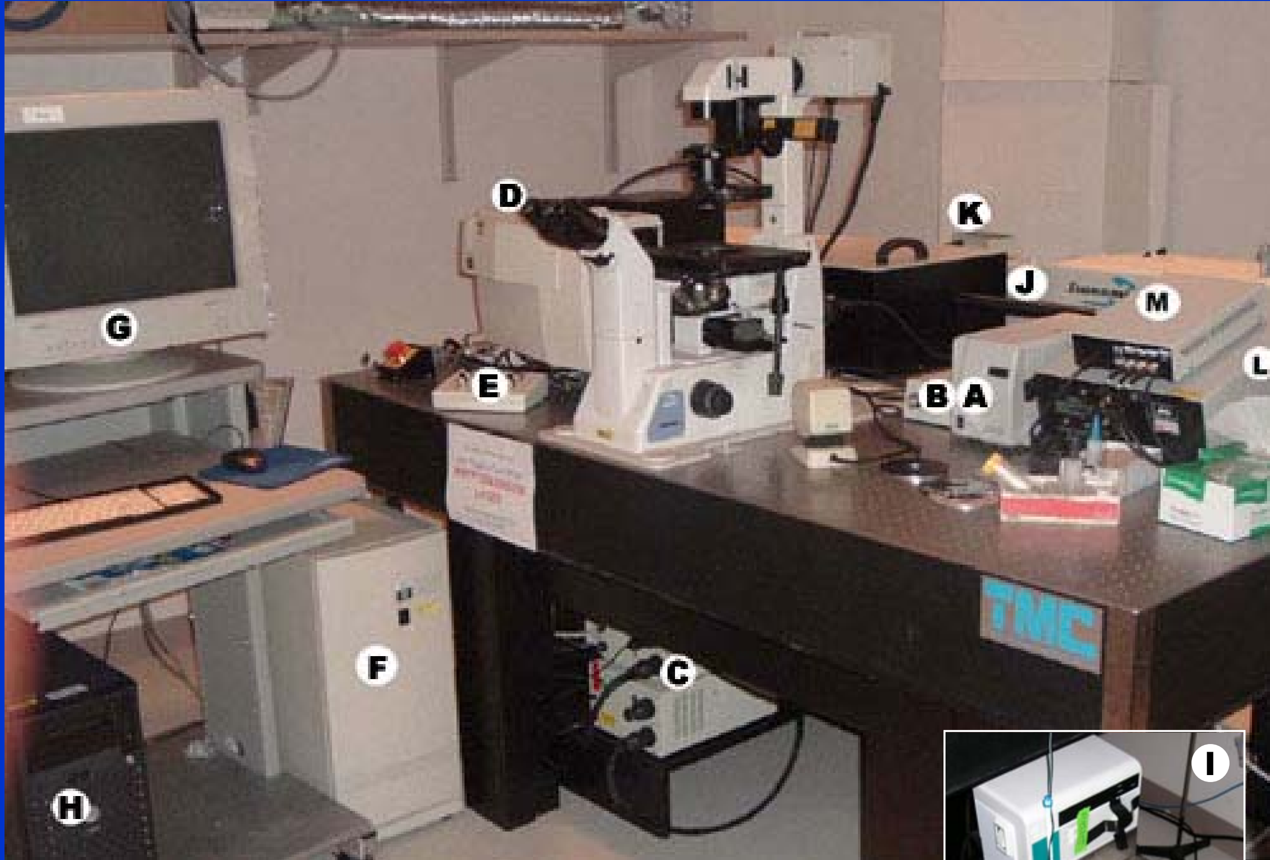
Indiana Center for Biological Microscopy

