



# LSM 510META Quick Guide

for systems in  
742A Light Hall and 4155 MRB 3

RECORD YOUR USE IN THE LOG BOOK

FOLLOW THIS GUIDE STEP-BY-STEP  
UNTIL YOU ARE FAMILIAR WITH  
THE SYSTEM OPERATION.



## I. STARTING THE LSM510

**If the system  
main power and  
Hg lamp are  
OFF, start here:**

1. Turn on the arc lamp power supply to the left of the microscope (742A) or below the microscope (4155.) (The power supply should be cool; if not, wait about 15 minutes.)

**If the system  
main power and  
Hg lamp are  
ON, start here:**

2. Using the main power switch next to the arc lamp power supply (742A) or on the shelf above the monitors (4155) turn on the microscope, scanner and computer system. Continue to Step 3.



Arc lamp power supply



Main power switch

3. Enter your VUnetID and password in the log-in window.

4. A black DOS window will open. Allow it to process and close on its own.

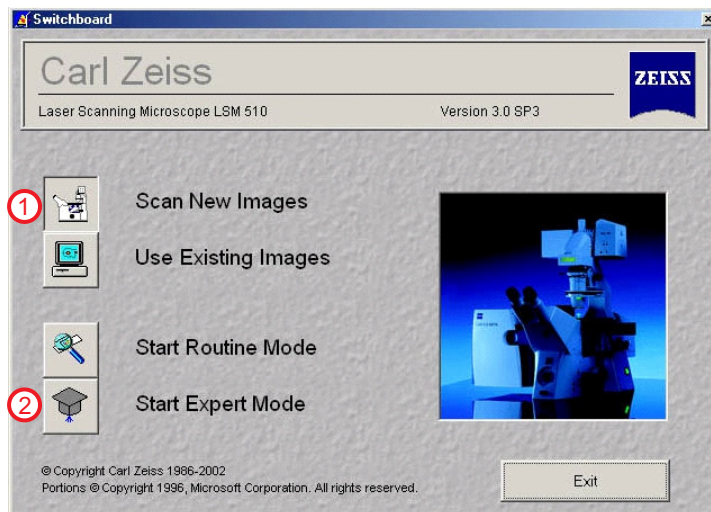
5. Double click on the **LSM 510** icon.

6. From the LSM 510 Switchboard menu, click the

“**Scan New Images**”<sup>①</sup> button and the

“**Start Expert Mode**”<sup>②</sup> button.

The LSM will go through instrument initialization and open a tool bar labeled “LSM Expert Mode.”

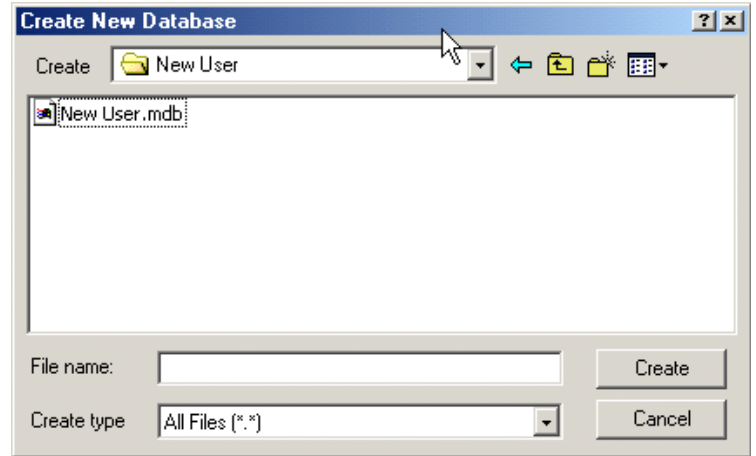


## II. CREATING A DATABASE FOR IMAGE STORAGE

1. Click the “**File**” ③ button from LSM 510 tool bar.
2. Click “**New**” ④ button.
3. Type in a data base name in the “**File Name**” field. Establish the location of the database to be created by setting the drive in the “**Create In**” field; open the appropriate folder by double-clicking on a folder icon from the list displayed.
4. Click the “**Create**” button. A window will open showing a blank database. Do not enter anything in the spaces at this time. *All images that are saved during your confocal session will be automatically saved in this database.*
5. Close the database window to reduce screen clutter.



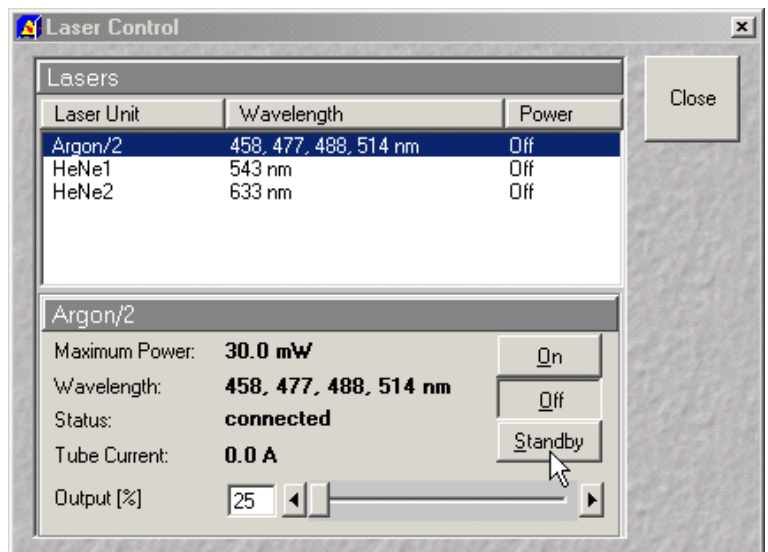
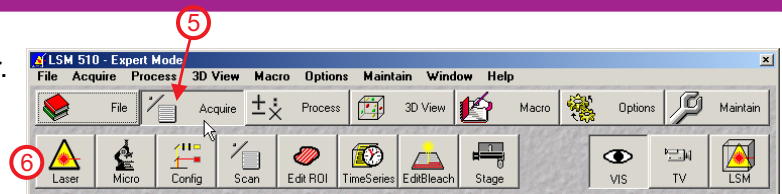
Selecting different buttons on the upper toolbar changes the buttons available on the lower toolbar.



