

UNIVERSITY OF MARYLAND FACILITY FOR CONFOCAL MICROSCOPY

USER'S GUIDE

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ZEISS LSM410 CONFOCAL MICROSCOPE 3976	2
I. SYSTEM START-UP	2
II. SYSTEM INITIALIZATION	2
III. SCANNING SINGLE IMAGES	4
IV. PINHOLE ALIGNMENT	5
V. RETURNING TO CONVENTIONAL MODE	6
VI. RETURNING TO LSM MODE	6
VII. OPTICAL SECTIONING	7
VIII. SAVING IMAGES TO DISK	8
IX. FTP (USING NCSA FTP)	9
X. SYSTEM SHUT-DOWN	9
XI. IN EVENT OF SYSTEM HANGUP (E.G., FROZEN MOUSE)	10
XII. NOTES	10
NP1600 CODONICS DYE SUBLIMATION PRINTER	12
I. NOTES	12
II. STANDARD PRINTS	12
III. PRINTING MULTIPLE COPIES OF THE SAME IMAGE	13
IV. CHANGING CONTRAST OR COLOR BALANCE	14
APPENDIX I. SIGNING ON TO THE CONFOCAL LISTSERVER	14
APPENDIX II. STANDARD FILTER COMBINATIONS	16

Notations in these instructions are:

- Bold** indicates clickable points in windows, or commands you type in with the keyboard;
- Italics* indicate variables;
- "Quotations" indicate example commands;
- ← means to press the enter key

ZEISS LSM410 CONFOCAL MICROSCOPE

I. SYSTEM START-UP

- A. Make sure the previous user turned the laser power control to minimum (all the way counter-clockwise).
- B. Turn on Kr/Ar laser power supply (Switch marked **AC POWER**--switch to **1**).
- C. Turn laser key switch beyond 90° clockwise to the spring stop, then let the key return to vertical (the laser ignites automatically after warming up a minute or so).
- D. Close the epi-illumination shutter on the microscope.
- E. If you need to locate specimens using conventional fluorescence, turn on Hg arc power supply (green switch). Don't start the system electronics for 60 seconds. While you're waiting, you can:
 - 1. Turn on the nitrogen gas supply to the optical table (should be at 80 psi).
 - 2. Write your name, starting time and date in the log.
 - 3. Load your optical disk into the Sierra drive (E:)-it will come to a stop with about 1/2" protruding. Or put a disk in the Zip drive (F:)
- F. After 1 min., start system electronics with key switch: turn clockwise beyond 90° to spring stop (until the relays engage), then let the switch return to vertical.
- G. Causes of persistent warning beeps during initialization:
 - 1. Reflector slider (beneath objective turret) not in confocal position.
 - 2. Illumination arm tilted back.
 - 3. Halogen lamp/transmission detector selector not pointed to detector.
- H. If you need the red (633nm) He/Ne laser, make sure it's power supply (green switch on front of electronics panel) is on. If you don't need it, make sure the switch is off.

II. SYSTEM INITIALIZATION

- A. Turn the coarse focus knob to lower the objective turret all the way (note that you can't do this until the LSM Program is loaded).
- B. Swing selected objective (63x, NA 1.4 40x, NA 1.2 water, 25x, NA 0.8, or 10x, NA 0.45 air) into position. Pull up on spring-loaded oil immersion lenses to make sure they are not retracted.

