Welcome to the Zeiss Confocal Laser Scanning Microscope

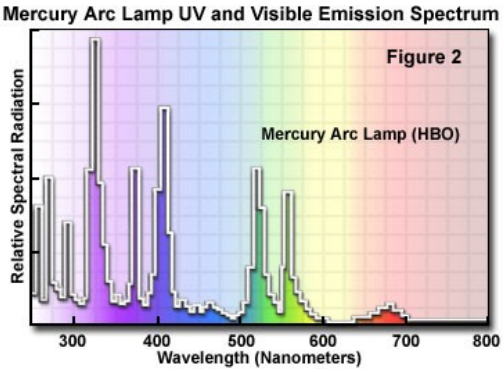
This set of instructions should help you through a scanning session and hopefully explain some of the different features of the system. This document will include descriptions of the common aspects of the system, facility rules and guidelines, as well as provide a set of step by step instructions to walk a user through a typical scanning session.
Good luck and good imaging! - Stephanie Moeckel Cole

Turning the system on

Depending upon when your session is scheduled, different parts of the system may be left on or the system may be turned completely off. The following instructions are for the system when it is turned completely off. Apply the necessary steps as needed for your session.

-Uncover the microscope, fold and store the cover.

-Turn on **Hg arc lamp** to the left of the scope and record starting hours in the logbook. (The arc lamp allows you to view fluorescence of DAPI, GFP, Fitc, Rhodamine etc. areas of the spectrum).



-Turn on the **remote control switch** on the stone table to the right of the scope. This switch powers the rest of the system: microscope, computer and the laser power supply.

Remote Control Switch

-If necessary turn on the hard drive to the computer and allow it to warm up.

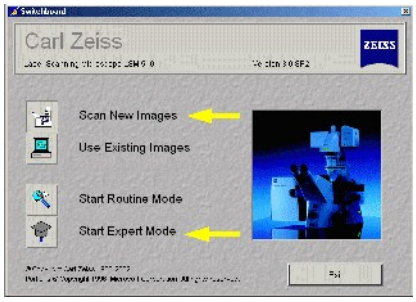
Signing into the system

All registered and trained users of the system have been given a user name and a password that allows access to the system. All users must be trained and approved for safe use by facility staff before they are allowed to use the system.

-**Ctrl/Alt/Delete** brings up the sign-in window. Enter your user name and password and log on.

-Look on the desktop for the ***LSM 510*** icon. Double click on LSM 510 icon to start the LSM software.

-A LSM window will appear, click ***scan new images*** and ***expert mode*** to start the software.

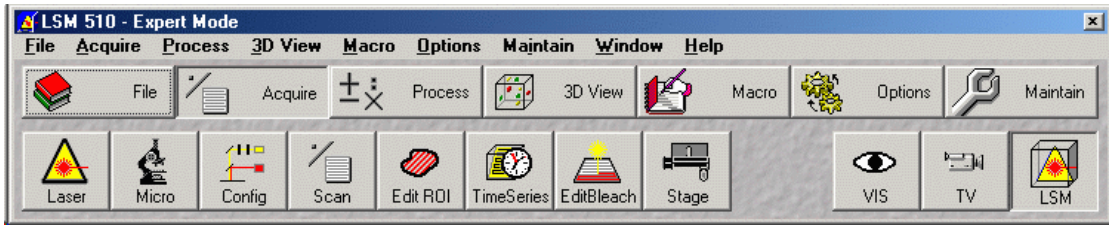


-Allow the software to initialize; this takes approx 30sec. to complete. If an error message appears, exit software, turn off computer and remote switch, wait 1 minute and then restart the computer, sign in again and restart the software. (No good reason why this occasionally happens, mainly because you have lots of data to collect!)

Main Zeiss Menu/Toolbar

This main menu/toolbar will remain open during your session; this toolbar has buttons/windows that control different aspects of the system. Some windows will be opened and used briefly and then closed while other windows will remain open during your entire session. Some windows are for operating features used during the scanning session while others are for imaging processing after images have been collected. A few windows have mainly administrative or system tools and settings and will not be opened or used during a routine scanning

session. Clicking on different windows will often bring up features on the toolbar not visible at the outset. The different buttons of the toolbar will be described briefly. The commonly used windows and their individual applications will be described separately in more depth.



<u>File</u>: The features for saving images and opening databases.

<u>Aquire</u>: The features for controlling different aspects of the system and performing scans.

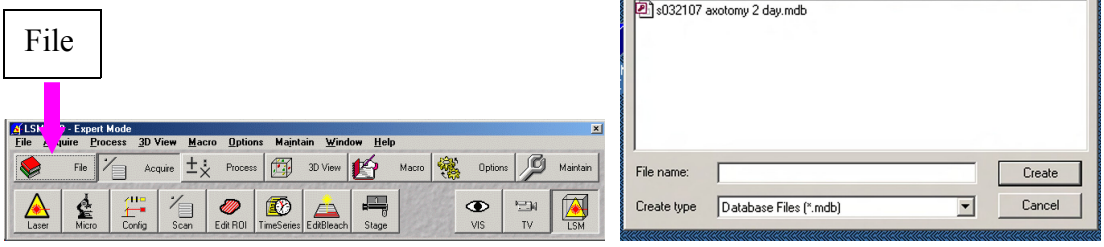
<u>Process</u>: Features for image processing after an image is collected.

<u>3D View</u>: Features for performing projections, panoramas and depth coding of an image.

<u>Macro/Options/Maintain</u>: Features for system administration, not used by the regular users of the system.

<u>File window</u>

This window allows the user to access old databases or create new ones. The zeiss system uses a database (.mdb) system to store files (.lsm). During each session, a user will generate a new database file to store images in or a previous database may be opened and used to store the images.



-A database may be created either before or after images are scanned, but must be created to save images.

-Files are stored in the ***E: / image temp*** in a folder named for the day of the week. Each user will click on the applicable days' folder and create a sub-folder with their name and the date for that day's session.