This will allow you to create a new database to store your images, experimental setup and comments from your confocal session. If you previously created a database, click the “**Open**” button instead of the “**New**” button.

## III. TURNING THE LASERS ON

1. Click the “**Acquire**” ⑤ button from the LSM 510 tool bar. The bottom row of buttons will change to the ones pictured at the right.
2. Click the “**Laser**” ⑥ button.
3. A “**Laser Control**” menu with a list of available lasers will pop up. Using the mouse, click on the laser(s) with the appropriate wavelength(s) to excite the specimen dyes. The laser(s) may already be on from the previous use. *See end of this section for common dyes and their appropriate lasers.*
4. If the “**Argon**” laser is off, click the “**Standby**” button. When status indicates “**Ready**”, set the “**Output %**” to 50% and then press the “**On**” button. When the laser fires a “**Tube Current**” value will appear. *A current of 5.5-6.5 is normal.* If the Argon laser status says “connected,” turn the output to 50% and press “On.”
5. For “**HeNe**” lasers, just press “**On**,” the lasers will go through a brief warm-up period.
6. Close the menu to reduce screen clutter.



The **Argon/2** laser should be used to excite green emitting dyes such as **FITC**, **Alexa 488** and **CY2** and is also used for **CFP**, **GFP** and **YFP** excitation.

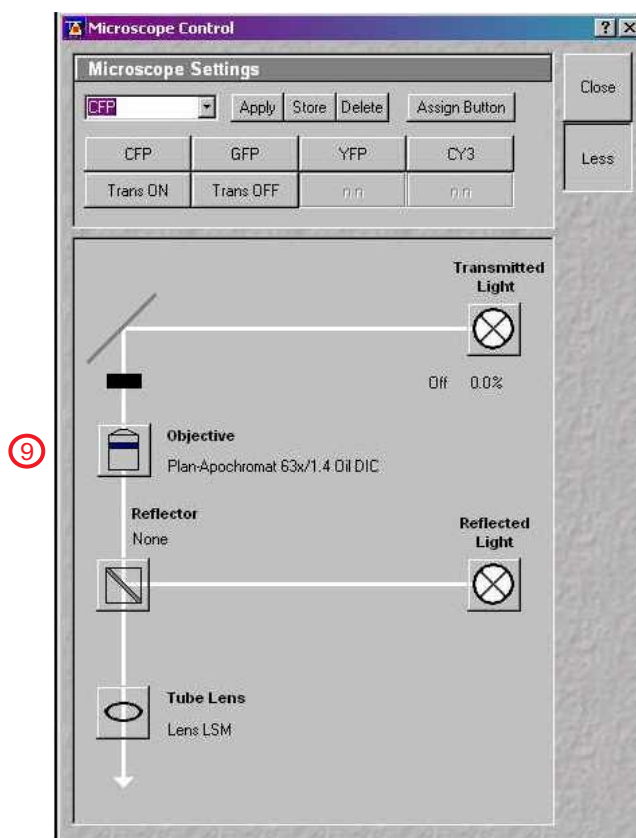
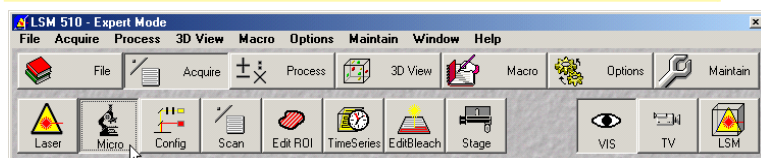
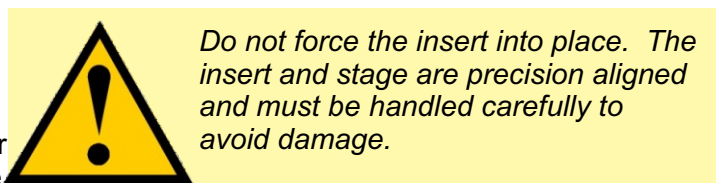
The **HeNe1** laser is used to excite red emitting dyes such as **CY3** and **Rhodamine**.

The **HeNe2** laser is used to excite far-red emitting dyes such as **Cy5**, **TOPRO3** and **Alexa 647**.

| Laser   | Wavelength         | Dyes                                |
|---------|--------------------|-------------------------------------|
| Argon/2 | 458,477,488, 514nm | FITC, Alexa 488, Cy2, GFP, CFP, YFP |
| HeNe1   | 543nm              | Rhodamine, Cy3                      |
| HeNe2   | 633nm              | Cy5, Alexa 647, TO-PRO 3            |

## IV. LOOKING IN THE MICROSCOPE TO VISUALLY SET UP YOUR SPECIMEN

- Check the objectives before proceeding.
  - Remove the stage insert and clean off any oil with a Kimwipe.
  - Clean each oil objective with lens paper.
  - 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.
- Press the **"Micro"** (7) Button from the **"Acquire"** menu of the LSM toolbar to see **"Microscope Control."**
- Select the **"VIS"** (8) button near the right side of the toolbar. This opens the shutter to the eyepieces.
- On the **"Microscope Control"** window, select the objective by clicking the **"Objective"** (9) button.
- Make sure the sample cover glass is clean, dry and sealed.** 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.
- 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.