BioRad MRC 1024 MP

Confocal & Multi-Photon Microscope

Microscope and the Attached Accessories



A: Mercury Lamp

B: Transmission Light

C: Kr/Ar Laser *(For Confocal Only)*

D: Detector

E: Toggle Switch Remote

F: BioRad Controller Box

G: Monitor

H: Computer

2 Photon Accessories:

I: Chiller

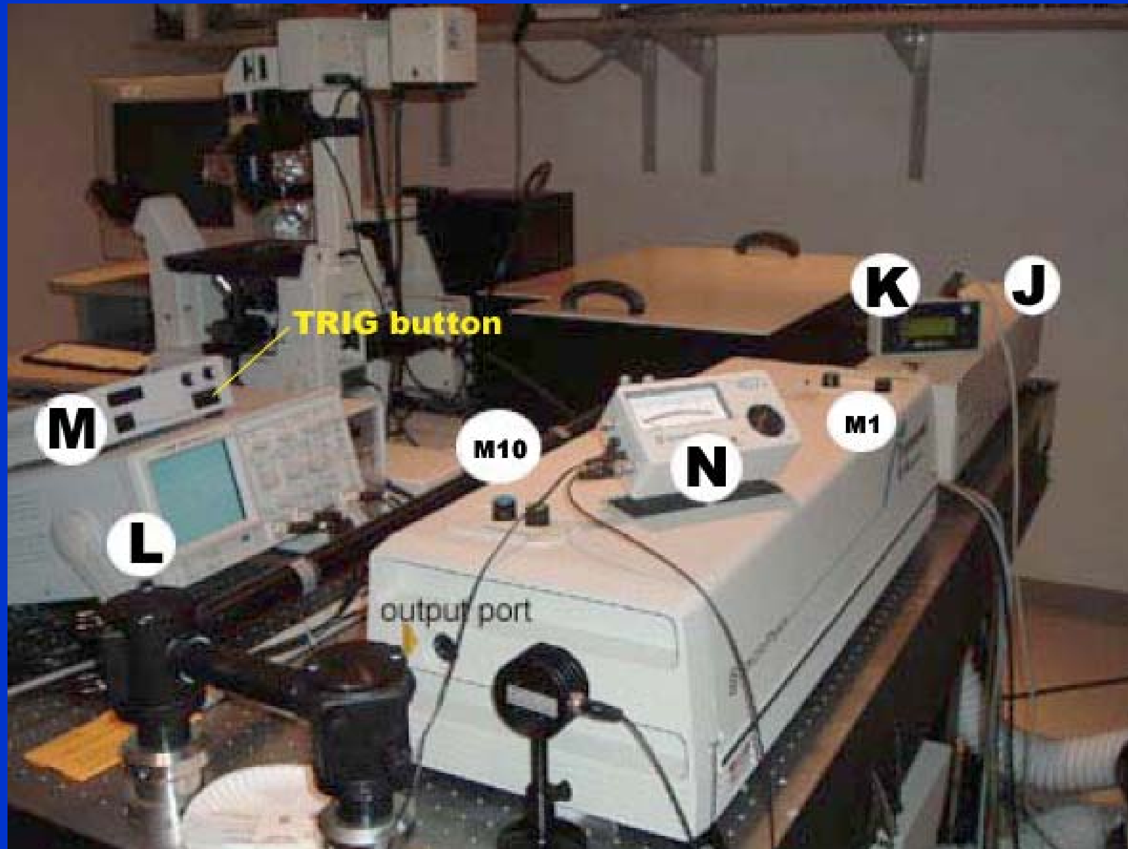
J: Pump Laser *(Titanium-Sapphire)*

K: Pump Laser Control

L: Oscilloscope

M: Reese Meter

Attached Accessories *(continued)*



2 Photon Accessories:

J: Pump Laser *(Titanium-Sapphire)*

K: Pump Laser Control

L: Oscilloscope

M: Reese Meter

- TRIG button

N: Laser Spectrum Analyzer

M10: Mirror 10 Knob

M1: Mirror 1 Knob

Starting Up
The Confocal (Single Photon) System

Starting Up The Confocal (Single Photon) System

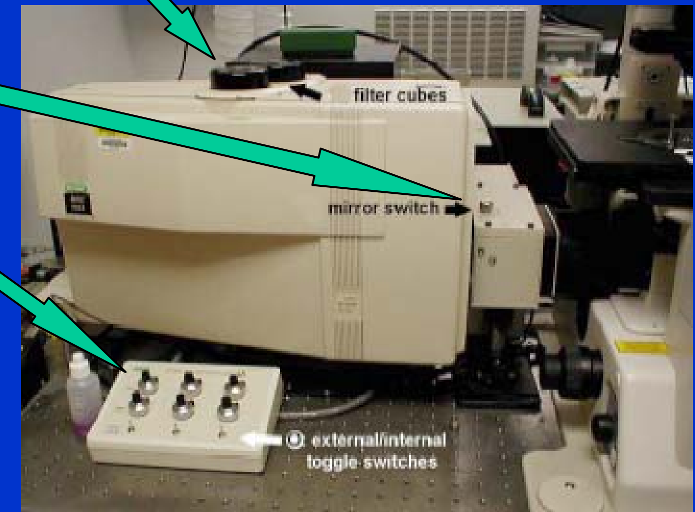
1. Turn on the **MERCURY LAMP** located on the table to the right of the microscope.
   (Once this lamp has been turned off, it should NOT be turned on again for 30 minutes).