-To create a database at the beginning of a session: Select ***File window--- New File***. Select from the drop down the ***E drive-: image temp ---day of the week*** folder. Create a subfolder with your name and the date. Double click on your subfolder and create the name for your database. This is where your sessions' images will be saved as you collect them.

-Databases can also be created after an image is scanned and it is about to be saved. In the image window select ***save as---New Mdb***. Select the ***E drive: image temp*** from the drop down. Select the ***day of the week*** and create a folder with your ***name/date***. Name first the database for the session then click ***create mdb*** and then name the actual image file. When saving files always check to be sure that it goes into the proper database. A list of databases will appear each time an image is saved.

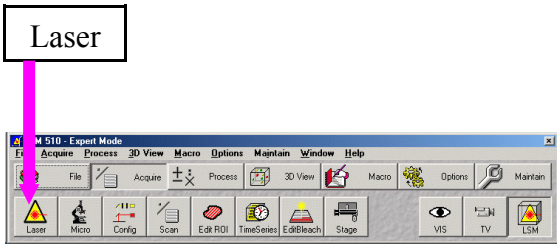
-To use settings from a particular image, open and load the desired image. In the image window look for the ***reuse*** button, click on ***reuse***. This restores most settings from that image. Gain/offset/scan speed/zoom/rotation/pinhole settings etc. will all be restored to what was used in that image. Objective used will not be restored, and must be selected.

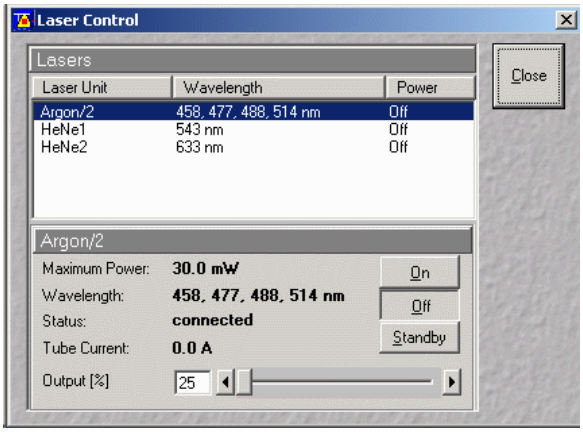
Files must be saved and removed or transferred within 7 days of generation. Files older than seven days will be deleted without notification by the facility staff.

<u>Laser Window</u>

This window is where the individual lasers to be used are selected and turned on/off. We have three lasers: Argon, HeNe1, and HeNe2. The argon laser has excitation lines at 458nm/477nm/488nm/514nm. The HeNe1 has a line at 543 nm and the HeNe2 has a line at 633nm. We do not have a UV laser on the system so DAPI type stains can only be viewed with the arc lamp and are not scan-able with the lasers.

Conversely the spectra required for the 643 laser is not visible with the arc lamp, but is scan-able with the laser.





<u>To turn on lasers</u>

- Select the ***Acquire*** option button from the main toolbar and click on the ***laser*** button.

-Select the individual lasers from the laser control window that will open (see above).
-To turn on the Argon laser: click on **Standby** then ***increase output to 5.9 -6.1 A Tube Current using the slider:*** laser will go to **Ready** automatically when used. (This used to state 50%, now the % value will be lower).

-To turn either of the HeNe lasers on, simply select the desired laser and select ***on.***

The laser control window can be closed until the end of session when it must be opened again to turn off lasers or leave the argon in standby mode. The argon laser requires approx. 1 hour to become stable before it can be turned on again, so please check to see when the next user is scheduled before turning it off.

<u>To turn lasers off</u>

- Select the ***Acquire*** option button from the main toolbar and click on the ***laser*** button.

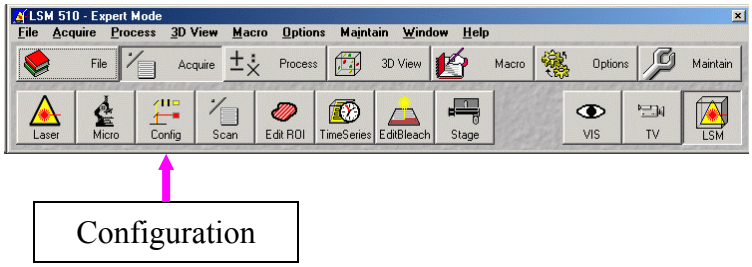
-Select the individual lasers from the list in the ***laser control*** window.

-To leave the Argon laser **on** for the next user at the end of a session, select the argon laser and ***decrease the output to 25%*** and select ***standby.*** Close the window, no such steps are required for either of the HeNe lasers and they can be left on.

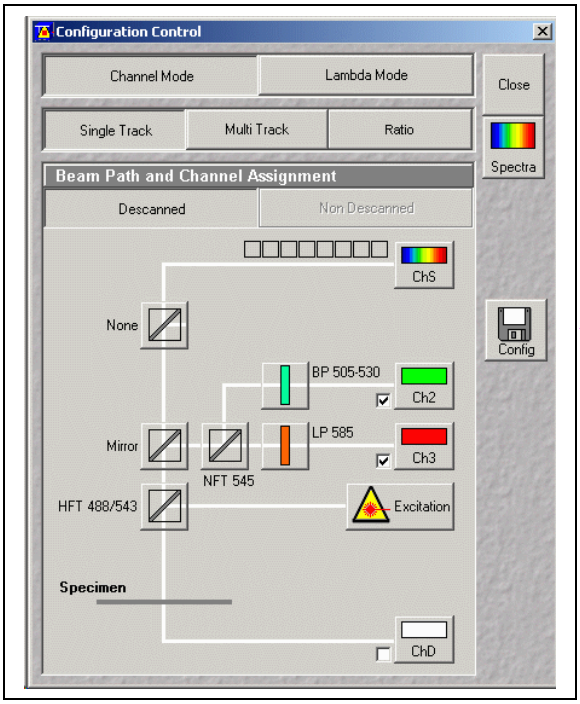
-To turn **off** the Argon laser, select the argon laser from the list and ***decrease output to 25***%, then click ***standby***, and ***off***.