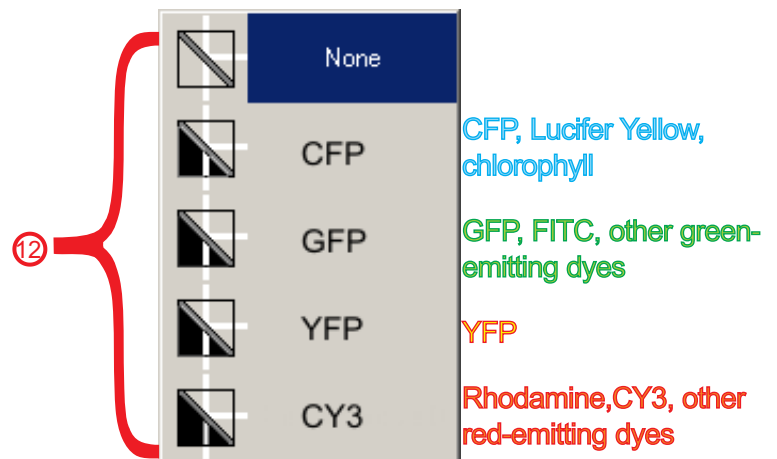
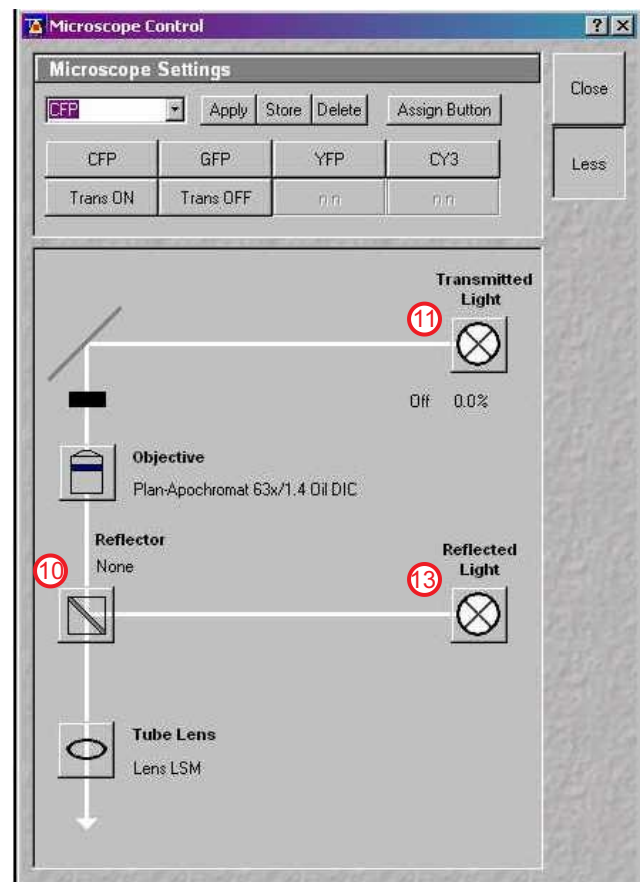
In the event of a spill, quickly wipe up all liquid. Notify a Cell Imaging Core staff member immediately. Timing is critical because expensive optics and electronics in the scan head can be damaged if liquid drips through the top of the microscope.



*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!*



7. To view the specimen in transmitted light (white light), set the “**Reflector**”<sup>10</sup> position to “**None**”.
8. Click the box for “**Transmitted Light**”<sup>11</sup> and click the **ON** button. Set the power to 1 or 2 using the slider. Or use the “**HAL**” switch on the side of the microscope.
9. Bring the sample into focus.
10. Turn off the transmitted (white light) using the “**HAL**” switch or by clicking the **ON** button again.
11. To view in fluorescence, click on the “**Reflector**”<sup>10</sup> button and select the appropriate filter set.<sup>12</sup>
12. Switch on the fluorescence excitation using the “**FL**” switch on the side of the microscope or click the box for “**Reflected Light**”<sup>13</sup> to **ON**. Reverse the switching process to turn off fluorescence excitation.
13. After the specimen is focused, the area of interest is selected, and fluorescence is confirmed, press the “**LSM**”<sup>14</sup> button on the right side of the tool bar to set the microscope for confocal imaging.
14. Close the “**Microscope Control**” window to reduce screen clutter.



