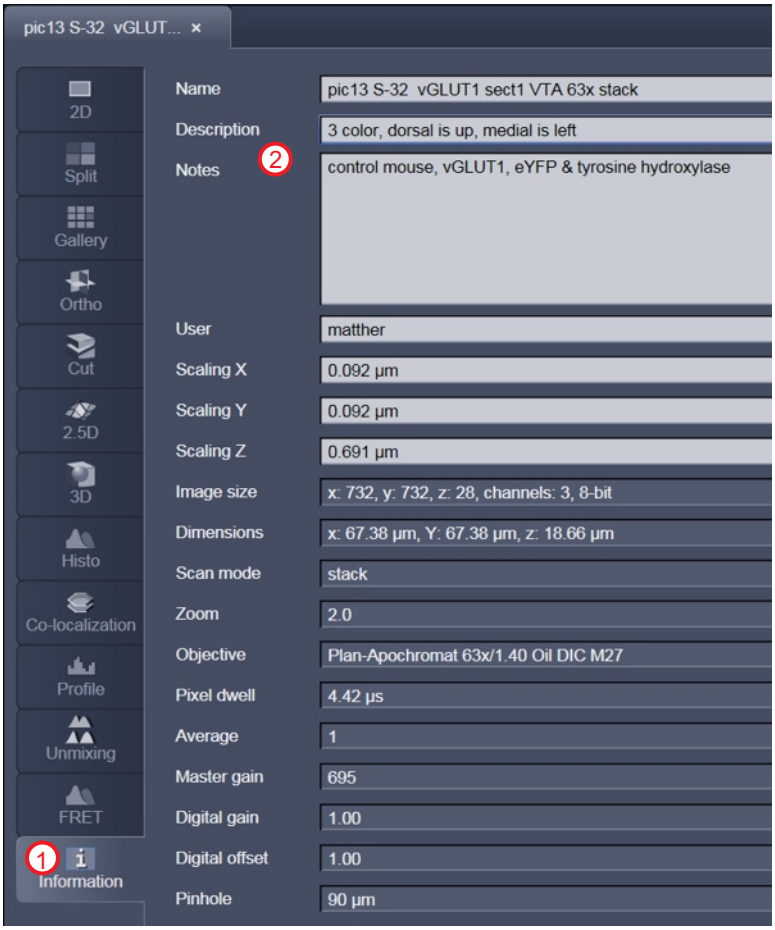


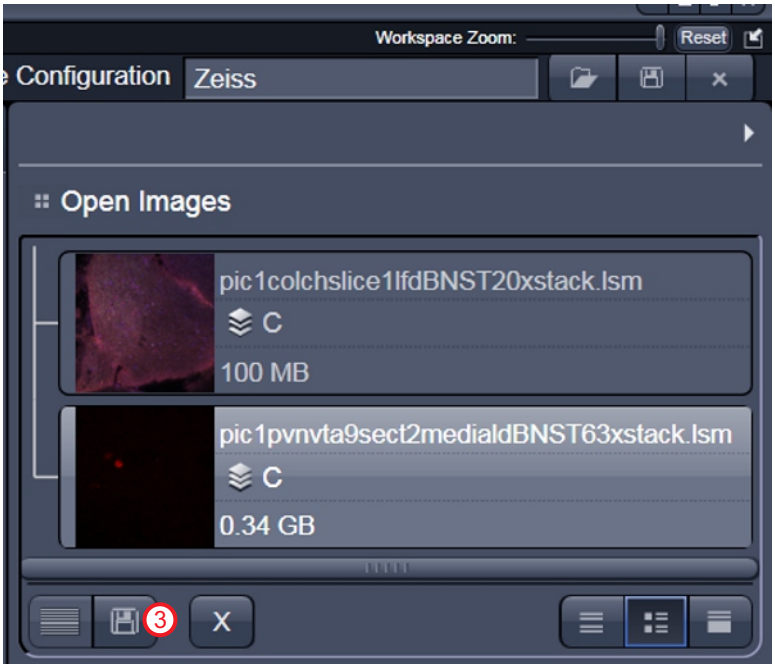
I. SAVING YOUR IMAGES

1. Before saving an image, you may want to annotate the image with special information. If so, click the "Information" button to the left of the image (1) and add your text to the "Description" and "Notes" boxes (2). Note that other technical info, for example scaling, objective used, etc., are already filled in and will be saved along with the image.

2. Click the save icon under the thumbnails of the open images (3) and fill in the file name in the new window (4). Browse for a destination for where the image will be saved (5). Save one copy as a .czi file type.

3. We recommend saving your images to our CISR server called **cisrstore.** Every CISR user is provided a folder with their VUnetID as the folder name, which can be used to store images (up to 50 gigabytes) for free. However, this is not to be use as an archive. Please backup your files elsewhere and erase files older than one year. Our server can be mapped as a new drive on computers throughout Vanderbilt for easy access to your data. Mapping instructions can be found on our website under the FAQ tab or ask a CISR staff member for help.





J. SHUT DOWN

1. Check the calendar on the CISR website to see whether you are the last user of the day. If you are using the scope during regular work week hours (until 6 PM) and there is another user scheduled before 6:30, please leave all of the equipment turned on, including the lasers. Close the ZEN software and log off.

2. If you are using the scope in the evening or weekend and someone is scheduled to come within 30 minutes, leave the equipment on for them and log off.

3. If you are the last user of the day or it is an evening or weekend where the next user is not due to arrive for at least 30 minutes, proceed to shut the system down.

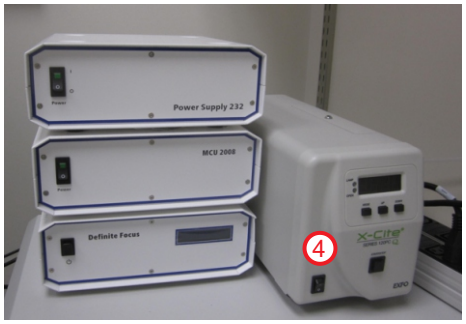
4. Save all images and configurations as appropriate. Close the ZEN software and log off.

5. Flip the toggle switch on the argon laser control module to “idle power”⑥.

6. Turn off the key and switch marked 5 & 4. The argon laser power supply (#5) must be allowed to cool off with the fan running before turning off the rest of the switches (about 2 minutes). When the cooling fan stops, turn off switches 3,2 & 1.

7. Clean off oil/water from immersion lenses and leave the condensor arm in the down position.

8. Sign out on the paper log. Come again soon.



DIC: Ocular Viewing & Kohler Illumination

This microscope can capture brightfield, monochrome images that overlay fluorescent images. These bright field images are DIC (**D**ifferential **I**nterference **C**ontrast) images. For optimal DIC images, light should be focused in the same plane with the sample. This is accomplished by adjusting the **condenser** and is called **Köhler Illumination.**

1. Focus the specimen using transmitted light activated by the DIC button ①.

2. Close the field iris ② until only a spot of light remains ③. If you can't see a spot of light, try raising or lowering the condenser with the adjustment knob ④.

3. Adjust the condenser knob until the polygon is in focus (edges as sharp as possible.)

4. Turn the "rabbit ear knobs" ⑤ to center the polygon.

5. Open the field iris completely ②.