-To turn either of the HeNe lasers off, simply select the desired laser from the list and select ***off.***

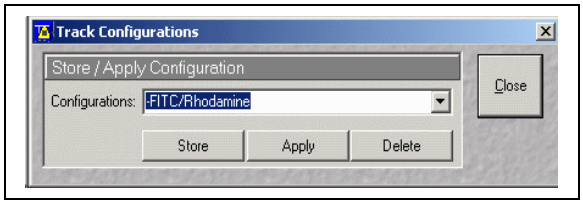
Configuration Window



This window is where different combinations of optical components (filters, mirrors and beam-splitters etc) are selected to configure the light path. There are many preset configurations available for different combinations of probes. Some of the default settings in the window will be left as is and some aspects will be changed by the user.



Small Configuration Window



Collection of signal from one or more of the basic fluorescent probes (GFP, FITC, Rhodamine, CY5 etc):

-Select ***Acquire*** from the main toolbar

-Select the ***config*** button from the main toolbar menu; this opens the separate configuration window.

-Default setting of ***channel mode*** is to remain. Select either the ***single track*** or the ***multi track*** options.

-Choose ***single-track*** (all lasers scan in one track and at the same time) if you have only one probe or if your multiple probes are well separated spectrally.

-Choose ***multi-track*** (lasers scan in separate tracks and sequentially) if you have multiple probes with the potential for crosstalk between signals.

-Choose the small ***config*** button on the right side (see arrow in figure above) of the configuration window; this will open a separate smaller drop-down type window. From the drop-down list choose the preset option that best suits your needs. Presets are named for the probes (for example EGFP, FTIC, FITC/RHOD, CY5/FITC etc). Select the desired configuration and click on ***apply***. This will change the optical components in the larger window to reflect the path you have selected. Close the window.

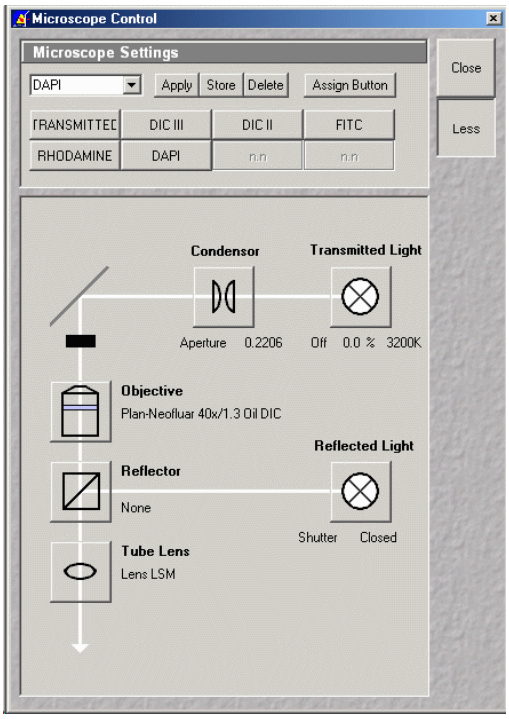
-The remaining default settings are all applicable for scanning with the more commonly used probes. For different probes or for special applications please see the facility staff for assistance in choosing the proper configuration paths or for further explanation of the individual aspects of the configuration window.

-To collect a bright field channel along with the fluorescent channels, simply check the box next to the ***channel D*** in the large configuration window.

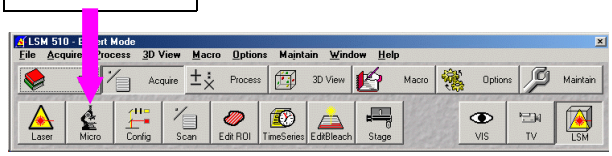
The configuration window can be closed for the remainder of the session unless a different setup is needed during the session in which case the window can be reopened and the previously described steps repeated to select the new configuration.

Microscope Window

This window contains the computer based controls for the microscope stand. Manual features on the microscope stand can also be controlled by accessing the buttons in this window. This window does not have to be opened if the user prefers to utilize the manual controls on the microscope stand itself.



Microscope



-Select ***Acquire*** from the main toolbar.

-Select the ***micro*** button from the main toolbar menu.

-Individual microscope features can be controlled either via the drop down selections as well as within the schematic of the window; and/or by manual buttons on the scope stand itself

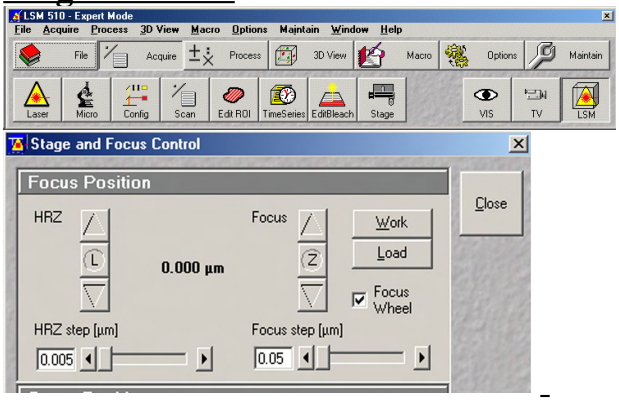
Reflector switches the reflectors on the scope allowing the light from the arc lamp to be attenuated. Reflector choices are Fitc, Rhod, and DAPI. There are two empty reflector slots in the circle. The manual controls for this feature are found on the bottom right side of the microscope stand behind the focus knob.

Objective allows for the user to select which objective they want to use. Objective choices are ***10x*** (dry), ***25x*** (water, oil and glycerin options on the correction collar on the objective), ***40x*** (oil) and ***63x*** (oil). The manual controls for this feature are found on the right side of the microscope stand behind the focus knob above the buttons for the reflector controls.