- C. IF YOU ARE USING AN OIL IMMERSION LENS, USE A MINIMUM AMOUNT OF OIL BETWEEN THE LENS AND THE SLIDE.
- D. Load the specimen slide: use the spring clips to hold the slide down. Make sure the slide carrier is stable--if not, remove, and reinsert the corner with the red dot first.
- E. Find a specimen via conventional microscopy: Click the **CONV↔LSM** button to activate a circle on the **CONV** side (or select a circle directly), select **T** (transmission) or **F** (fluorescence) or **Ov** (both). Open the epi-illumination shutter to the middle position for fluorescence.
- F. Close the epi-illumination shutter.
- G. Set the following parameters:
1. **Lens**: set for the objective in use.
 2. **Atten** (attenuation): The three columns correspond to (left to right) the He/Ne, UV, and Kr/Ar lasers. 3 is the normal starting point with laser power at 90%. Bigger numbers mean more attenuation (Transmission=100%/A, so 1=100%, 3=33%, 10=10%, etc.).
 3. **Bandw** (bandwidth): 0 (no low-pass filtering)
 4. **t** (time): 1 sec

NOTE: **Em** and **D1** are not active on our system
 5. **Function**: Frame
 6. **Laser**: 488 (for fluorescein), 568 (for rhodamine), 633 (for Cy5 or reflection), or combinations for simultaneous imaging.
 7. **Size**: 512x512 is standard
 8. **Contrast** (Gain): start at a setting of 100
 9. **Brightness** (Blacklevel): 9999
 10. **Pinhole**: default for objective:
 - a) 18 for 63x/1.4
 - b) 13 for 25x/0.8
 - c) 10 for 10x/0.45
 - Make sure an X is in the **CONFOCAL** box under **EXTRAS**
 11. **ZOOM**:
 - a) **1** to locate specimen
 - b) **3.2** for high resolution with 63x/1.4 objective at 512x512 (0.124 μm/pixel for Size=512x512)

- c) **4.6** for 25x/0.8 objective
- d) Notes:
 - The current pixel dimensions are indicated next to x dimension in the parameter window-this parameter is not updated until the specimen is scanned.
 - Pixel dimensions are affected by both the **ZOOM** and **SIZE** settings
 - Pixel dimensions should be 1/2 the distance you want to resolve (Use the pinhole calculator, opened by clicking the unmarked button to the right of the pinhole size slider, to determine the resolution for the current conditions)
- H. Click the **CONV↔LSM** button to activate a circle on the LSM side (or click the circle directly: use **FL** for fluorescence).
- I. Position a filter combination that is appropriate for your sample (see Appendix II for standard filter combinations, and refer to the Zeiss filter sheet)

III. SCANNING SINGLE IMAGES

- A. Select appropriate PMT (**R/FL 1-3**) in LSM column of Laser Control Panel-be careful not to confuse PMT #3 and #2.
- B. Make sure the illumination arm is forward and the halogen lamp/detector selector is pointed towards the detector.
- C. Move the reflector slider all the way to the right.
- D. Open the epi-illumination shutter to the far left position (no heat filter in the laser pathway).
- E. Increase laser power to 50% (indicator at 12:00).
- F. Click **START** to begin scanning.
- G. Verify scan at specimen; locate problem if it's missing (check screen for error message; if none, you probably need to open the epi-illumination shutter).
- H. Press F9 for automatic adjustment of PMT gain ("Contrast").
- I. Optional: define **ROI** (region of interest).
- J. Adjust the focus
 - 1. Manually: pay attention to the direction arrows next to the focusing knobs
 - 2. Motorized
 - a) Click the **Mot** button near the bottom of the control panel window. A motor control window appears.

- b) Set the z step size (use 0.25 μ m for high-resolution work).
 - c) Focus by clicking the top (objective moves up) or bottom (down) arrow buttons
 - d) Click **Done** when you're finished.
- K. Click **COLOR** in the Display Panel one time to load the Rangefinder LUT.
- L. Adjust **BRIGHTNESS** down to get some blue pixels in the dimmer areas (hold down the right mouse button to drag the slider).
- M. Adjust **CONTRAST** up to get some red pixels in the brighter areas.
- N. If necessary, re-adjust **BRIGHTNESS**.
- O. Image averaging methods:
 - 1. Select **TIME**, pick a value >1 second
 - 2. Select **AVERAGE, FRAME**, pick a value >1
 - 3. Select **AVERAGE, LINE**, pick a value >1
- P. Click **SINGLE** to obtain an averaged image (clicking **START** causes the system to average the image repetitively).
- Q. ***TURN THE Kr/Ar LASER DOWN TO IDLE WHEN NOT SCANNING***

