Zeiss 780
Training Notes

780 Start up sequence

Turn on Main Switch, System PC and Components Switches

Do you need the argon laser (458, 488, 514 nm lines)?

Yes

Turn on the laser's main power switch and turn the key ¼ turn to the right, to the on position.

Please note, all other lasers are turned on within the software.

No

Turn on X-cite

This is the fluorescent light source for viewing your sample through the oculars. You will regulate the bulb intensity via the software.

Turn on the PC

Are you using the argon laser?

Yes

Push the toggle switch to the run position.

Is the green light on?

No

Yes

Increase power until red light comes on; if red light is on power is too high.

No

Log on, initiate Zen software

780 Training Notes

User Computer

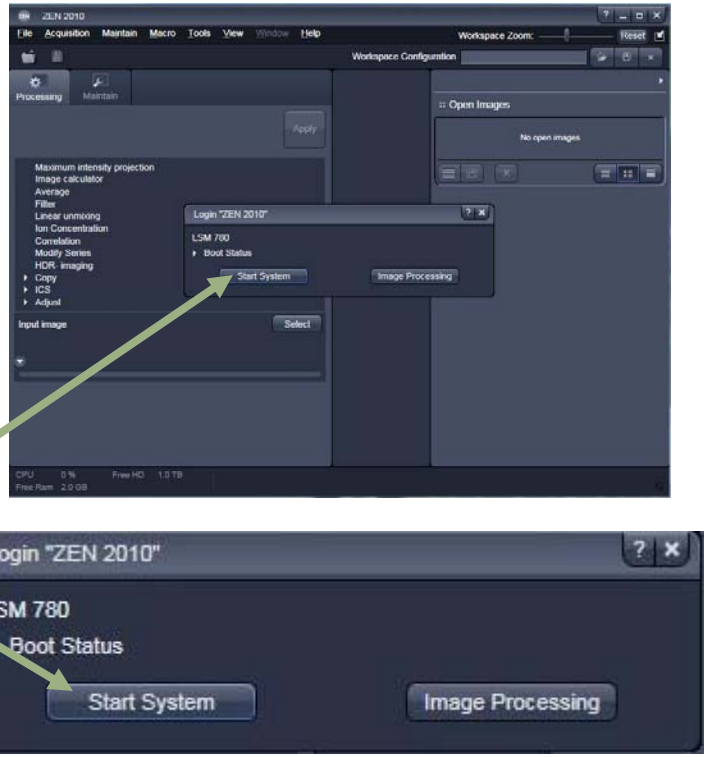
Turn on the computer; power button is on the top left of the tower.

- 64-bit operating system
- Windows Vista Ultimate

Please wait for Vista to finish initializing before launching the Zen software, the short-cut should be located on your desktop.

Zen 2010

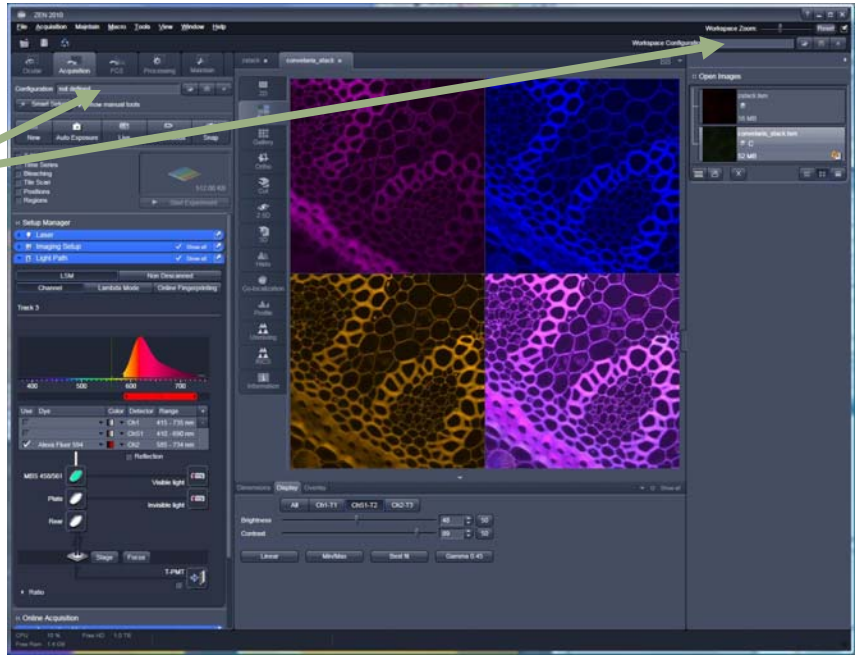
- Double click the Zen icon on the desktop.
- Select Start System from the Zen 2010 login window. This will start up the hardware and software. Alternatively, Image Processing is for using the system offline, and this mode will ignore all the hardware components.



780 Training Notes

Zen workspace

The workspace is very large so give yourself room. It is designed to step you through initially looking at your specimen via the Oculars tab, to acquiring images via the Acquire tab. The remaining tabs add advanced features, FCS, off-line Processing and Maintenance options.



The workspace configuration and your sample configuration can be stored separately. In addition, your sample configurations can be easily retrieved from the Reuse function when you have an image loaded in the workspace.

780 Training Notes

Transmitted light Source and shutter

Fluorescent light Source and shutter

Environmental Controls

Objectives

Ocular Tab

The Ocular tab gives you access to the microscope for previewing your sample. Select Online to make the microscope functional.

At the top of the panel, you can open and close the Fluorescent Shutter. in the Configuration pane, you can create shortcut buttons for using transmitted and fluorescent light.

Ocular panel

This panels allows you to configure the microscope so you can view your image under transmitted or fluorescent light; DAPI, FITC, or Rhodamine filter sets.

Objectives

There is a dedicated set of objectives for the 780 however it is possible to use other objectives that are available in the center.

Image

Once you have located you image, be sure to center it in the field of view as the scan area on the system is very small and you want to be sure to capture the area you are interested in.

780 Training Notes

Acquisition Tab

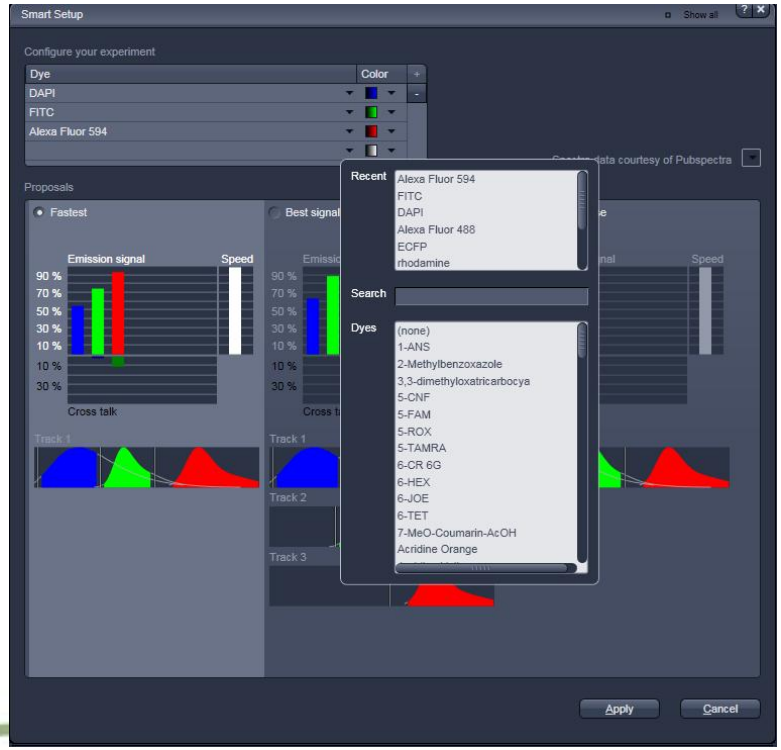
When using the Acquisition tab, always be sure to first select View>Show All, and check Show Manual Tools. The Zen software tends to hide information that is useful for capturing your images so by making these selections you will have all the tools necessary for your work. For example, the Setup Manager, which gives you access to the lasers will be hidden unless Show Manual tools is selected