Halogen displays the % of halogen light illuminating the specimen. The manual on/off switch for this feature is on the right side of the stand below the florescence control button. The manual attenuation slider for the halogen is on the front of the microscope stand.

Fluorescence controls the on/off of the fluorescent light. Specimens can be viewed with either halogen or fluorescent illumination or with both light sources as desired by the user.

Stage Window



Stage

This window contains the software based control of the stage. This window will remain open throughout the session and be used repeatedly during a session. The controls in this window are used to focus through a sample while scanning with the lasers and to set the starting and ending points of a scan.

-Click on ***acquire*** in the main toolbar and then on the ***stage*** button.

-The stage is computer controlled by clicking the ***up/down arrow*** buttons in the small window.

-The amount that the stage moves is determined by the ***focus step.*** The focus step is not the same as a ***Z interval/step*** (which is in the z settings section of the scan window). The ***focus step*** is the user determined distance that the

stage moves per click of the up or down arrows while focusing through a sample; while the ***Z step/interval*** is the distance between stack slices during image acquisition.
-While in continuous scanning mode, click on either the up or down arrows to move up or down through the sample. You can use either the ***x/y continuous*** scanning mode or the ***fast x/y*** scanning mode**.**

-To zero out the stage readings, click on the ***Z*** button (between the up and down arrows). The micro-fine (***Hz***) stage control buttons (on the left of the window) is not an option that we own, so it is not enabled.

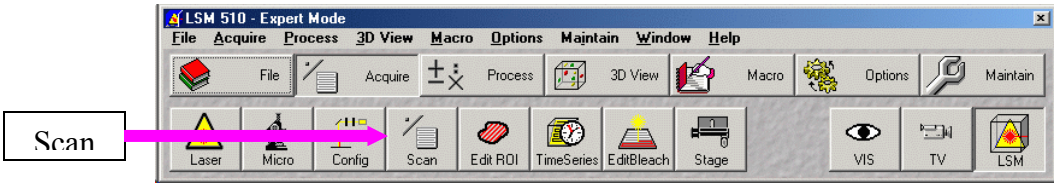
-The instruction steps for setting the start and stop points for an image acquisition scan will be explained under the ***z settings*** window description; but will use this ***stage window.***

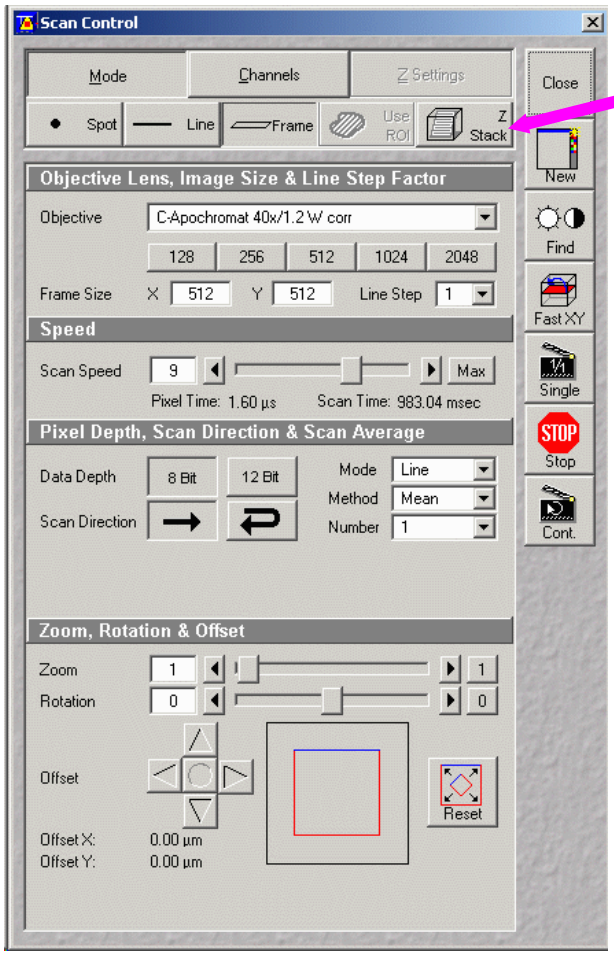
Scan Control Window

This window contains the bulk of the controls that are used during a scanning session. This window is where most image specifications: physical (scanning speed, pixel size, data depth etc), light parameters for individual channels (pinhole size, gain and offset levels), and z specifications (start, stop, interval, slice # etc) are determined by the user and specified prior to actual image acquisition. This window will be opened by the user and remain open during a session and the user will cycle back and forth through the various menu options as they setup and complete individual scans during a session.

-The three main buttons/windows (**mode, channels, and Z settings**) across the top will each be described separately and individually. Each of those buttons opens a different view of the scan window with the pertinent controls.

-The buttons on right hand side of the window are a series of buttons that control the type of scan performed (***new, find, fast xy, single/start, stop, and cont)*** will be open at the startup.





Z stack Button

New: opens a new window.

Find: looks for the brightest object in a plane of focus and sets initial light levels (explained further below).

Fast XY: a continuous scanning mode at highest scanning speed and full frame, useful only when focusing through a sample.

Single/Start: starts an official scan at user set determinants (intensity levels

Stop: stops any scanning mode.