IV. PINHOLE ALIGNMENT

- A. Set up parameters and get an image of the sample.
- B. Turn off averaging, set time=1
- C. Reduce **Pinhole** to 10.
- D. Reduce **Contrast** to ~50 (note original value so you can reset it).
- E. Remove barrier filter (set to free position)
- F. Start scanning, adjust contrast until the specimen is just visible.
- G. Note the z encoder value so you can return to the specimen plane of focus.
- H. Carefully focus away from specimen (z encoder value decreases) until a maximal reflection from the coverglass/glycerol interface is obtained. Adjust contrast until reflection is medium gray; optionally decrease attenuation to reduce noise.
- I. Set **Zoom** to 8.
- J. Stop scanning, select **Function; Line Scan**.
- K. Adjust focus until the trace is maximized and flat.
- L. Adjust **Contrast** until the trace is approximately half maximum.

- M. Turn top pinhole alignment knob clockwise, then counter-clockwise, about 1/4 turn, and locate the position that maximizes the trace. If the trace goes off-scale, reduce **Contrast**.
- N. Double-check focus (adjust to maximize trace).
- O. Repeat for the bottom alignment knob.
- P. Repeat for top and bottom alignment knobs until the trace is maximal. Check focus again.
- Q. **Cancel** Line Scan, reset contrast, attenuation, zoom and pinhole values, and replace barrier filter.
- R. Repeat for all channels that will be used during your session.

V. RETURNING TO CONVENTIONAL MODE

- A. Turn laser power to idle.
- B. Close the epi-illumination shutter.
- C. Move the reflection slider away from the confocal position.
- D. Click on **Ov** in the mode box.
- E. Open the epi-illumination shutter (to the middle opening, with the heat filter) to observe the specimen.

VI. RETURNING TO LSM MODE

- A. Make sure specimen is in focus and located just to the right of field center.
- B. Close the epi-illumination shutter.
- C. Click on the **R/FI** corresponding to the PMT you will use.
- D. Check the three things the computer keeps track of:
 1. Move the reflection slider to the right (LSM) position.
 2. Make sure the illumination arm is down.
 3. Make sure the tungsten illumination/transmission detector selector is pointed towards the detector.
- E. Check the two things the computer ignores.
 1. Open the epi-illumination shutter all the way (no heat filter).
 2. Turn the laser power up half way.
- F. Check Control Panel options:
 1. **Averaging** off

2. **ROI** off
 3. **Confocal** checked
 4. Centered scanner (no tick marks on arrows in LSM Control Panel)
 5. **Contrast** setting not too high for current sample
 6. Optional: **Zoom**=1
- G. Click **Single** and watch the specimen to verify laser function.
- H. Press F9 for automatic contrast adjustment.
- I. Optimize the image:
1. Adjust **Zoom**
 2. Adjust focus
 3. Load rangefinder LUT and fine-tune **Contrast** and **Brightness**
 4. Select averaging method

VII. OPTICAL SECTIONING

- A. Click **START** to scan the specimen, adjust the focus to find the brightest level, adjust **CONTRAST** and **BRIGHTNESS**.
- B. Adjust focus to the lowest position in the specimen, click **Mot**, click →**0** to set z-axis position to zero
- C. Focus to the top of the specimen and note the z-axis position, reflecting the specimen thickness in microns. Note the position of the highest level in the sample.
- D. Focus to the center of the specimen (z indicator at 1/2 of thickness from above), select **ROI**, select **Set ROI**, adjust size and position of box to enclose area of interest, click the right mouse button and select **OK**.
- E. Select an averaging method (**Frame**, **Line**, or **T>1**)
- F. Select **Z**, **Z Sectioning**
- G. Set the **Z interval** to the step size between sections
1. For square voxels, make the interval equal to the pixel size set by **ZOOM** and **SIZE**
 2. For maximum resolution with the 63x/NA 1.4 objective, set the interval to 0.53μm
- H. Select **Host Memory** (system RAM) or **File** (disk) to store the images. If you select File, specify a subdirectory (use an IM-*n* subdirectory under C:\image, or your optical disk) and filename. Use no more than 5-6 characters in the filename to leave room for 3-2 digits of numbers that the LSM will automatically add to the name for each file in the series. *Warning*: selecting the **Screen** option will only display each optical section; they will not be saved in memory.
- I. Set the **Number of Sections** you will collect. The **Max** button will provide an indication of the maximum number you can collect into RAM or disk. Thickness