Smart Setup

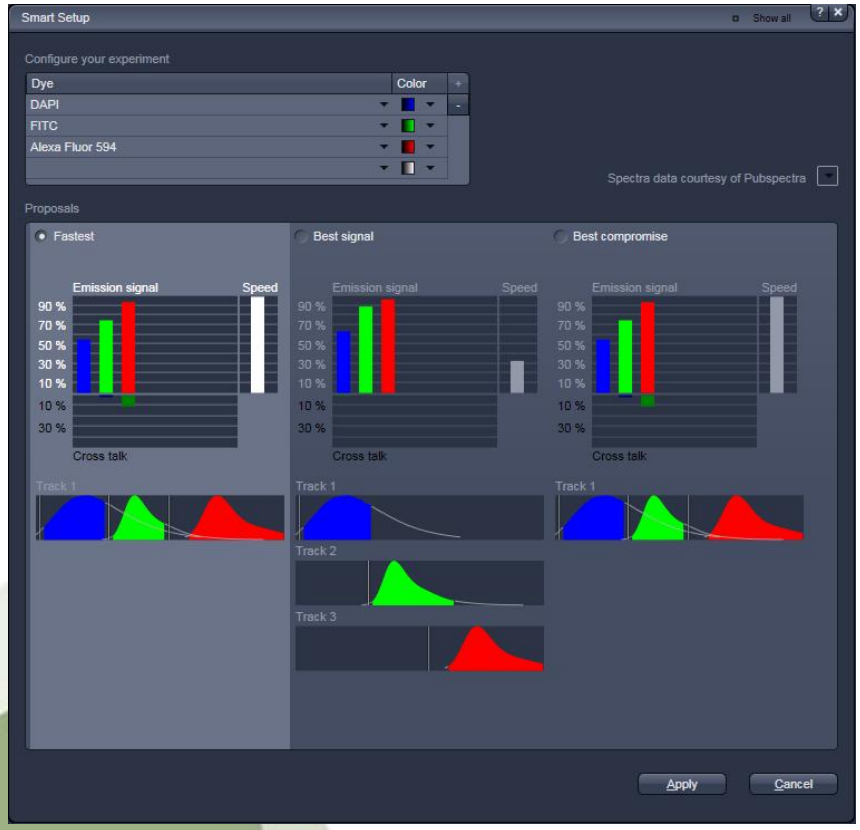
Until you are familiar with the workings of the 780, the best and easiest way to get started is to use the Smart Setup tool. Select your dye(s) from the drop down menu. The software will automatically pseudo-color your dyes; you can change the suggested color from the right down arrow.

Depending on your imaging needs, the Smart Setup will offer you up to four different configurations for imaging your sample: Fastest, Best Signal, Best Compromise, and Linear Unmixing. Whatever configurations you are presented with, you can always manually modify the configuration in the Light Path.

Make your selection and press the Apply button to implement the configuration.



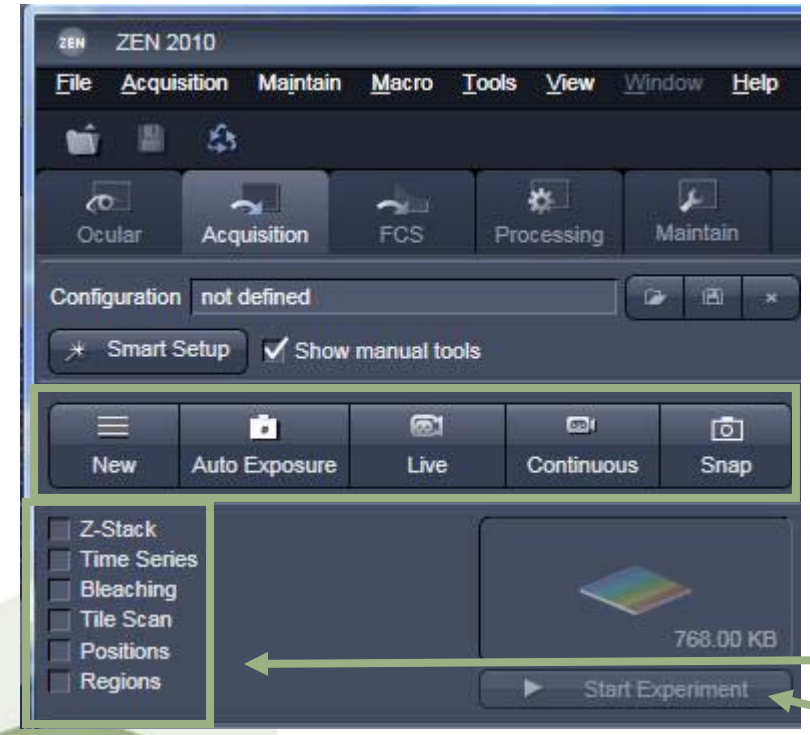
780 Training Notes



Smart Setup (continued)

- Fastest: Acquires the image with simultaneous excitation and detection of the different probes. This option may produce bleed through between the different channels.
- Best Signal: This will usually configures the system such that each probe is scanned one at a time, and then over lays the images as one at the end of the scan routine. This will prevent bleed through between the probes however, this subjects your sample to be exposed to the laser light for “X” times, depending on the number of probes you are using, which can lead to bleaching or other laser induced damage.
- Best Compromise: This option will often combine two probes into a signal track and image the other probe(s) separately. This may or may not avoid bleed through between the probes depending on the nature of the probes being used and it may also be the same option as the Fastest configuration, it all depends on the probes.
- Linear Unmixing (not pictured): This option creates a confirmation for computationally subtracting bleed through between channels after you acquire pure spectral profiles of the probes you are using.

780 Training Notes



Acquisition Tab

You can work your way down the acquisition tab as you explore its functions.