Cont.: Continuous scanning mode

-Clicking on the ***z stack*** button near the top of the scan window (red arrow in picture above) will bring up more buttons on the right hand side of the window: ***single xy, xy continuous***, ***line select*** and ***range)***

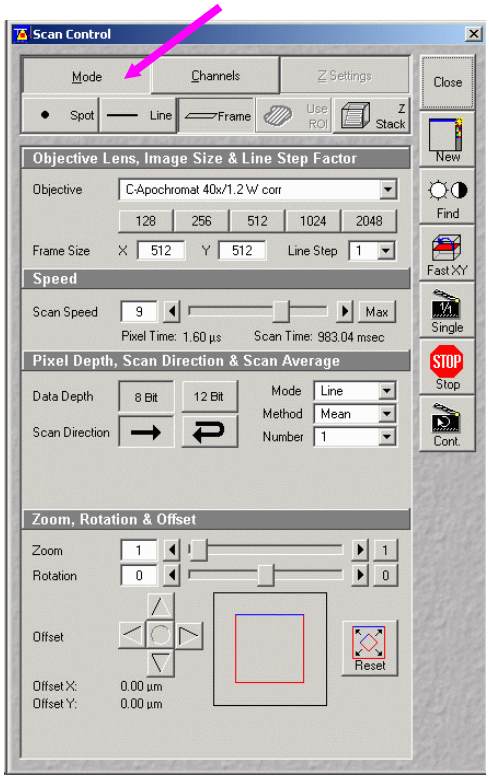
XY Scan: a single scan through the current plane of focus.

XY Continuous: a continuous scanning mode at the current user determined levels (scan speed, crop/zoom, frame size, offset and light intensity levels). This is the scanning mode used to set the light intensity levels (gain/offset) etc) prior to image acquisition.

Line Select: used to define a scan line for the line scanning mode. This control can define either a straight cutline or a free shape curve cutline.

***Range*:** displays the range of the XZ series, allows the user to set the range of the Z series and to change the focus by using the XZ scan view in the image window.

Mode



This button/window opens a view of the scan window where the different physical parameters of an image/scan are set. Some default settings will be left as is, while other aspects and features will be selected and changed by the user. Aspects that will be routinely changed are described individually.

Frame size:

-Number of pixels x pixels of a square frame ex. 512 x 512

-Larger numbers = smaller pixels and better resolution

-Select the number of pixels that best suits your sample.

-***Optimum*** gives a non-standard number of pixels ex 824 x 824, (can be problematic when comparing different images, better to stick with the standard frame sizes).

- The frame does not have to be a square frame; by using the crop tool on the image window or by typing in a number (ex 225 x 512) will change the frame to a rectangle, but will not alter the zoom as long as one set of parallel sides remains the original frame value.

Data Depth:

8 bit = 2^8 possible grey scale values of a pixel (0-255)

12 bit = 2^{12} possible grey scale values of a pixel (0-4095)

-Choose the depth that you need. For some of the more advanced image processing or analysis the larger data depth is needed, but files will be **very** large so use the larger depth only if really needed.

Direction:

This button indicates that the lasers will scan only in one direction and is the default setting.

This button indicates that the lasers will also scan on the reverse as well. Selecting this option will cut down the amount of time it takes to scan, but corrections (***interpolation)*** must be made so that the edges of the frame are not scanned twice.

Mode, Line, Number

This control allows for the removal of noise from a scanning frame. **Through a series of algorithms, pixels along a line are summed and averaged a number of specified times which removes random noise from the image.**

-Line and Mode will remain the in the default settings, but the number will be changed from 1 to a desired number by the user repeatedly on/off during a session.

-The desired ***number*** of times this operation is performed is selected from the drop down (1, 2, 4, 6 etc).

-It is advised to have the ***number*** at one when focusing or making adjustments to light levels so that the changes will be more quickly reflected

in the image. Right before a scanned image is to be collected, it is advised to then select the desired number and start the official scan.

Zoom, Rotation, Offset

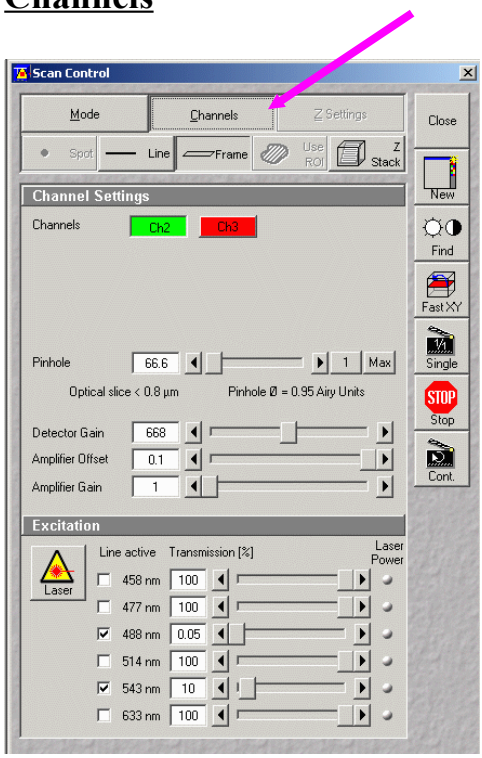
These controls allow the user to manipulate a frame prior to scanning. From simple magnification changes to centering an object within a frame this region of the scan control window has controls to allow image manipulations before scanning of the frame. Features can be combined with the crop button functions from the image window itself.

-***Zoom*** increases the magnification of the frame while maintaining the frame size/resolution. Zoom values can be entered manually or by using a slider. It is possible to go down to a .7x zoom with this tool.

-***Rotation*** allows the frame to be rotated, allowing the alignment of the desired image to be optimized by the user (ex: object always entering the frame from the left to the right).

-***up/down/left/right*** arrows allow for the frame to be moved in the x/y directions allowing centering of the object within the frame.

Channels

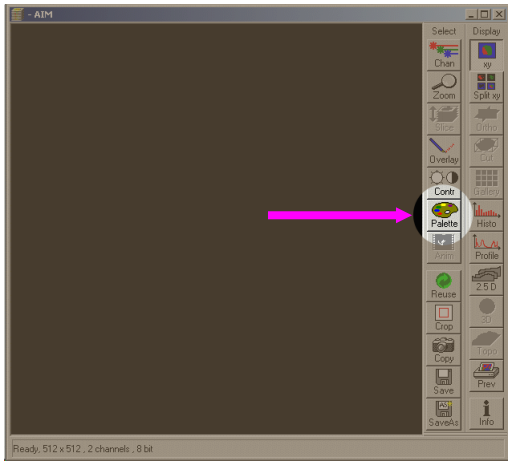


This button opens the channel section of the scan control window. This is where the light parameters will be set prior to imaging. Depending upon what configuration is chosen, the different channels will appear at the top of the window. There will be a small color coded button for each channel. The pinhole settings for each channel will be set here and the gain/offset for each channel adjusted individually here as well.