of specimen divided by Z interval equals the number of sections required to sample the entire specimen.

- J. If the current focus position is half way through the specimen (Step 4), set the **Current Section Pos** to half the **Number of Sections**
- K. Set **Refractive Correction** to 0.97 if your specimen is mounted in glycerol and you're using an oil immersion objective. If the specimen is in water, the correct value is 0.88, but you should be using the water immersion objective. For dry objectives, use 1.47 for specimens in glycerol, and 1.33 for specimens in water.
- L. (Optional) Select **Z Scan** and use the mouse to move the line overlay to the thickest area of the specimen. Click the left mouse button and an extended Z scan through this area will appear on the image monitor. The dashed line indicates the current section for the xy plane, the upper solid line indicates the starting section (away from slide), and the lower line indicates the final section (towards the slide). Position the mouse anywhere between the solid lines, hold the left button and move the start/final section lines together so they include the area of interest. If necessary, reset the number of sections or Z interval to increase or decrease the width between these lines. Press the right mouse button to return the pointer to the command screen.
- M. (Optional) **Interpolation** enables an automatic section-by-section increase in PMT gain, which is important for thick specimens. You should turn off averaging to set interpolation, and turn it on again before collecting the series. Checking **Interpolation** causes the microscope to first focus to the final position in the series; a window opens inviting you to set **Contrast** and **Brightness** for this position. **Autoadjust** is the same as F9. After the settings are correct, the microscope focuses on the first position in the series, and you repeat the process. Don't forget to turn **Averaging** back on.
- N. Select **OK** to start collecting the optical section series.
- O. Remember to idle the laser before you analyze the image stack.
 - 1. Use **Z, Animate** to check image stack (click **From Host**)
 - 2. Use **3D, Stereo Images** to make red-green 3D anaglyphs.
 - 3. Use **3D, Projection** and **Z, Animate** (click **Host RAM** off) to make a rotating 3d rendering

VIII. SAVING IMAGES TO DISK

- A. Make sure the image you want to save is displayed on the monitor. If the rangefinder or another color LUT is active, the system will store the image with these colors.
- B. Click **FILE, STORE IMAGE**
- C. CLICK AN X IN THE **CHUNKY RGB** BOX IF YOU ARE SAVING COLOR IMAGES.
- D. Click E: for optical disk, or select an empty subdirectory (e.g., \images\im-1; \images\im-2; etc.) on C:, the LSM hard disk. If there are no empty

subdirectories, find the one with the oldest files and delete them with file manager.

- E. Type in a file name (8 characters maximum, no special characters like :/\=;).%#@, etc.). Leave the .tif extension
- F. Click **OK** or press enter.
- G. To save z-sections or time series:
 - 1. Click **FILE, STORE SERIES**
 - 2. Select disk and subdirectory, type in a filename-leave room for the system to add a section number (e.g., use 6 letters or less for 11-100 sections; 5 or less for >100)
 - 3. Click **HOST MEMORY** if you collected the images into system RAM
 - 4. Click **OK** or press enter to store the series
 - 5. Click **FILE, LOAD SERIES** and check the file list to verify storage
 - 6.