### 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, HD15052, DK59637 and Ey008126).

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

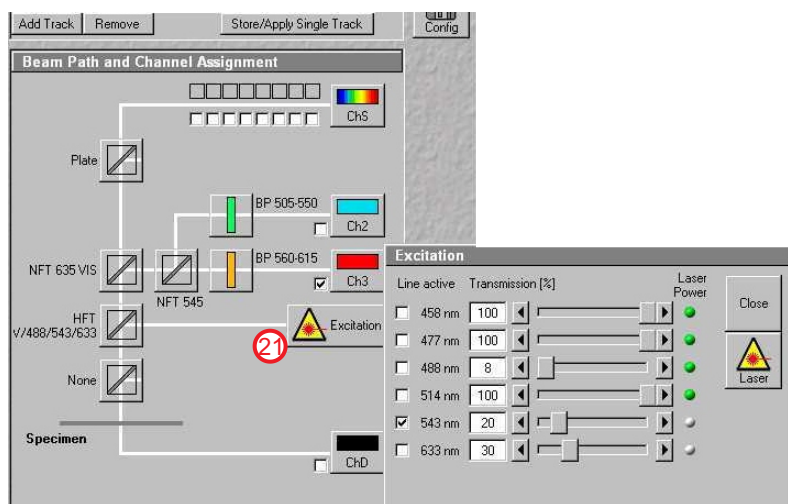
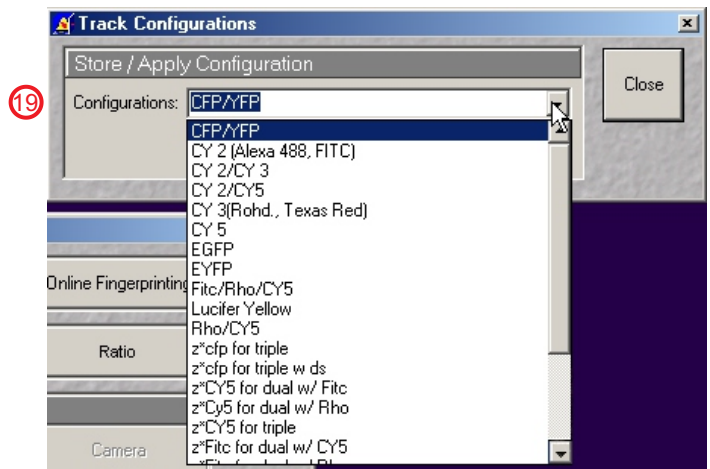
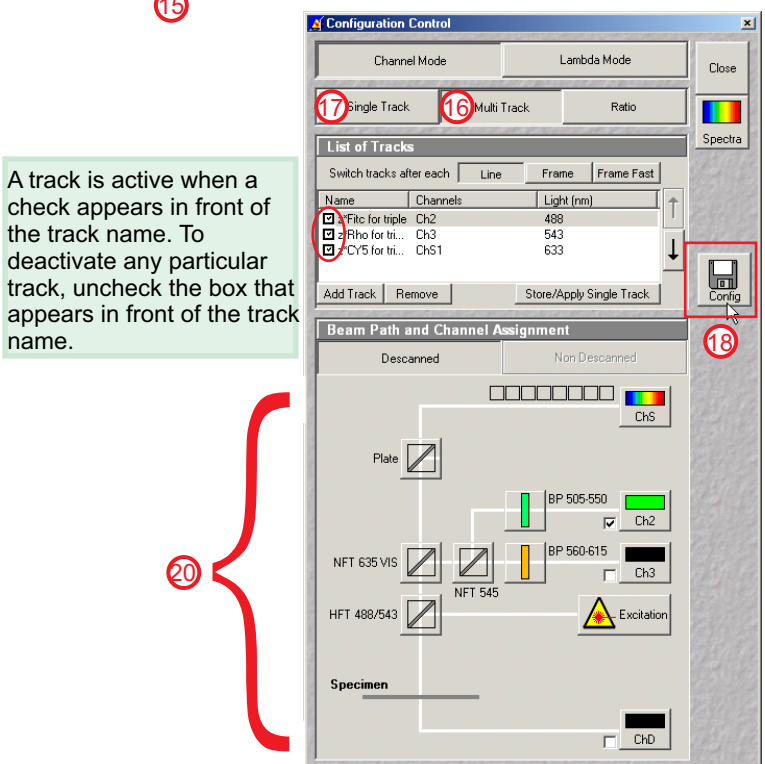
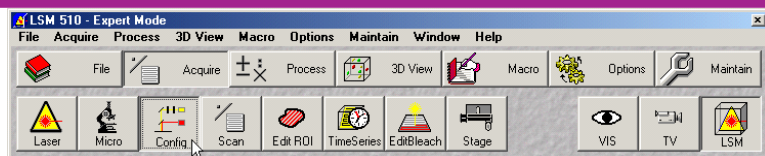
## V. CHOOSING THE CONFIGURATION

1. Press the “**Config**”<sup>15</sup> button from the Acquire menu in the LSM 510 toolbar.
2. Click on “**Multi Track**”<sup>16</sup> - used for collecting 2 or more signals *separately* or “**Single Track**”<sup>17</sup> - used for collecting 1, 2 or 3 signals *simultaneously*.  
  
Generally, it's best to use Multi Track for samples with more than one fluorophor.
3. Click on the “**Config**”<sup>18</sup> button on the Configuration Control window.
4. A “**Track Configuration**”<sup>19</sup> menu will appear. Open the pull-down menu and select the filter configuration for the dye(s) you are using (e.g. FITC/Rhodamine)

5. Click the “**Apply**” button.  
Don't click “**Store**” or “**Delete**” for any of the default configurations.
6. The correct settings and tracks for the configuration you have chosen will appear in the “**Beam Path and Channel Assignment**”<sup>20</sup> window. Do not alter these settings unless you are an expert with these properties.

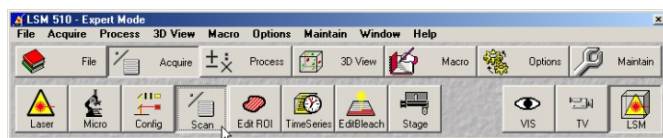
7. Now is a good time to check the laser setting for each channel. Click the excitation button<sup>21</sup>. It's better to start low and raise the laser strength as needed while optimizing (see section VI-5) than to start too high and risk bleaching or damaging your sample.