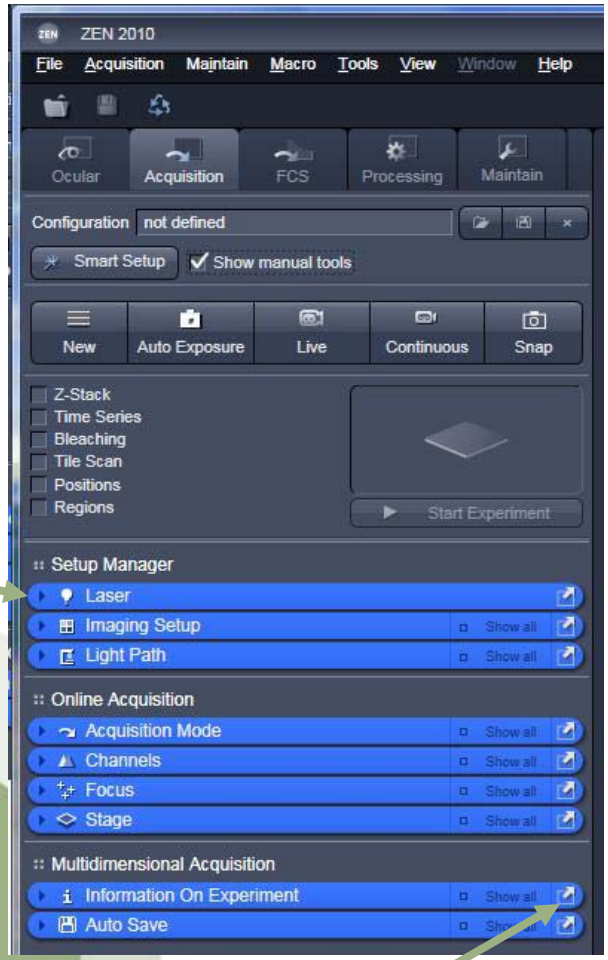
- New - opens a new image capture window.
- Auto Exposure - Computer will optimize the master gain and offset for the given laser power and pinhole size.
- Live - fast scan for previewing image.
- Continuous - scan that can be regulated fast or slow.
- Snap - Single scan.

Note, when you initiate a scan, that particular button will be converted to the Stop button.

Advanced Features

Select from Z-stack, Time Series, Bleaching, Tile Scan, Positions, and Regions. You must initiate the scans using these functions with the Start Experiment button.

780 Training Notes



Open/close Submenu with down arrow.

Click arrow to detach/attach panel and open it in main workspace.

Acquisition Tab

The Acquisition tab initializes with the submenus collapsed; be sure to select the arrow to expand each submenu.

Setup Manager

- Laser
- Imaging Setup
- Light Path

Online Acquisition

- Acquisition Mode
- Channels
- Focus
- Stage

Multidimensional Acquisition

- Information on Experiment
- Auto Save

780 Training Notes

Acquisition Tab

Setup Manager

Laser

The 780 offers the following laser line selections:

Diode 405nm (30mW)

Diode 440nm (15mW)

Argon 458nm, 488nm, 514nm (25mW)

Helium-Neon 633nm (5mW)

Chameleon (tunable two-photon laser) 690-1020nm (1W)

The argon and Chameleon lasers are the only lasers powered on outside the software.

The software is smart enough to prompt you to turn on a laser when you select it from the Smart Setup option, however if you manually need to turn on a laser you should do so with this panel.

780 Training Notes

Acquisition Tab

Setup Manager

Imaging Setup

- Mode
  - Channel Mode - x-y scanning configuration.
  - Lambda Mode – Used to create spectral profiles of dyes
  - Online Fingerprinting – applies spectral profile configuration to dye, to correct for bleed through issues, as it is scanned
- Switch track every
  - Line – Tracks are switched line by line. Laser line, laser intensity and channels can be changed between tracks.
  - Frame – Tracks are switched frame by frame. Laser line and intensity, filters, beam splitters, gain and offset, and pinhole setting can all be changed between tracks.
  - Frame Fast – scanning procedure can be made faster. Only the laser line intensity and Amplifier offset can be changed between tracks.

780 Training Notes

Acquisition Tab

Setup Manager

Imaging Setup (cont.)

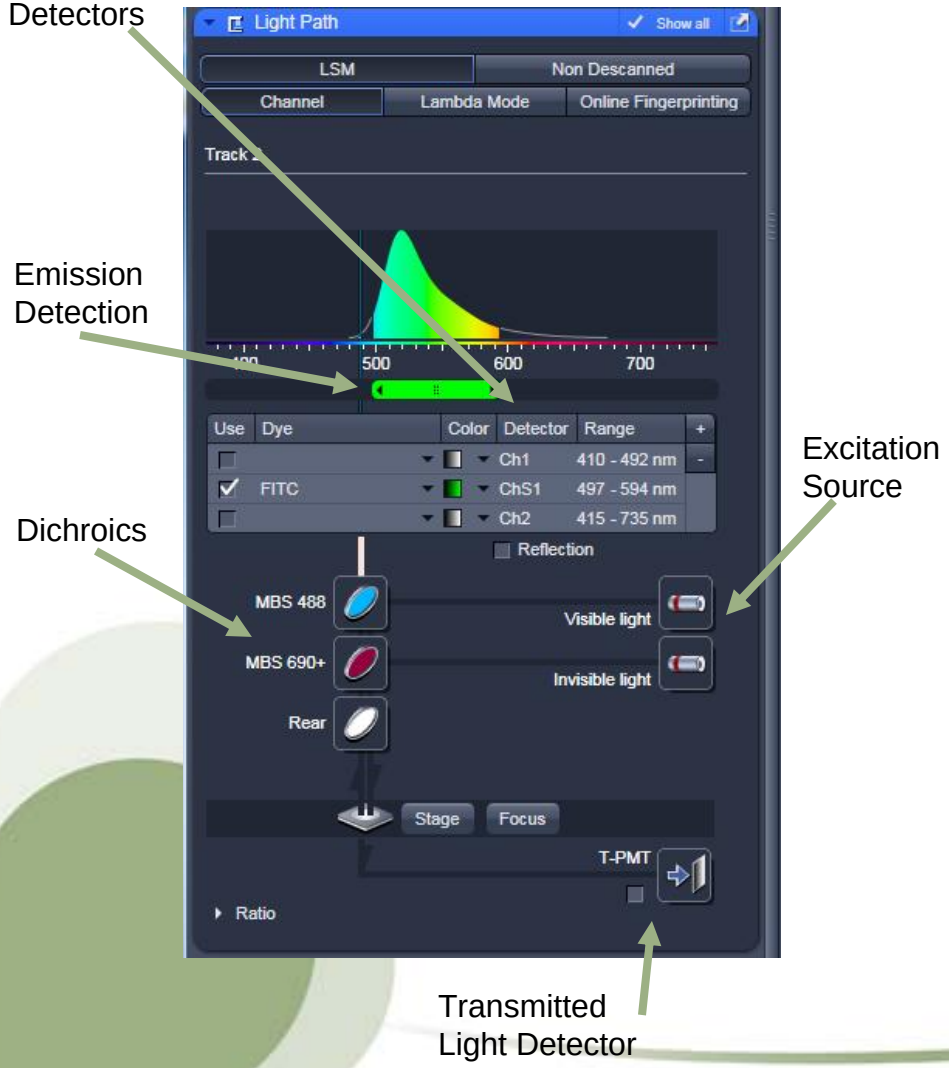
Track – refers to the dye(s) that are being detected and enables or disables them by their selection. A maximum of four tracks can be created.

The track displays the dye, laser line excitation and the emission detection range. The actual configuration is defined in the Light Path panel, which is found below.

Light Path

- LSM – Single Photon Mode
- Non Descanned – Two Photon Mode
- Channel Mode - x-y scanning configuration.
- Lambda Mode – Used to create spectral profiles of dyes
- Online Fingerprinting – applies spectral profile configuration to dye, to correct for bleed through issues, as it is scanned.