IX. FTP (USING NCSA FTP)

- A. The target system needs to be in server mode. If you're using NCSA Telnet, just start the program.
- B. Exit from windows by pressing ALT-F4 until you get the exit message; click OK or press enter
- C. Type **cd\tcp←**
- D. Type ftp **134.192.xx.yyy←**, where the variables indicate the target machine's IP address
- E. Type **bin←** to set the transfer type to binary
- F. Type **lcd \images\im-x←** where the variable indicates the subdirectory containing the images you want to transfer.
- G. You can type **lls←** to get a directory listing
- H. Type **cd \subdirectory←** to change to the appropriate subdirectory in the target system
- I. Type **mput *.tif←** to send all of the image files in the current subdirectory to the target system
- J. When the transfer has finished, type **bye←**
- K. Please erase your files from the hard disk

X. SYSTEM SHUT-DOWN

- A. If someone is scheduled to use the microscope within an hour, idle the laser and leave it on. Go to step D.

- B. Turn laser power down.
- C. Turn the laser key switch counter-clockwise. Leave the red switch on for 5 minutes to cool the laser down.
- D. Get rid of your image files on the hard disk.
- E. Exit Windows
- F. Retrieve your optical disk by pressing the button on the right front of the Sierra disk drive (*WARNING*: don't press this button when the blue or yellow LEDs are lit, or you'll lose data).
- G. Turn the key switch counter-clockwise to shut down system electronics.
- H. Turn off the Hg arc power supply (If someone is scheduled to use the microscope within an hour, leave it on).
- I. Sign out of the log book. If you experienced problems with the system, indicate them in the remarks column..
- J. Clean the oil off of the objectives-use dry lens paper.
- K. Turn off the nitrogen gas supply to the optical table.
- L. Clean up your mess. Leave nothing. Use the trash can.
- M. Put the cover on the microscope. Don't let it contact the Hg arc lamp, or you get to buy us a new cover.
- N. Turn off the **AC POWER** switch on the laser power supply.

XI. IN EVENT OF SYSTEM HANGUP (E.G., FROZEN MOUSE)

- A. Hold down ALT, press I
- B. Use arrow key to select **Reset Image System** and press enter
- C. If this fails to clear the problem, hold down ALT, press I, Use arrow key to select **Reload Image Shell**

XII. NOTES

- Laser must run >20% power to avoid noise
- Idle the laser when not scanning
- The barrier filter slider for PMT-1 is inverted relative to 3 and 2
- Transmission detector is sensitive to ambient light: turn room lights off when you use it
- Be sure to utilize slide clips on stage to stabilize position for Z motor control
- **BANDWIDTH**: larger number eliminates more high frequency data. No one uses >2; 0 is high-resolution, no noise attenuation; 1 is Zeiss' default, 2 can be used for coarse objects in noisy fields.

- **T**=time of scan. Value on the left is the time per frame for 512x512, value on right is actual time of image acquisition based on size, ROI, averaging, and storage to memory or disk.
- Pixel dimensions for 512x512 Zoom=1: 10x=2.5 μ m; 25x=1.0 μ m; 40x=0.625 μ m; 63x=0.397 μ m. For higher zooms, multiply the pixel dimension by the zoom factor. For 1024x1024, divide the pixel dimension by 2; for 256x256, multiply by 2; for 128x128, multiply by 4; etc.

NP1600 CODONICS DYE SUBLIMATION PRINTER

I. NOTES

- A. This printer is only available through the network
- B. **WARNING:** LSM images must be saved using the CHUNKY RGB option or color images will not print correctly.
- C. Supported file types include:
 - .TIF
 - Postscript Levels I & II
 - JPEG
 - Windows .BMP
 - Targa .TGA
 - PC Paintbrush.PCX
 - GEM Image
 - Portable Bitmap (PBM)
 - Portable Graymap (PGM)
 - Lisp Machine Bitmap
 - Amiga ILBM (IFF)
 - GIF
 - Portable Pixmap (PPM)
 - Silicon Graphics Raster (RGB)
 - Sun Raster
 - X11 Window Dump
 - .XWD