**Suggested laser power ranges for imaging are:**  
**Argon - 1-10**  
**HeNe1 - 1-30**  
**HeNe2 - 1-30**

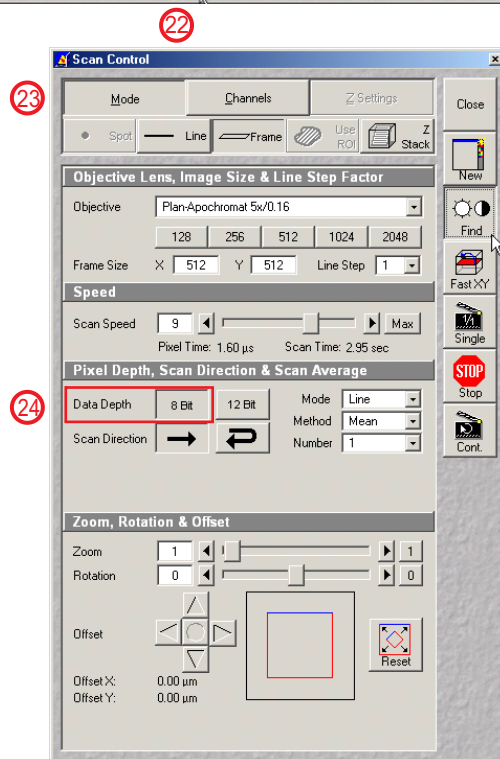


## VI. LASER SCANNING

1. Click the “Scan”<sup>22</sup> button from the LSM510 tool bar to get the “Scan Control” window.



2. The first window to appear is “Mode”<sup>23</sup> which allows the user to choose the size of the image and zoom level as well as bit depth<sup>24</sup>. The default is 8-bit or 256 shades of gray from a value of 0 for black to 255 for saturated. To have the best quality image, we want to use that entire range.



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.



3. To optimize your image, use the controls in the **Channels** 25 window:

A. Adjust the pinhole slider 26 so all channels have the same optical slice thickness and no channel has a pinhole value less than 1.0 Airy unit.

B. Click **"Fast XY"** 27 to compose your image and select your desired initial focus.

C. For multi-labeled specimens it is easier to view the image in split screen where each label is laid out side by side. The **"Split XY"** 28 button is located on the right side of the image window.

D. Under **Channel Settings** there are buttons for each dye in the configuration you have selected. The first channel listed will be shown in the upper left-hand corner of **"Split XY"** display.

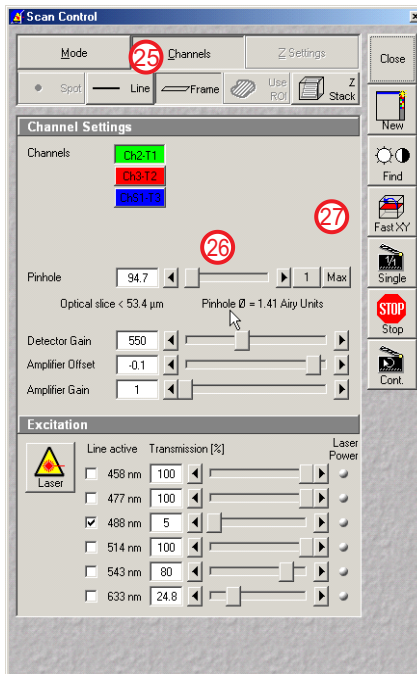
E. Click **"Palette"** 29 and select "Range Indicator." A specialized look-up table is applied so that all of pixels with a value of 0 are bright blue and all the of the pixels with a value of 255 are bright red. A good quality image will have only a few blue and a few red pixels.

F. Turn off all but one channel by clicking in the boxes to the left 30 of the channels in the Configuration Control window.

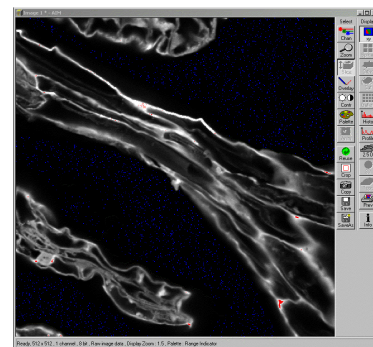
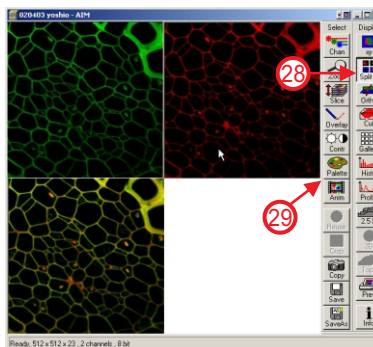
G. To make the image brighter or dimmer by increasing or decreasing the saturation (depicted by red pixels,) adjust **"Detector Gain"** 31. The lower the detector gain, the less noise in the image.

**Suggested gain range: 500 - 700**

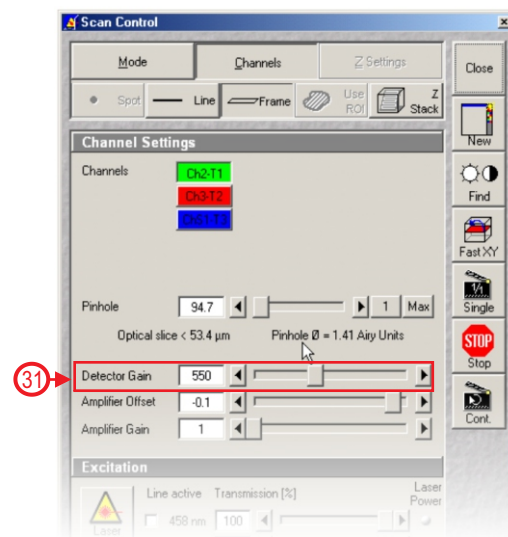
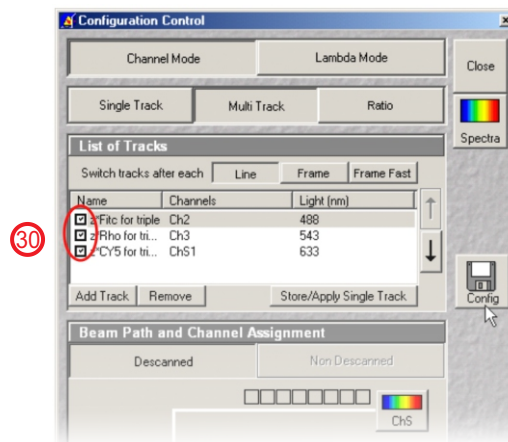
**Absolute limit: 900 for Channels 2 and 3, 750 for Channel S1**