780 Training Notes



Acquisition Tab (cont.)

Setup Manager (cont.)

Light Path (cont.)

Options for Configuring the Light Path

- Dye and pseudo-color display
- Detector – select from Ch1 or Ch2 (standard PMTs) QUASAR detectors ChS 1-8, or transmitted light detector ChD (not confocal).
- Emission Range
- Laser – either visible or invisible (UV) and set attenuation values (% transmission)
- Main dichroic beam splitter

780 Training Notes

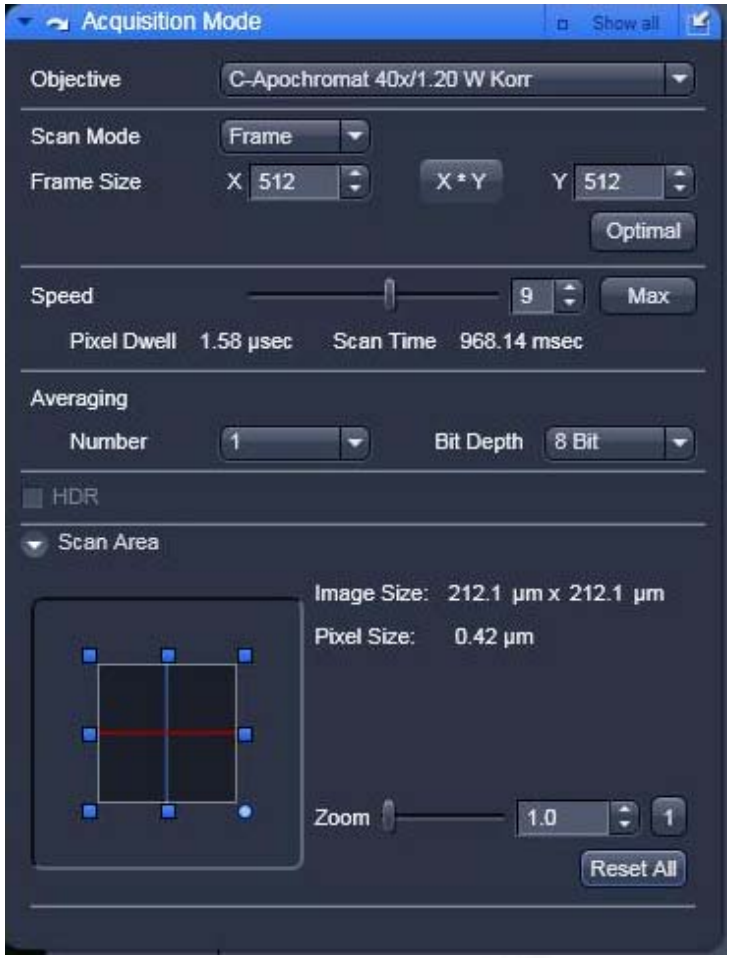
Acquisition Tab

Online Acquisition

Acquisition Mode

- Objective – change objectives from here
- Scan Mode – Frame, Line, Spot
- Frame Size – defaults to 512 x 512. Select Optimal for appropriate number of pixels depending on numerical aperture and excitation wavelength.
- Speed – adjust scan speed. A higher speed with averaging yields results with the best signal-to-noise ratio. Use slower scan speeds for better images, but be aware of photobleaching.
- Averaging – improves image by increasing the signal-to-noise ratio. Select from Line or Frame averaging and select the number to average by.

  Averaging helps to reduce photobleaching.
- Bit Depth – select from 8 bit (256 gray levels), 12 bit (4096 gray levels), or 16 bit (65536 gray levels). Be sure your software can handle the information. 12 and 16 bit images are recommended for publication and quantitative measurements.
- Scan Area – set the microscopic zoom



780 Training Notes

Acquisition Tab

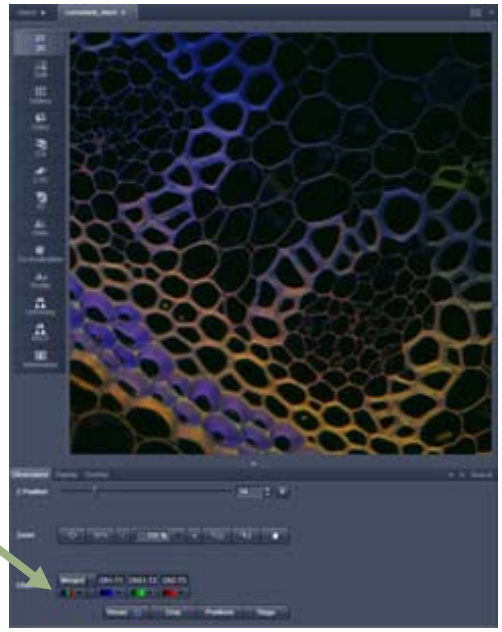
Online Acquisition

Channels

The track for the Channel must be active and selected in order to make changes.

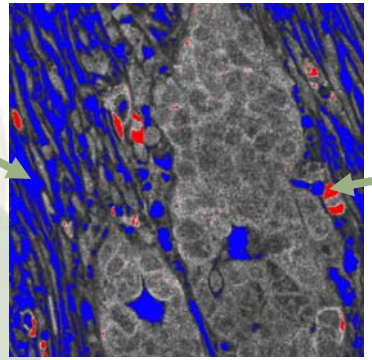
- Lasers – turn lasers on and off and modify laser power.
- Pinhole – set pinhole to 1 AU (airy unit) to achieve best z resolution based on the excitation wavelength and objective numerical aperture. Be sure to match the section thickness when scanning multiple channels.
- Mode – Integration and Photon Counting
- Gain (Master) – amplifies the voltage on the photomultiplier tube; regulates brightness of the image
- Digital Offset – sets background/threshold level of the image
- Digital Gain – post acquisition enhancement of the gain signal.

780 Training Notes



Select range indicator

Minimum intensity



Maximum intensity

Acquisition Tab

Online Acquisition

Channels (cont.)

Remember, when acquiring your images, you want to be within the dynamic range of the detector. This is especially critical when collecting images that will be used for quantitative analysis.

- Use the Range Indicator to adjust the gain and offset. Blue= minimum, Red= maximum (0 and 255 for 8 bit image, 0 and 4095 for 12 image, 0 and 65536 for 16 bit image.)
- Adjust Detector Gain and Amplifier Offset to remove red and blue pixels. Minimum should be ~ 10-20 gray levels above 0 and Maximum should be 10-20 gray levels below 255 for an 8 bit image. You can scale this accordingly for 12 and 16 bit images.