-Select a channel (usually green/red/white/blue boxes labeled with channel 2, channel 3, etc.) and set the pinhole to 1 (**click on 1**); repeat this step for each channel. The bright field channel (white) will not have a pinhole setting.

-After locating the desired area on the sample to image, click on the ***Find*** button found along the side of the scan control window. This will open a separate image window. The ***Find*** feature causes the scope to set the gain/offset on the brightest signal in the plane of focus. This allows the computer to preset the gain/offset levels for each channel and prevents damage to the detector. Sometimes ***Find*** does not work find all channels and those can be then set manually. The levels from ***Find*** are not always ideal and will most likely be further optimized by the user. Find is generally only used once at the beginning of a session. Intensity levels may have to be tweaked between samples, but repeating find before each scan is an unnecessary step.

-To optimize the signal after the ***Find*** feature has been performed and/or to set the levels manually, click on the ***palette*** button in the image window (the red arrow in the picture below) and select ***range indicator***. The ***palette-range indicator*** tool will display the image in grey scale and assign a red or blue color to saturated pixels. ***Red*** = white saturated pixels while ***Blue*** = the black saturated pixels. While scanning continuously, adjust the ***gain and offset*** for each channel. ***Gain*** = the white levels or brightness, ***Offset*** = the black levels or background. Try to get as much signal as possible while avoiding saturation. To turn this tool off, select ***palette*** in the image window again, and then select ***no palette.***



Hint:
To remove red---turn down the gain!

To remove blue--turn up the offset!

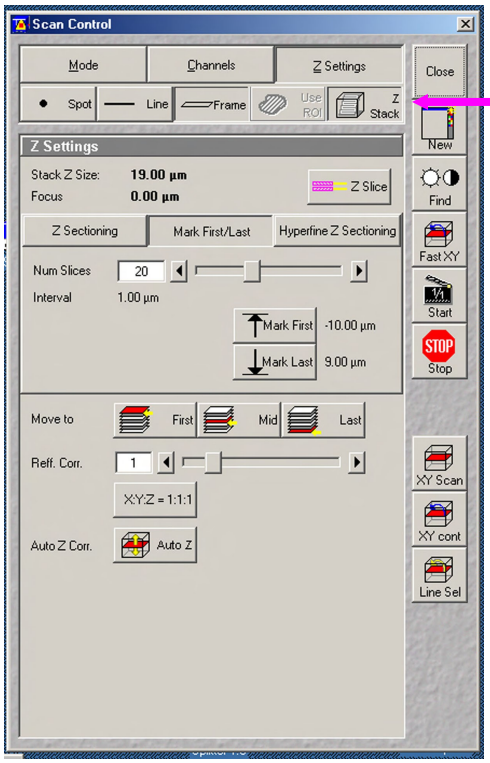
-Leaving the ***palette-range indicator tool*** on while collecting an image will not make any difference in the colors in the final image; and is advisable to leave on while focusing through a sample to avoid being surprised by a sudden increase in signal while in the middle of collecting a scan.

-Try to set your intensity levels in the brightest plane of focus in the sample and in a part that you do not care about in case your prep is sensitive to photo bleaching.

-When switching to a different objective the user will have to reset the pinhole settings to 1 airy unit. The optimal pinhole size is partly determined by the numerical aperture (NA) of an objective lens, and different objectives have different value NAs.

-If you use the ***fast XY*** scanning option, the image is scanned at the highest speed with a full frame size; this will affect the appearance of the image temporarily. When the official scan is started the image should be at the user determined settings.

Z Settings



This section of the scan control window controls the z information about your image. The number of slices, the interval between slices, and the beginning and ending points of an image scan are all controlled via this window. To enable this feature, click on the *Z stack* button.

-Two main options: ***Z sectioning*** and ***Mark First and Last.*** Either of these options can be used to generate a stack of images. The ***hyperfine Z sectioning*** button is not an enabled feature on this system and is grayed out.

Z sectioning: Allows the user to determine (n) number of slices with (n) interval in between the slices with the starting slice specified by the user.

-The system will scan for (n) slices until all specified slices are done, starting at the current focus position slice or at a slice specified by a number.

-The user will focus using the ***stage control window*** to a desired starting point and then specify how many slices are desired from that point onwards.

Mark First and Last:

Allows the user to specify the range to be scanned, starting and ending at a user determined point.

-Using the up and down arrows in the ***stage control window***, while scanning continuously, focus down through a sample until the desired starting point is reached and then click ***mark first*** in the z settings window, this will set the beginning point of the scan. Then focus through in the opposite direction until the desired ending point is reached and then click on ***mark last,*** this will set the ending point of the scan***.*** Unless ***mark first or last*** is clicked again, the specified range will remain intact. If needed, the image may be adjusted as to orientation or cropped, or the light intensity levels adjusted within the channels window. Neither will affect the selected range when the scan is officially started.

-The ***Z interval*** that is used will determine how many slices are taken through the specified range. A larger z interval will result in a smaller number of slices as the range is being divided by a larger number.

-Move to: ***First/ Mid/ Last*** will scan the image either at the fist slice of the range/ middle slice of the range/ or the ending slice of the range.