The pinhole value determines the thickness of the sample from which light is gathered. Higher pinhole values= more light reaching the detector. However, a larger pinhole means that z-sections and projections will have less definition (fuzzier) because each slice will be thicker. Pinhole sizes need to be matched across channels to insure accurate signal level comparison between channels. Matched pinholes are also requirement for z-stacks.



blue = 0  
red=255



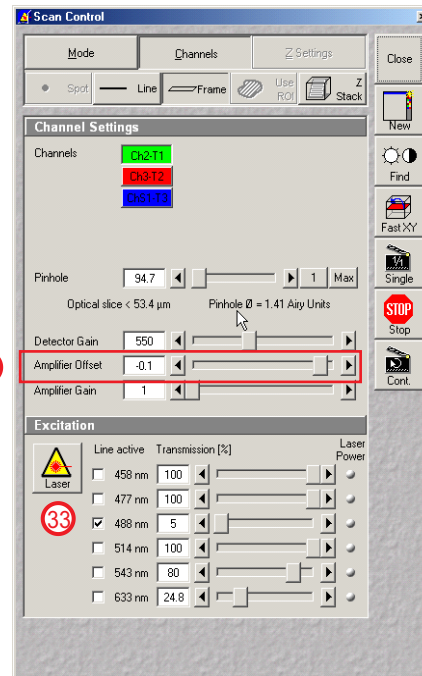
Options for *acquiring* an image are available from the Scan Control window. Options for how the image is *displayed* are available on the toolbar to the right of the image.

Values in all windows can be adjusted by using the sliders, typing in a value, or clicking on an arrow button. Hold down the CTRL key while clicking for fine adjustments.

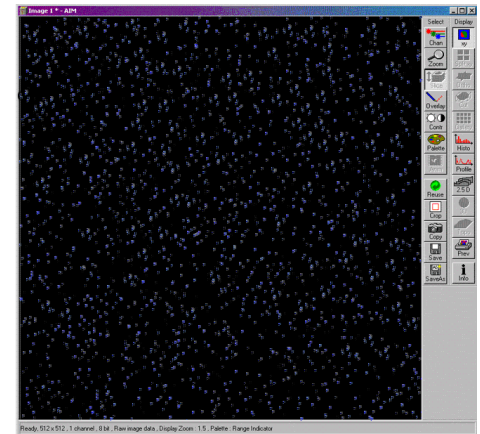
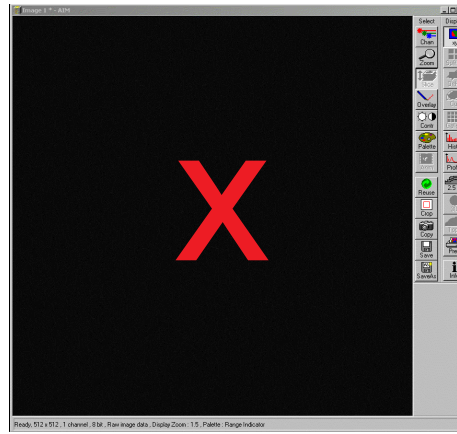
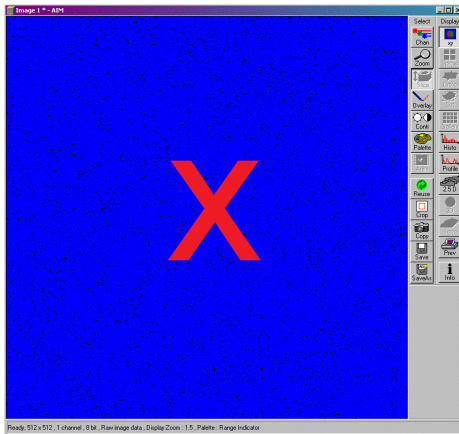
#### 4. To adjust the Amplifier Offset: 32

A. While still in Range Indicator, turn off the laser by clicking in the checked box to the left of the wavelength 33. (Remember which one you turned off so you can turn the right one on again!)

B. Adjust the Amplifier Offset slider to achieve the appearance of a starry night - many blue dots on a field of black. Since the slider is very sensitive, it may be best to use the arrows at either end. At some point, one click in either direction will make a big change in the amount of "stars." If neither of those settings is satisfactory, press the Control key while clicking with the mouse. This will give you fine control.

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C. When you are finished adjusting the Amplifier Offset for a channel, turn the laser back on.



The image will have a great deal of contrast, but low-level signal will be lost.

The resulting image will have low contrast.

**This adjustment is correct.** The image will have good contrast and will preserve sample detail.

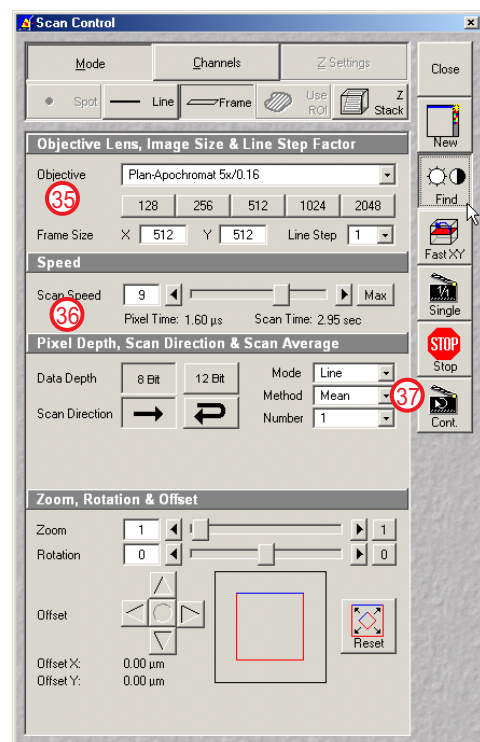
5. If the signal intensity is weak, you can increase laser strength 33, but be careful! A high laser setting can bleach your sample or create phototoxic conditions in live specimens.