780 Training Notes

Acquisition Tab

Online Acquisition

Focus

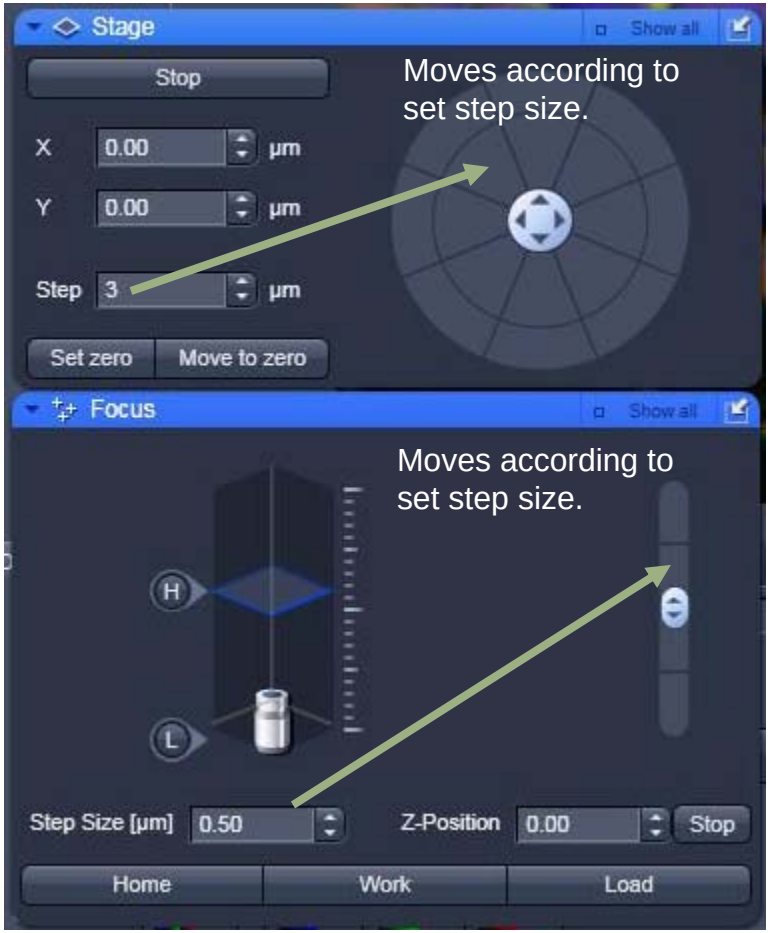
The 780 offers the ability to manually control the focus or to use the software to control the focus.

- Step size – set a fixed step size, in microns. Move up or down in the focal plane based on that step size.
- Z-position – specifies location of focal plane.
- Home – zeros the focal position.
- Work – returns the objective to the desired focus, from the previous sample.
- Load – lowers the objective so the user can change the sample.

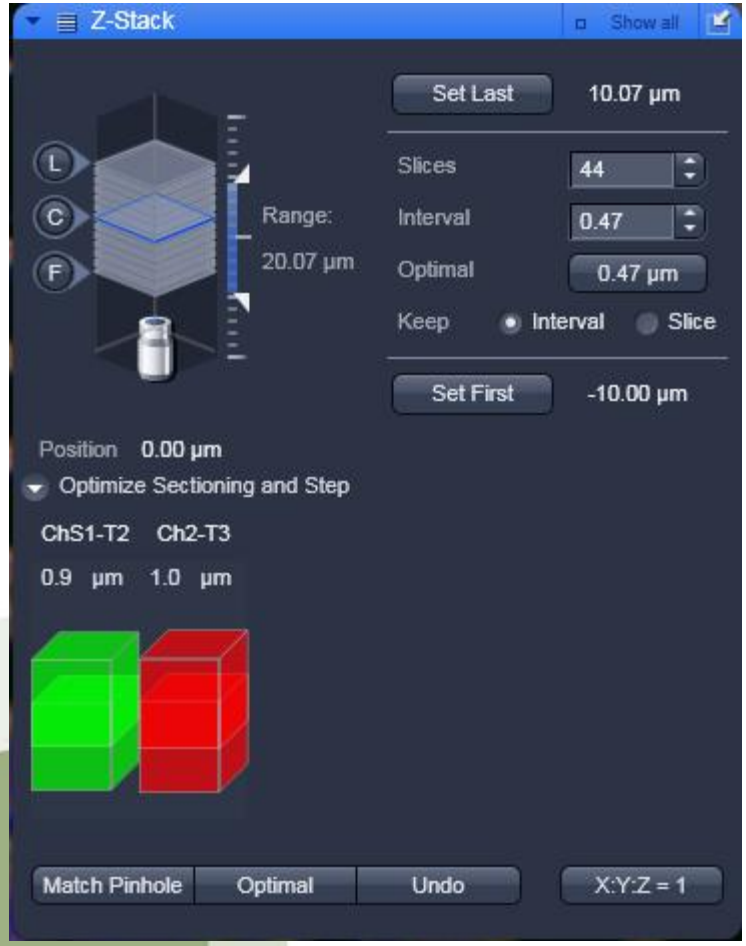
Stage

The 780 allows you to manually control the stage via the joy stick or to use the software to control the stage.

- x-y coordinates – indicates stage position.
- Step – move specified step size, in microns.
- Set zero – establish a zero point (0,0) on the stage
- Move to zero – move to the zero point on the stage.



780 Training Notes



Acquisition Tab

Online Acquisition

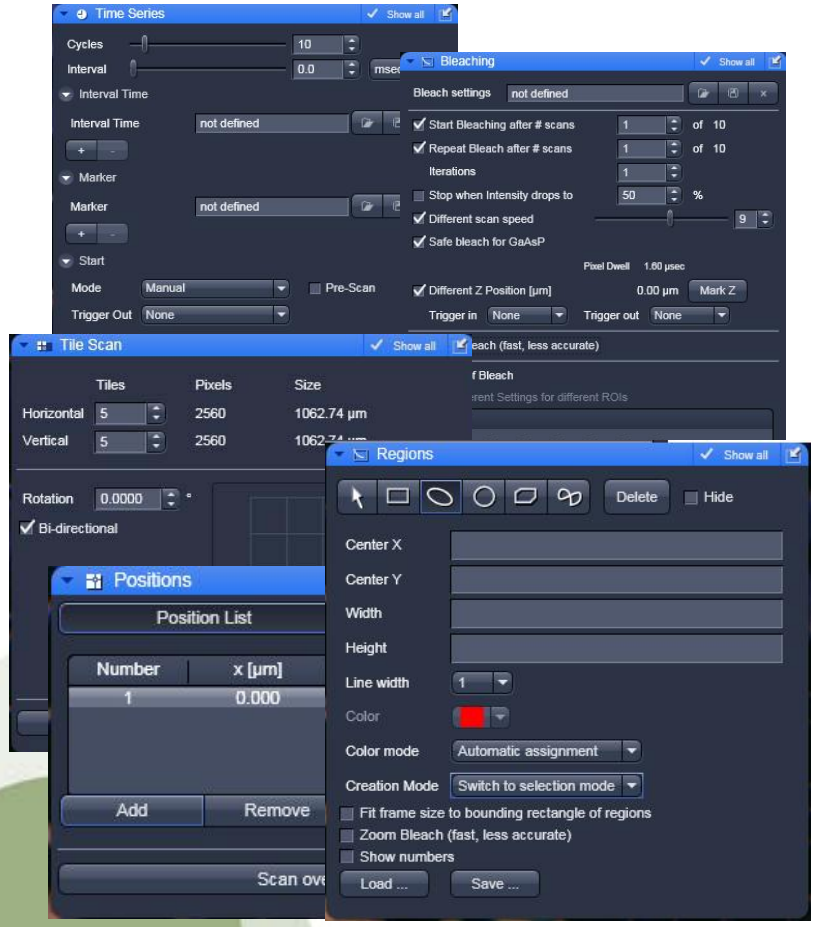
Multidimensional Acquisition

Z-stack

Collect multiple images of different focal planes within a single file for 3-D analysis/display.