II. STANDARD PRINTS

- A. FTP to 134.192.23.135
- B. Use your unique UserID to logon
- C. Respond to the password prompt by entering a number indicating the printer mode you will use:
 - nn←***
 - *nn=1* is Fixed format: image is printed without scaling (300 pixels/inch)
 - *nn=2* is Scaled format: image is scaled to fill the 8«x11 print
 - *nn=11 to 99* is Fixed Multi-format: several images are printed with scaling in an array of x columns and y rows, where x is the first number and y is the second (e.g., 23 prints 6 images in two columns and three rows). The Codonics will not produce the print until you have sent it the appropriate number of images.
- D. Set transfer type to binary:
 - bin←***
- E. Change to the directory containing the image(s) you want to print:

lcd \directory←

e.g., "lcd d:", "lcd \images\IM-2"

F. Send the images to the printer:

send filename.ext← (for single images)

mput *.ext← (for multiple images)

e.g., "send image.tif" sends the image.tif file to the printer
"mput im*.ps" would send im-1.ps, im-2.ps, im-3.ps, etc. to the printer
"mput *.*" would send all files in the current directory to the printer

G. Log off the printer:

bye←

H. When you pick up your prints, be sure to sign them out on the log sheet.

III. PRINTING MULTIPLE COPIES OF THE SAME IMAGE

A. For fixed (#1) and scaled (#2) modes, append a number to the send command:

send filename.ext nn←

where *nn* indicates the number of copies
e.g., "send image.tif 12" gets you 12 copies of image.tif

B. For variable multi-format modes (#11-99):

1. Use an ASCII text editor (e.g., MS-DOS edit or Windows NotePad) to create a file with the following single line:

SETUP COPIES nn

where *nn* indicates the number of copies. The capital letters are mandatory. Give the file a name you'll remember (e.g., 3copies.txt)

2. Create a second ASCII file with the following single line:

CANCEL

The capital letters are mandatory. Name the file (e.g., cancel.txt)

C. Log on to a multi-format mode (#11-99) and set the transfer to binary.

D. Send the text file to the printer:

send filename.ext←

e.g., "send 3copies.txt"

- E. Send the appropriate number of images for the selected mode to the printer
- F. Return the settings of your userID to normal by sending the second text file:

send filename.ext←

- G. e.g., "send cancel.txt". Failure to do this means you will get *nn* copies of the next image you send using your UserID.
- H. Log off the printer

IV. CHANGING CONTRAST OR COLOR BALANCE

- A. Use an ASCII text editor (e.g., MS-DOS edit or edlin) to create a file with the following two lines:

**GAMMA=nn
TCR=nn**

where nn indicates a value for contrast (gamma) or color balance (TCR). The capital letters are mandatory. The default for gamma is 1.00, and larger numbers result in higher contrast. A value of GAMMA=1.80 works well for black and white fluorescence images. The default for TCR is 18, and smaller numbers produce images with more vivid colors. The default value works well, but may not match the color balance of your monitor. Give the file a name you'll remember. In the facility, these files are in the \TCP subdirectory and are named x-x--yy.np where x-x refers to gamma and yy refers to TCR (1-8--15.np sets gamma to 1.8 and TCR to 15)

- B. Logon to the system (password=0) and set the transfer to binary.
- C. Send the text file to the printer:

send filename.ext←

e.g., "send 1-8--15.np"

- D. You have to log on again to change to the printer mode you want to use:

user userid←

- E. Respond to the password prompt with the printer mode number.
- F. Send the image files as usual.
- G. The printer will remember the gamma and TCR settings for your userID. To reset them to the default, send the file 1-0--18.np to the system printer mode (password=0) after you logon with your UserID.

APPENDIX I. SIGNING ON TO THE CONFOCAL LISTSERVER

1. Use an e-mail program like PROFS or PINE to send a message to:

LISTSERV@UBVM.CC.BUFFALO.EDU.