6. Other options for improving image quality are available from the "Mode" 34 window under Scan Control:

A. Increase the resolution, called "Frame Size" 35 in the Scan Control window. This will result in larger files and slower acquisition times.

B. Set the "Scan Speed" 36 based on the appearance of your image scanned on the "single" or "continuous" setting. A scan speed of 7 or 6 works well for bright fluorescence.

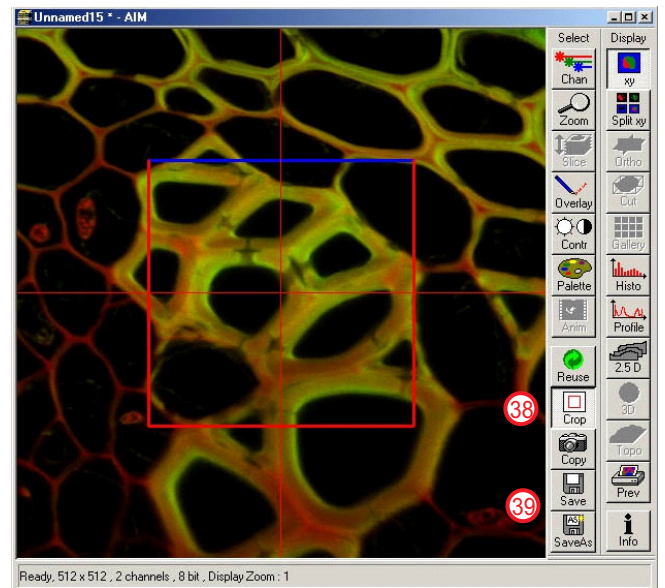
C. Averaging 37 (mode set on "mean") of 4 - 8 should improve signal-to-noise; however, the image acquisition rate will be slower and photobleaching will be greater.

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## 7. To zoom into an area of interest:

- Click the **"Crop"** <sup>38</sup> button on the lower part of the toolbar. Zooming will enlarge an area of interest by scanning the laser into a smaller area of the field of view. This function can actually increase the X,Y resolution of an image.
- A red box will appear on your image.
- To reposition the box, click and drag the center.
- To resize, click and drag a corner of the red box.
- To change either the horizontal or vertical dimensions, click and drag a side of the box.
- To rotate the box, click and drag one of the bisecting lines that extend past the box's perimeter.
- Changes take effect once the **"Single"** button is clicked, and they remain in effect for subsequent images unless the reset button in the **Scan Control** window is clicked. Note that zoom and rotation can be controlled from that window also.



After an image is zoomed, it may be necessary to readjust the Detector Gain.

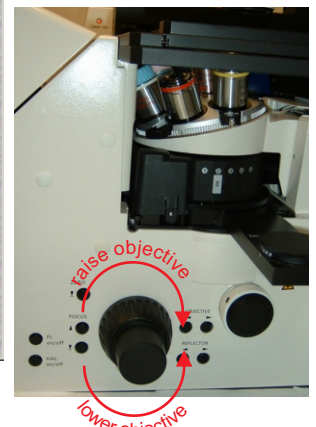
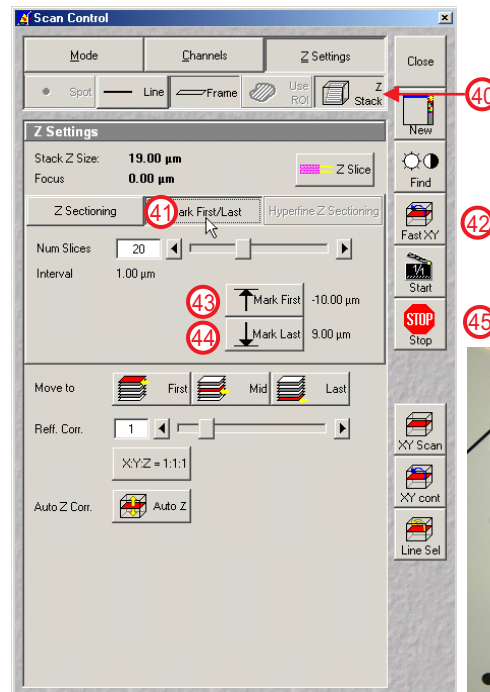
- To save your image, click the **"Save"** or **"Save As"** <sup>39</sup> button on the right side of the image. The default location for saving the file will be the database you created after you opened the LSM program.

## VII. Z-SECTIONING

Once you have set up your image as defined in the above section, you can collect a series of confocal images through all or any of the focal planes of your specimen. The focus motor must work against gravity to accurately accomplish Z sectioning, which means that the objective must move through focus from the lowest point to the highest point.

- Press the **"Z Stack"** <sup>40</sup> button on the "Scan Control" menu. The **"Z-Settings"** window is now active.

- Click on the **"Mark First/Last"** <sup>41</sup> tab.
- Click **"Fast XY"** <sup>42</sup>.
- Move the focus manually down (towards the microscope user) until the image of the specimen begins to disappear, then click the **"Mark First"** <sup>43</sup> button in the Z settings section.
- Now move the focus in the opposite direction (away from the user) until the image of the other side of the specimen begins to disappear. Click **"Mark Last."** <sup>44</sup>
- Click the **"Stop"** button. <sup>45</sup>