- Set Last – mark ending point of stack
- Set First – mark starting point of stack
  - Slices – displays how many slices will make up the stack
  - Interval – distance between each slice
  - Optimal – calculates each slice to be ½ the z-resolution for proper Nyquist sampling.
  - Keep Interval/Slice – decided if the stack should be based on the interval thickness or the number of slices.
- Optimize Section and Step
  - Match Pinhole – for multiple wavelengths ensure that the pinholes are yielding the same section thickness.
  - Optimal - calculates each slice to be ½ the z-resolution for proper Nyquist sampling.
  - Undo – clears selection

780 Training Notes



Acquisition Tab

Online Acquisition

Multidimensional Acquisition – additional options

- Time Series - Collect a series of images based on a time interval.
- Bleaching – module for FRAP (fluorescent recovery after photobleaching.)
- Tile Scan – create larger images by tiling smaller images together.
- Positions – record stage coordinates to return to
- Regions – drawing tools used to isolate particular scan areas of the sample.

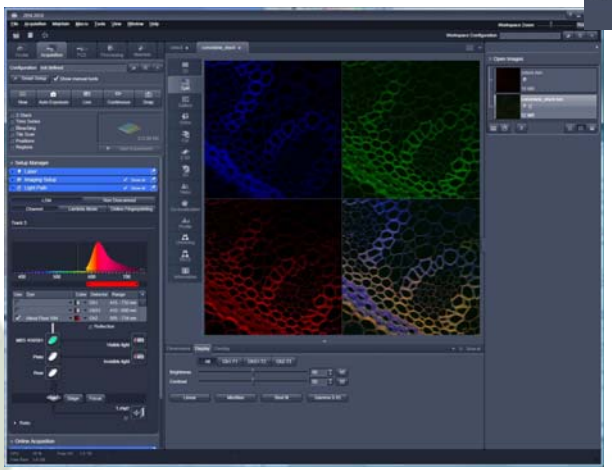
780 Training Notes

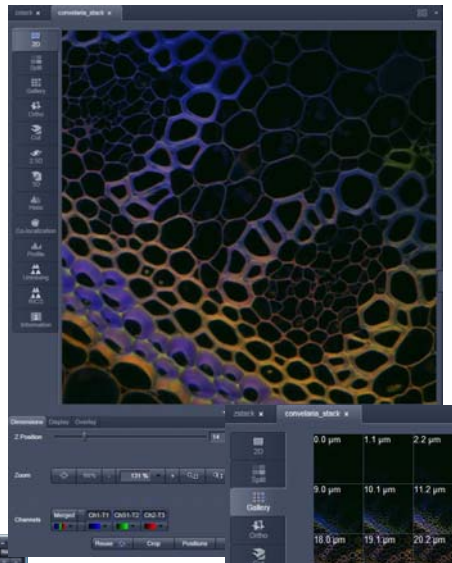
Image Display

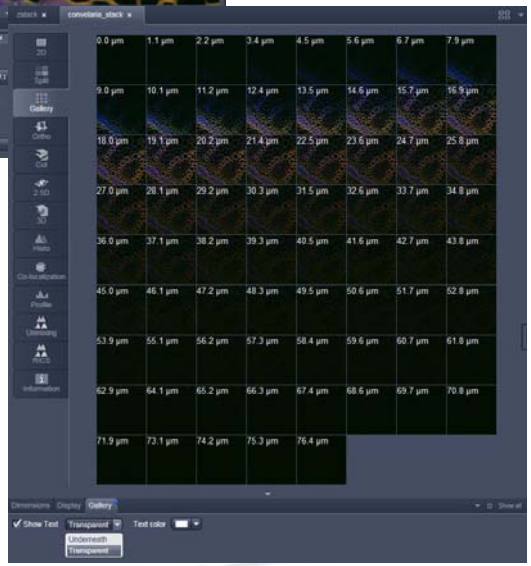
Images that are acquired are displayed in the central field of the software and their display settings can be modified.

Options for viewing your images, depending on how they are acquired include:

- 2D – x-y view of image
- Split – view multi-channel images as individual and overlay displays.
- Gallery – display of z-stack or time series images.







780 Training Notes

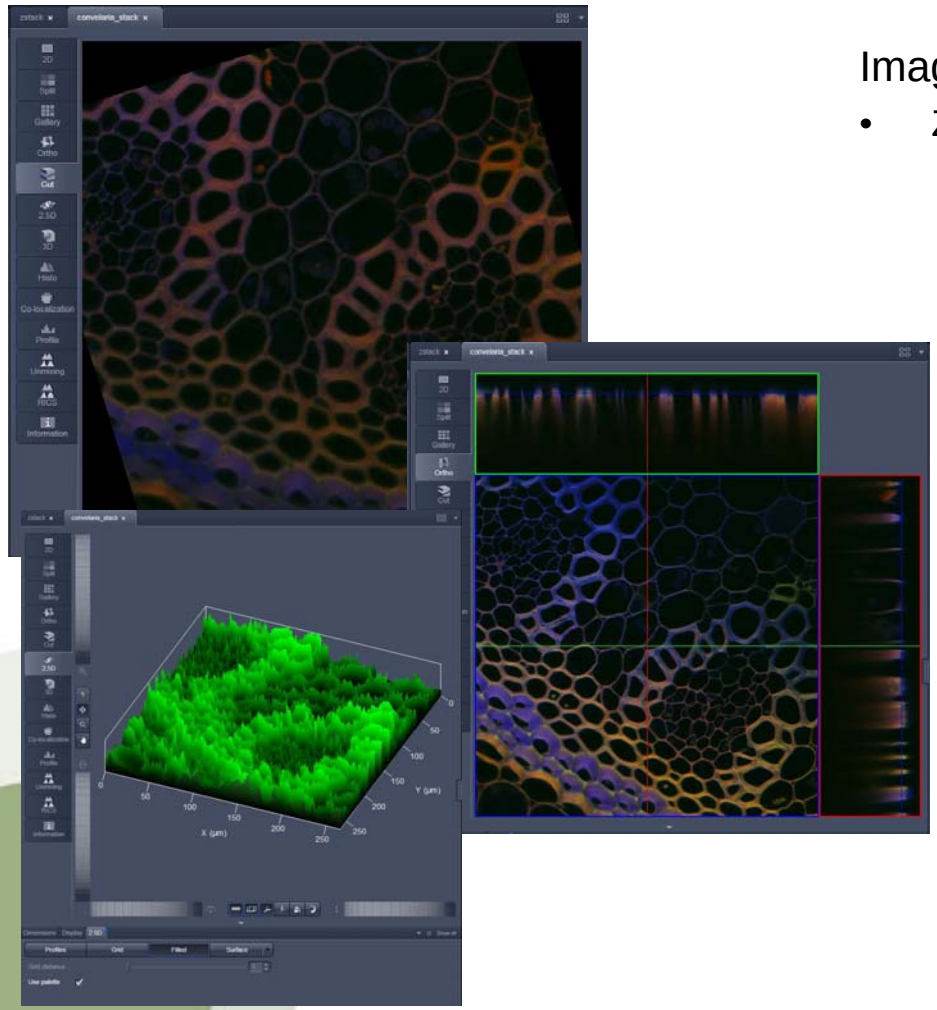
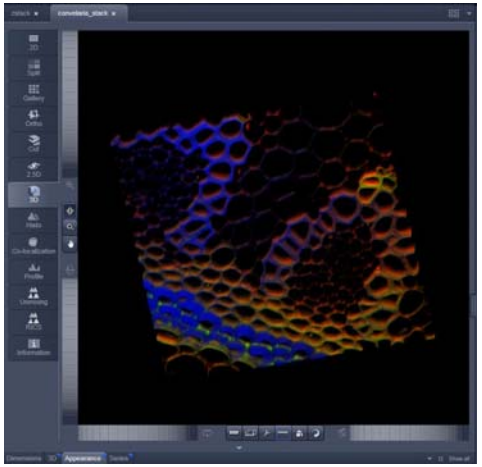


Image Display (cont.)