Include the following line:

SUBSCRIBE CONFOCAL *firstname lastname*

Note that this message will be read by a machine, not a human.

2. You'll receive a standard message from the listserver explaining how to send a confirmation message, after which you'll be on the list.
3. The archives of the confocal listserver, as well as example images, vendor information, etc. is available from the Multidimensional Microscopy World Wide Web server at

<http://arts.eng.buffalo.edu/index.html>.

APPENDIX II. STANDARD FILTER COMBINATIONS

- I. For detecting Fluorescein only (with PMT-3):
 - A. Use the 488nm Kr/Ar laser line
 - B. DBS-1: #1 (FT 488/568)
 - C. DBS-2: #2 (FT 630)
 - D. DBS-3: #1 (mirror)
 - E. Em-1: (PMT-1 not used)
 - F. Em-3: #1 (LP 515)
 - G. Em-2: (PMT-2 not used)

- II. For detecting Rhodamine only (with PMT-1)
 - A. Use the 568nm Kr/Ar laser line
 - B. DBS-1: #1 (FT 488/568)
 - C. DBS-2: #3 (Free)
 - D. DBS-3: (PMT-3 not used)
 - E. Em-1: #1 (LP 590)
 - F. Em-2: (PMT-2 not used)
 - G. Em-3: (PMT-3 not used)

- III. For detecting Cy5 only: (using PMT-1):
 - A. Use the 633nm HeNe laser line
 - B. DBS-1: #2 (NT 80/20 mirror)
 - C. DBS-2: #3 (Free)
 - D. DBS-3: (PMT-3 not used)
 - E. Em-2: Remove this slider from the PMT2 slot, invert it, and insert into the PMT1 slot: #1 (BP 670-810)
 - F. Em-1,3: (PMT-2, 3 not used)

- IV. For detecting Fluorescein (PMT-3) and Rhodamine (PMT-1):
 - A. Use the 488 and 568nm laser lines (either simultaneously or sequentially)
Warning: simultaneous excitation permits fluorescein signals to cross into the rhodamine channel
 - B. DBS-1: #1 (FT 488/568)
 - C. DBS-2: #1 (FT 560)
 - D. DBS-3: #1 (Mirror)
 - E. Em-1: #1 (LP 590)
 - F. Em-2: (PMT-2 not used)
 - G. Em-3: #3 (BP 515-540) *Warning:* #2 (BP 515-565) will allow 488nm-excited rhodamine emissions to cross into this channel.

- V. For detecting Fluorescein (PMT-3) and Cy5 (PMT-1):
 - A. Use the 488 and 633nm laser lines (either simultaneously or sequentially)
 - B. DBS-1: #2 (NT 80/20)
 - C. DBS-2: #2 (FT 630)
 - D. DBS-3: #1 (Mirror)
 - E. Em-2: : Remove this slider from the PMT2 slot, invert it, and insert into the PMT1 slot: #1 (BP 670-810)
 - F. Em-1: (PMT-2 not used)
 - G. Em-3: #2 (BP 515-565).

- VI. For detecting Fluorescein (PMT-3), Rhodamine (PMT-2) and Cy5 (PMT-1):
- A. Use all laser lines
 - B. DBS-1: #2 (NT 80/20 mirror), or switch between #2 for Cy5 detection and #1 for rhodamine and fluorescein detection
 - C. DBS-2: #2 (FT 630)
 - D. DBS-3: #2 (FT 560)
 - E. Em-2: Remove this slider from the PMT2 slot, invert it, and insert into the PMT1 slot: #1 (BP 670-810)
 - F. Em-1: Remove this slider from the PMT1 slot, invert it, and insert into the PMT2 slot: #3 (BP 590-610) *Warning:* #2 (BP 575-640) will allow 568nm-excited Cy5 emissions to cross into this channel.
 - G. Em-3: #2 (BP 515-540)