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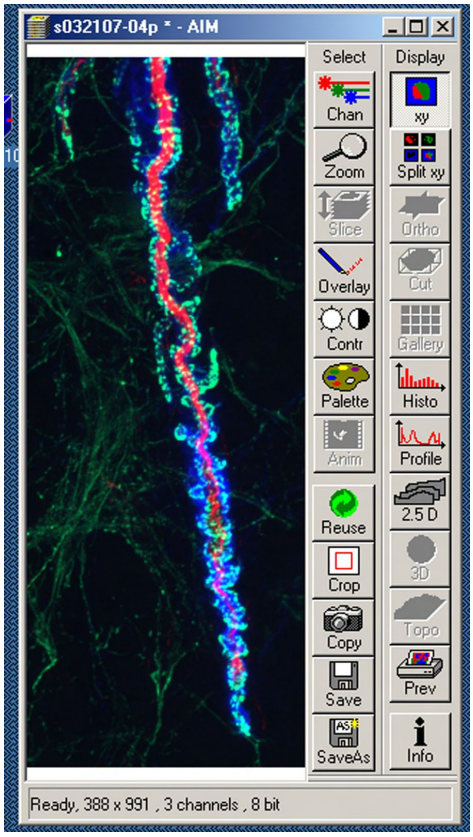
Image Window



When any scan is performed (Find / XY single / XY continuous / Fast XY, and or Start) it will open a separate image window. This window will have an image displayed initially in the merged (XY) view with all the channels displayed together. The image window contains the button controls for changing the display of the image (XY, split XY, gallery) as well as options and tools for analyzing the image.

Channels: allows the user to select different colors that the channels will be displayed in, standard preset options: green, red, white, blue; are available or custom colors may be selected. The color choice does not really matter, as images are ultimately recorded in the computer as grey scale and colors are added secondly.

XY: Displays the image in the merge view with all the channels combined together.

Zoom: Opens a series of buttons that allows the user to zoom in or out of an image.

Split XY: Displays the image in the split view with a separate section for each channel. For example you are scanning a sample labeled with FITC and Rhodamine. The ***Split XY*** display will have three views: FITC alone, Rhodamine alone, and the combined merge. If only one label is used then ***XY*** and ***Split XY*** display will look the same.

Slice: Displays a slider bar to move through the slices in an image stack.

Ortho: Displays the image in an orthogonal display mode with movable lines that select the XZ and the YZ views of the image stack to be displayed on the sides.

Overlay: Has drawing element tools for creating scale bars, arrows and text on the image as well as options for selecting and extracting regions of the image and opening them as separate image windows. The blue wastebasket button will remove drawing elements that have been added.

Cut: Provides options to manipulate and section the image in the different planes.

Contr: Allows the brightness and contrast to be changed for the individual channels separately, as well as all the channels together.

Gallery: Displays the image as slices. The total number of slices will be displayed, slices that have been scanned will show an image, and those that are not yet scanned will be black. The progress of the scan can be observed from this display option.

Switching back and forth between these different display views is possible while scanning and will not affect the image. Sometimes the scan may need to "catch up" to the new view, so be patient. Options that are not allowed while scanning will be grayed out and unavailable for use.

Palette: This tool allows for different ways of viewing the image.

-***No Palette***: a normal display without anything added

-***Range Indicator:*** displays the image in gray scale and assigns a red or a blue value to saturated pixels.

-***Rainbow and Glow scale***: Displays the image with funky colors added to sections of the image. (Seriously though, there is a reason and if you are interested there are usable data contained within the cool colors!)

Histo: Displays the image in conjunction with a histogram for each channel that shows the signal intensity in graphical form.

Anim: Allows for the control of animation schemes (time lapse or panoramic projections).

Profile: Provides tools for the definition of a user selected line and the signal intensity measurements along that line.

Reuse: Reuses the settings from that image and makes changes in the scan control window for the next image.

2.5D: An option that displays the image in a 2D graphical representation of the signal of the image.

Crop: Allows the image to be cropped, available only in the XY view.

3D: Feature not enabled

Copy: Copies the image to the clipboard

Topo: Feature not enabled

Save/Save as: Saving options for the image

Preview: Shows the current printing setup of the image

Info: Provides all the information about the image.

For more detailed explanations or for specific higher applications please consult the facility staff for clarification. Following are step by step scan instructions as well as facility rules and guidelines.

Basic Scanning Instructions

-Turn on arc lamp (located to the left of the microscope stand)

-Turn on system via remote control button (located on the table on the right of the scope)

-Start LSM software-Scan new images-Expert mode

-Main Zeiss Toolbar
- ***Acquire***

-***Laser***: Open the laser window and select appropriate laser(s) and turn on.

-***Config***: Open the configuration window
-Select either single or multitrack scanning options
-Click on small config button
-choose a configuration from the dropdown window and click apply.
-For bright field collection-check channel D in the big window.
-Close the window

Scan: Open the scan window by clicking on scan in the main toolbar
-***Mode***: -Choose objective, frame size.
-***Channels***: -Select each channel and adjust pinhole
-Click on 1 to set the pinhole to 1 airy unit.

-Apply oil if necessary to objective or slide (oil kept in small countertop incubator)

-Place sample on stage, cover slip down

-Click on ***VIS*** (eyeball button in main toolbar)
-Turn on either the fluorescence or halogen light to view your sample (buttons on scope or use micro control window)

-While looking through the eyepieces, focus on the sample.

-Turning the focus knob away from you brings objective up to the sample, turning knob towards you lowers objective down away from the sample.

-Turn off either the fluorescence or halogen light.

-Click on ***Find*** or ***XY scan*** in the scan window to bring up an image window.

-Click on Channels in top of scan window

-Use ***split XY*** to separate the image window view to view each channel separately.

-Adjust Gain/offset for each channel

-Use the ***Palette*** tool in the image window to help you adjust your light intensity levels.

-Click on ***XY cont.*** to scan continuously…

-Focus up/down, using the stage control window, through the sample to find brightest plane.

-Increase/decrease the ***Gain/Offset*** values until desired image quality is reached.