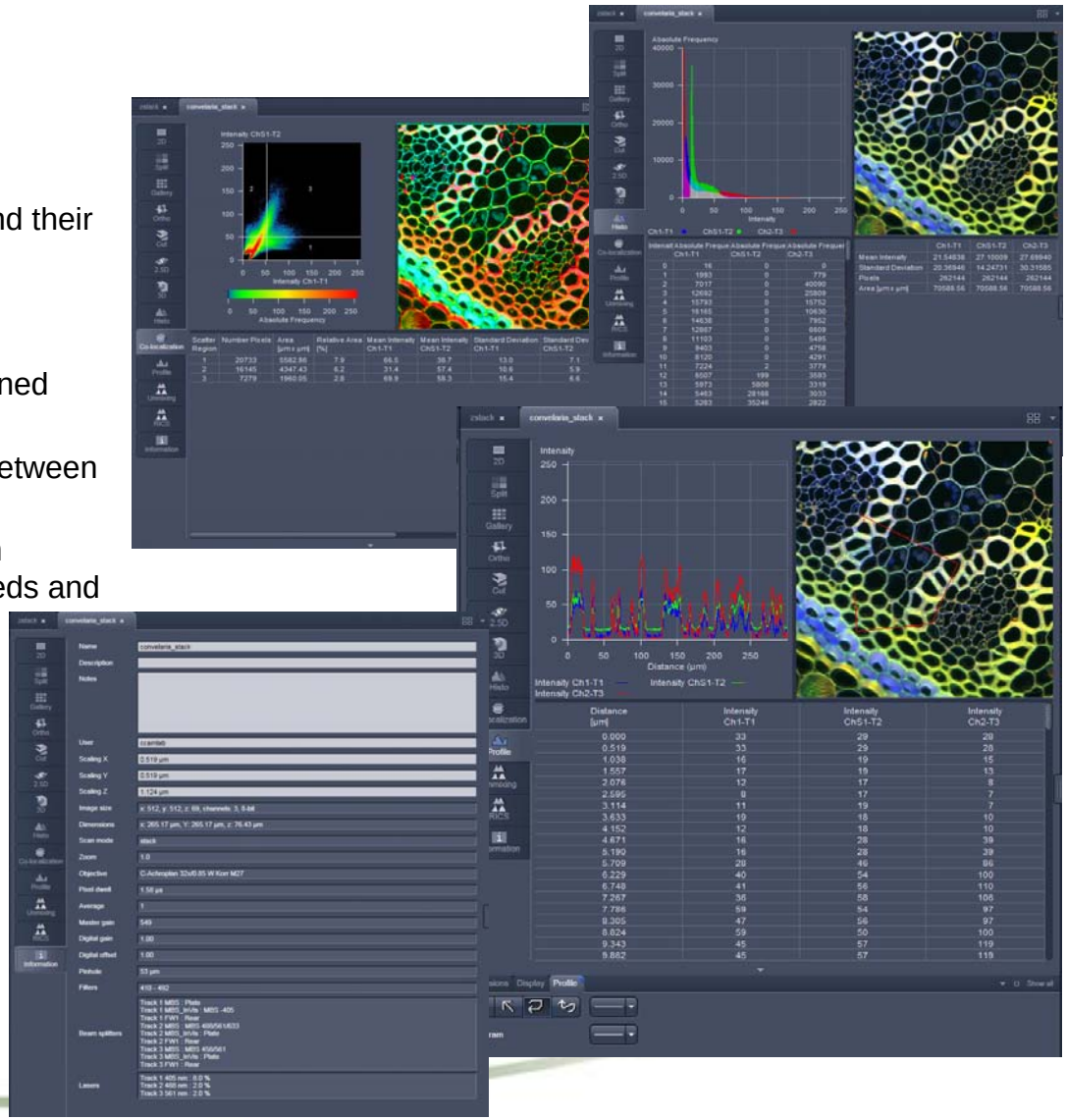
- Z-Stack Display options
  - Ortho – orthogonal display, xy, yz and xz options
  - Cut – display a particular slice of stack and have options for manipulating that slice
  - 2.5D – layered depth display
  - 3D – reconstruction of z-stack



780 Training Notes

Image Display (cont.)

- Histogram – display of image intensities and their frequencies within the image.
- Co-localization –display spatial overlap of fluorescent regions in different channels
- Profile – display pixel intensities along defined regions or lines.
- Unmixing – separate fluorescent overlap between channels
- RICS (Raster Scanning Image Correlation Spectroscopy) – determine molecular speeds and concentrations.
- Information – summary of image file.



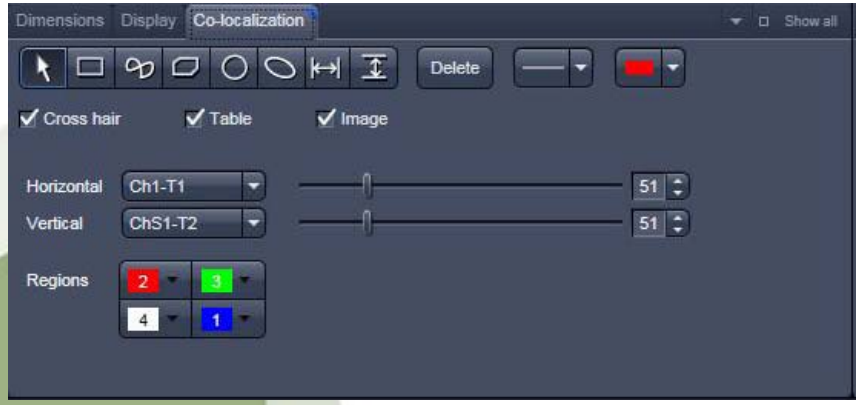


Image Display (cont.)