2. Click the pink and yellow “Z Slice”<sup>46</sup> button.

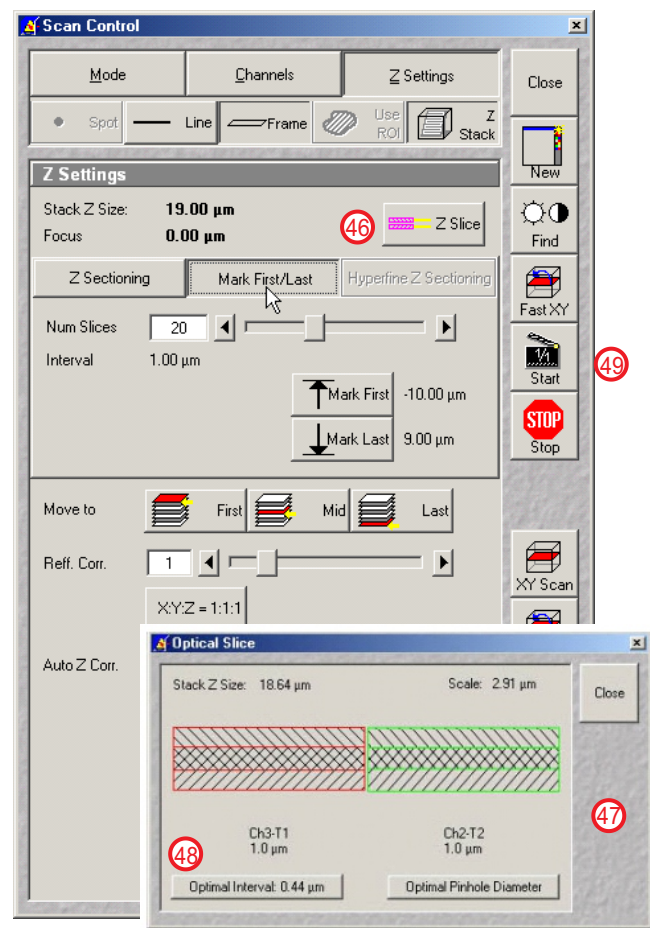
3. The “Optical Slice”<sup>47</sup> window will open. Click the “Optimal Interval”<sup>48</sup> button to set the number of Z-slices based on your pinhole size.

4. To start the z sectioning click “Start 1/1”<sup>49</sup> button. The system will automatically begin Z sectioning. Be careful not to bump the air table or the microscope until z sectioning is completed.

All the acquired z slices can be viewed by changing into “Gallery”<sup>50</sup> mode. This button is located to the right of the image.

A black bar will be shown under the image and will move from left to right, showing that the 510 is in the process of z sectioning. The laser will stop scanning automatically when z sectioning is done.

5. The entire stack of images can be saved with “Save” or “Save As” buttons<sup>51</sup> on the right side of the image.



## VIII. SHUTDOWN

If the system will *not* be used again before 6:00p.m.:

1. Click the Laser button within the LSM program and turn off all lasers.

2. Exit the LSM program.

3. Back up your data now or, if you have saved it on CISRstore, later from your lab.

4. Log out of the computer session.

Click “Start” on the Windows task bar.

Click on “Log Off”.

**DO NOT** use the computer power switch to turn off the computer.

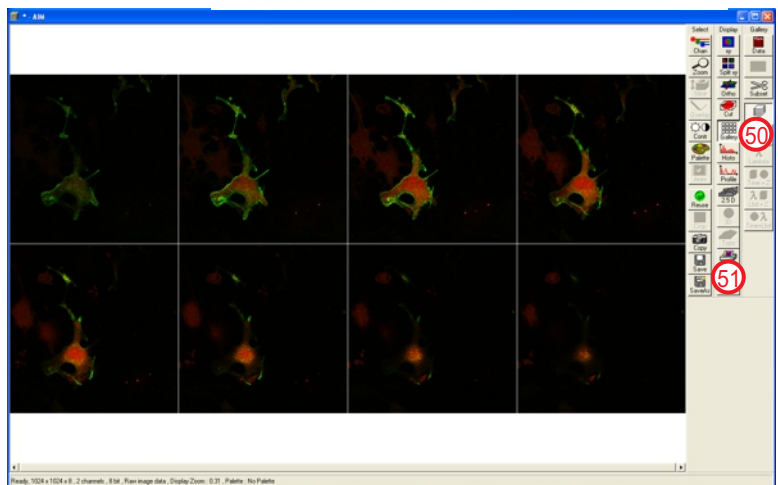
5. Clean any oil off objectives and stage.

6. Sign out on the log sheet.

7. When the cooling fan for the Argon laser cuts off (about three minutes *after* the laser is turned off,) **and not before**, turn off both power switches.

If the system *will* be used again before 6:00p.m.:

Follow steps 2 through 6 above.



As long as the “Z series” button remains depressed, the movie clap board button will initiate a series rather than a single image. (Note that the icon is labeled “start” rather than “single”.) To return to single mode, you must de-select “z-series” by clicking on it again.

## **Protocol for Access to 742 Light Hall**

Cell Imaging Shared Resource, June 23, 2004

### Room Access

You must use your VU ID card to unlock the main door.

- Your VU ID card must be authorized to open the door (see authorization below.)

After entry the door must be closed and locked – DO NOT prop the door open.

Do not lend your card to anyone for entry – you will be responsible for loss or damage.

If your card is lost or stolen, report it immediately to a CISR staff member (see contact below.)

You will be photographed by a security camera upon entering the room.

### Authorization

Authorization of your card will require registration and proof of training.

### Useful Links

CISR's homepage: <http://cistrweb.mc.vanderbilt.edu/>

Free version of the LSM software for opening and exporting .ism files:

<http://www.zeiss.com/> Under "Products" select "Microscopes" then "confocal", then "Download software". Scroll down to Laser Scanning Microscope"; select either "Zen" or LSM Image Browser" or both. Or go the FAQ's section of our website, "Where do I get relevant software?" for a link.

Molecular Expressions – FSU's encyclopedic microscopy site

<http://micro.magnet.fsu.edu/primer/index.html>

### Contact Information

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