-To remove red (white saturated) turn down gain

-To remove blue (black saturated) turn up offset

-Switch the image display view back to ***XY*** and adjust image orientation as desired using the crop tool

<u>Setting the Z Setting range for the scan</u>

-While scanning continuously (***fast XY*** or ***XY cont***.) set start/stop by focusing to the starting point and click on ***mark first*** (in the ***scan window-Z settings)***, then focus in the opposite direction to the ending point and ***mark last***.

-Select the desired z interval under ***interval*** in Z settings.

-Stop the scan

-Select the number of times to ***average*** from the drop down window in ***mode*** of the scan window

-Start the acquisition scan with the ***start*** button in scan window.

-Save the image in a new or old database under the appropriate day of the week folder in the ***E Drive: Temp Data***.

Projections of stacks

-To make a simple projection of an image, select ***3D view*** from the main toolbar and select ***projection***.

-In the projection window that will open up select the ***X or Y*** option and enter the following information in the boxes:

0 = ***first angle***
1= ***# of projections***
0 = ***difference angle***

-Make sure the desired image is displayed in the projection window and click apply. The projection will open as a new image and must be saved.

-Panorama images are also easily created. Select ***panorama*** and the desired **number of projections.** The degree of complexity is controlled by the difference angle and the number of projections. Experiment, the results are usually fun, and as long as your original stack image is saved nothing will be harmed!

Ending a Session: Check online to see if anyone is scheduled after you. Whether or not to leave the system on (in standby) was previously a complicated decision because some people cancelled their reservation late, some finished early, or others came late for their appointment; all of these issues left the possibility of having the system left on, but unused, for extended periods. The choice is now simple - **If** the next user is not standing there looking at you, shut it down. **If** they contacted you and told you to leave it on, write this in the logbook, leave it in standby (see below) for them, and they are responsible for all usage time (at regular rates) from your logoff until they finish and log off (or shut down). **If** you leave it on when you shouldn't, YOU are responsible for all run time at regular rates; be sure you pay attention to these rules. Be punctual for your reserved time and communicate with other users if you need the system left ON.

- **The Mercury Arc lamp needs less than 5 min to reach operating condition, and approx 15 min. to cool down before restarting.**
- **The HeNe lasers are solid state and are ready withing a minute and do not require cool down period; they may be switched as needed.**
- **Argon laser is a gas laser and needs a few minutes to become "Ready" at startup and about 5min to cool down - until the fan stops.**
- **All lasers have optical feedback to stabilize output power and do not require extensive warmup for normal work; they are ready when they say "Ready". Only for the most exacting measurements on 12-bit data might a longer warmup possibly be useful, but it is much better to have internal brightness standards with your sample than to depend on laser stability.**

To leave the system in standby (when appropriate!):

- Select the ***Argon laser*** from the laser panel (Laser button in the main toolbar) and ***decrease power to 25%*** and click ***standby.***
- The HeNe lasers may be left on or off depending upon time before next user: OFF if it will be more than 15min.
- The software windows may be exited and the remote control switch left ***ON***.
- The arc lamp is turned OFF unless the next user is present or has contacted you.
- Clean objectives, cover the microscope.

- Record your usage in the log book.

To shut down the system:

- Select the ***Argon laser*** from the laser button in the main toolbar and then click ***off.***
- Select the other lasers and choose off.
- The software windows may be then be exited. A warning message will appear asking the user to wait for the fan to shut off.
- Cooling takes approx. 5 minutes so archive data and clean objectives while waiting.
- Once the fan shuts off the computer may be shut down and the system may be completely turned off via the remote control switch.
- Turn off the arc lamp.
- Cover microscope and store oil in the incubator.
- Sign out of logbook.

Microscopy Facility Policies

Any use of any system must be logged in the logbooks with the user's "project#" and the user's name (not the Lab, or PI name).

ZeissPC ("Meta")

This computer is not a place to store your data longterm, there are too many users, it would fill up, and failures can happen at any time!

Files will not be deleted for seven (7) days only, no exceptions.
Files may be saved to the following area only:
E-drive in the appropriate "Day of Week" folders. Create an "mdb" folder with your name and date - store your files there.
Files found in other locations (like the C: drive) will be removed.
Talk to the staff if you have trouble with archiving your data.

Exception:
Each active user is permitted a personal “Keeper” folder on the Data-drive to hold sample images, settings or notes. This folder may be no more than 50MB per person, and should be clearly identifiable with your name.
Folders larger than 50MB will be deleted without notice!

Data files may be archived by burning a CD, using a USB flash drive or portable HDD, transferring to our server IMGPC5, your UDRIVE space, or other network storage.

The Zeiss computer (META) should not be used for "computer use only"; use "IMGPC5" (Room 1) next door; It has a full licensed copy of the Zeiss AIM software we purchased for this purpose. All use of the image collection workstation is billable. *The system log files are periodically audited for logbook compliance.*

IMGPC5

- **IMGPC5 is not another black hole for your files.**
- **Files are safe here for seven (7) days only too.**
- IMGPC5 should always be ON - the monitor power button is on top of monitor; Do not shutdown - just Log Off and turn off monitor. Files copied from the Zeiss system to IMGPC5 can be retrieved later without having to power on the Zeiss confocal workstation.
- To transfer files from the ZeissPC to IMGPC5: My Network Places | Computers Near Me | IMGPC5 (or "ImageTemp on IMGPC5" shortcut on some desktops.....)
- (username and password for login will be provided)
- Use/create a folder with your name (recognizable) in the ImageTemp folder.
- People actively working on deconvolution or other projects on Imagepc5 may keep current work files here; please clean up when you finish.

Reservations.

- Instrument reservation information and link is on our webpage at http://www.bio.umass.edu/microscopy/
- Please read and know the reservation policies. They are meant to be fair.
- Users of the confocal system will be given login information.
- Reserve time you expect to actually use.
- Be punctual for your reserved time and communicate with other users; with the arc lamp and laser (leave on? turn off?) issues, we can all work more effectively if we treat this responsibly and communicate.
- Remember that you are required to leave contact info with your reservation for it to be valid. Reservations with no contact info are not valid.