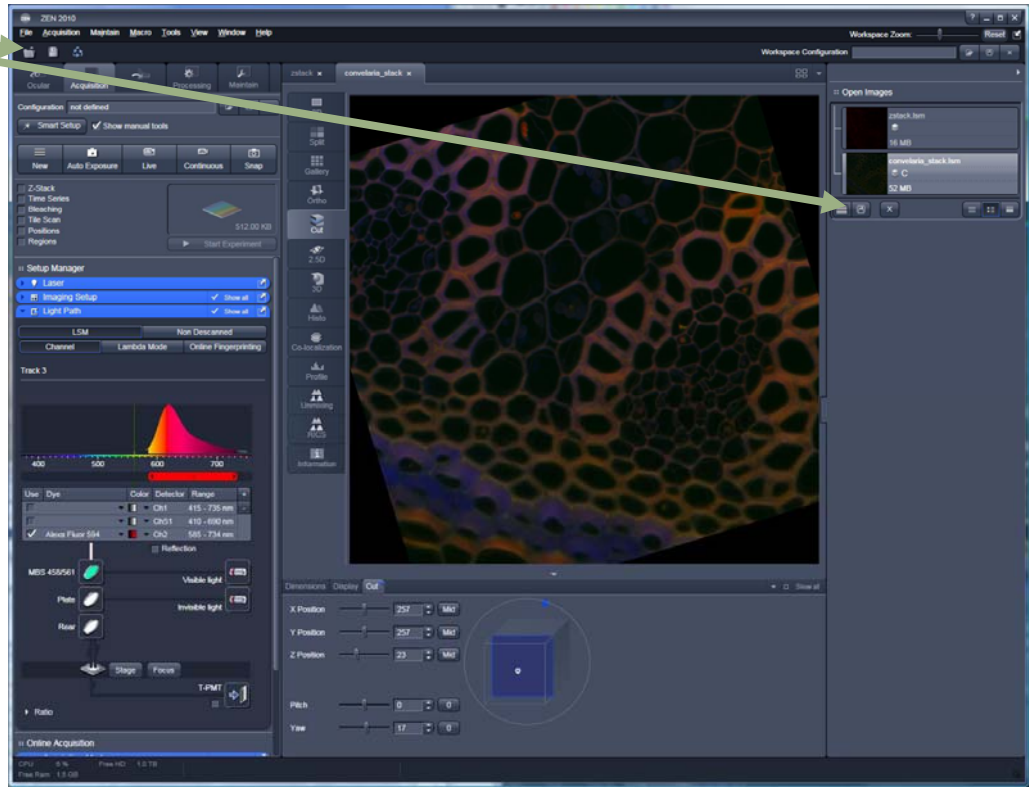
Tabs

The display options will vary depending on the image data and the advanced features being displayed.

- Dimensions
  - Z position – for stack
  - Zoom – digital zoom
  - Channels – range indicator, pseudo-colors, turn on/off channels displayed
- Display
  - Adjust screen display with brightness, contrast and gamma
- Overlay
  - Image overlays, i.e. measuring, drawing elements.

780 Training Notes

File save

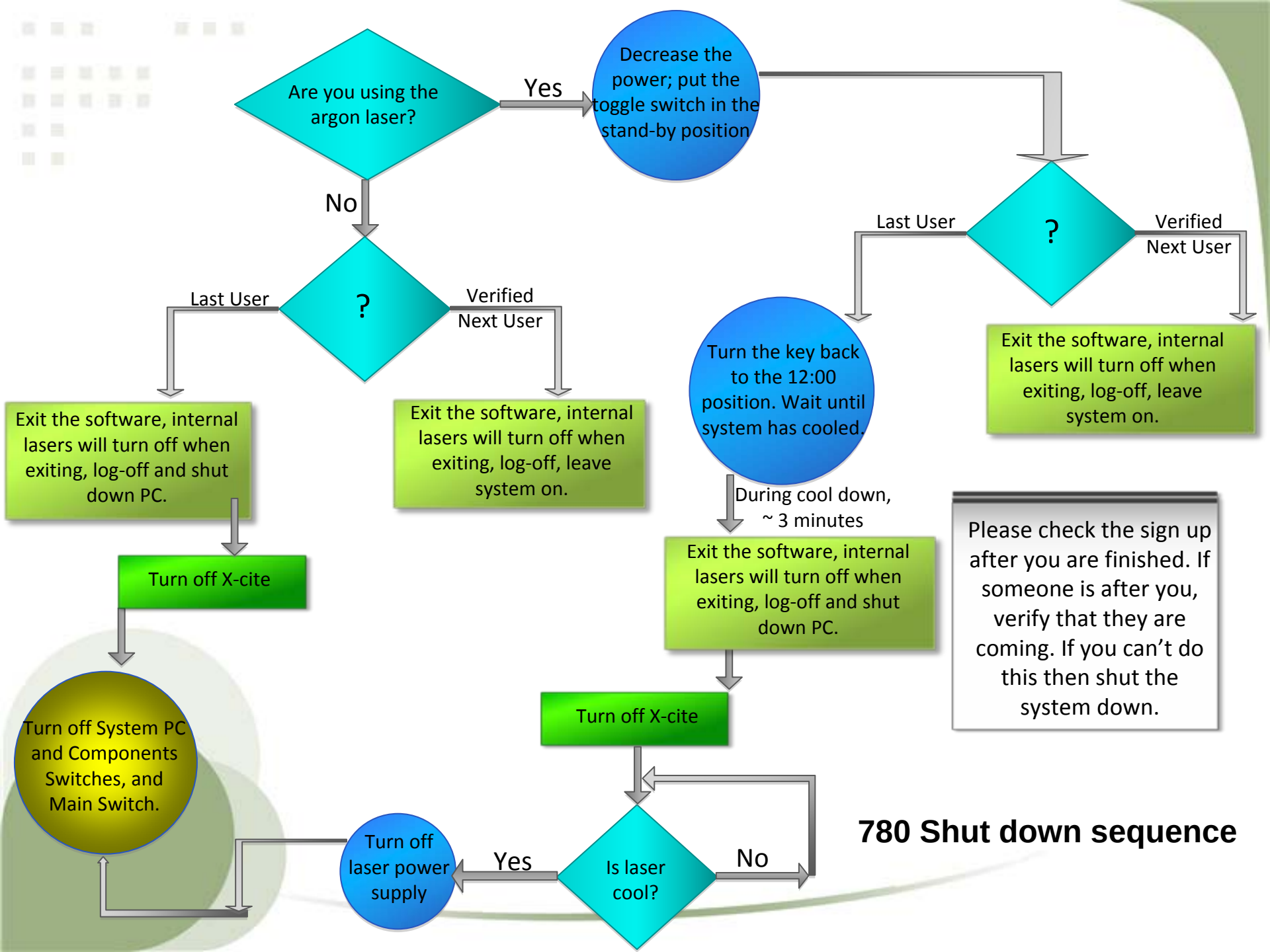


Saving Images

Images are saved as .lsm files. The files are tif files that contain the acquisition parameters stored in the header of the file. You can always export the files as different file types however it is recommended to always save the .lsm file, your raw image data file.

Images are to be saved to your lab's partition on the remote server:

\\cfs02.cam.uchc.edu\home\CAM\account_username


Are you using the argon laser?
Yes
Decrease the power; put the toggle switch in the stand-by position
No
?
Last User
Verified Next User
Exit the software, internal lasers will turn off when exiting, log-off and shut down PC.
Exit the software, internal lasers will turn off when exiting, log-off, leave system on.
Turn off X-cite
Turn off System PC and Components Switches, and Main Switch.
?
Last User
Verified Next User
Turn the key back to the 12:00 position. Wait until system has cooled.
Exit the software, internal lasers will turn off when exiting, log-off, leave system on.
During cool down, ~ 3 minutes
Exit the software, internal lasers will turn off when exiting, log-off and shut down PC.
Turn off X-cite
Is laser cool?
Yes
No
Turn off laser power supply
Please check the sign up after you are finished. If someone is after you, verify that they are coming. If you can't do this then shut the system down.
780 Shut down sequence