2. Turn on the **TRANSMISSION LIGHT** located next to the mercury lamp.

3. Make sure that the **1P *(Single Photon)* FILTER CUBE** is inserted into the detector to the left of the microscope.

4. Set the **MIRROR SWITCH** to **INTERNAL,** as well as the **TOGGLE SWITCHES** on the remote.

5. Turn on the **Kr/Ar LASER** *(turn the key **CLOCKWISE)*** located on the floor just below the microscope.



Starting Up The Confocal (Single Photon) System

6. Turn on the rocker switch to the BIORAD CONTROLLER BOX located on the floor to the right of the computer table.

7. Turn on the MONITOR and the COMPUTER (left button on front of computer).

8. Next, LOGON to the computer, using your USERID and PASSWORD.

9. Launch the LaserSharp 2000 icon on the desktop.

10. Select your USERID and enter your PASSWORD.

Starting Up
The 2 Photon System

Starting Up The 2 Photon System

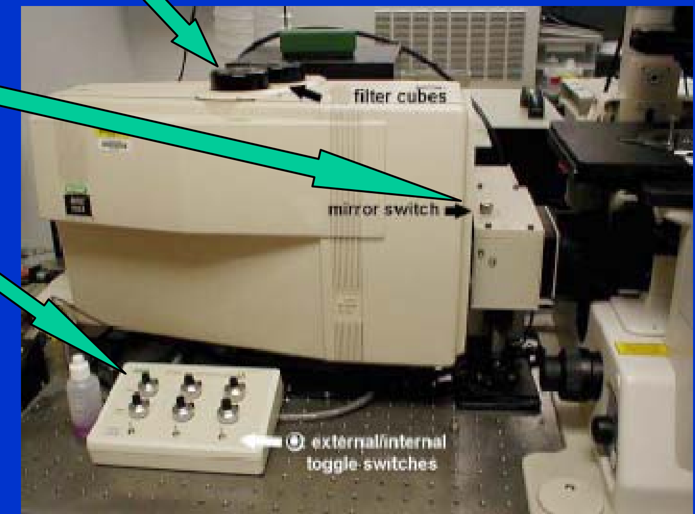
1. Turn on the MERCURY LAMP located on the table to the right of the microscope. *(Once this lamp has been turned off, it should NOT be turned on again for 30 minutes).*

2. Turn on the TRANSMISSION LIGHT located next to the mercury lamp.

3. Make sure that the 2P *(2 Photon)* FILTER CUBE is inserted into the detector to the left of the microscope.

4. Set the MIRROR SWITCH to EXTERNAL, as well as the TOGGLE SWITCHES on the remote (*depends on how many channels you are using).*

5. If you think you will need the Confocal Laser (Kr/Ar LASER) and it is currently off, turn it on by turning the key CLOCKWISE.



Starting Up The 2 Photon System

6. Turn on the rocker switch to the BIORAD CONTROLLER BOX located on the floor to the right of the computer table.

7. Turn on the MONITOR and the COMPUTER (left button on front of computer).

8. Next, LOGON to the computer, using your USERID and PASSWORD.

9. Launch the LaserSharp 2000 icon on the desktop.

10. Select your USERID and enter your PASSWORD.

11. Turn on the CHILLER for the Pump Laser control, located on the floor just below the Titanium-Sapphire Laser.

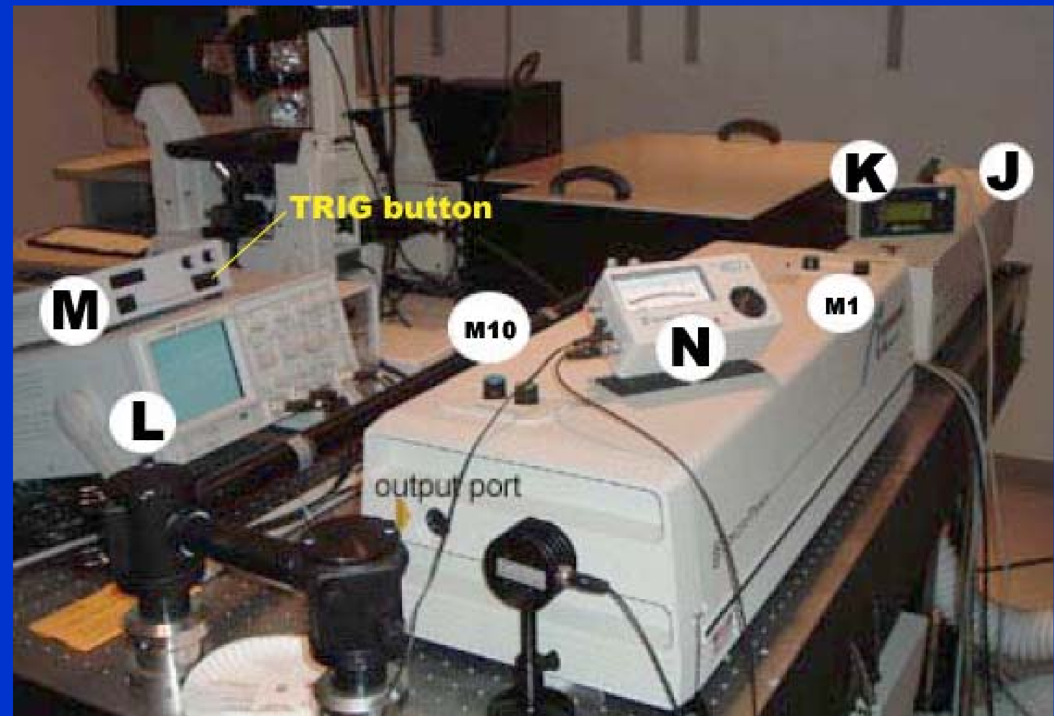
Starting Up The 2 Photon System

12. Press the power button on the PUMP LASER control (K). Message will state, "System Warming Up." *(It takes approximately 5 minutes to warm up).*
    a. Once the system has warmed up, PRESS AND HOLD THE POWER KEY until the laser starts *("laser emission" light will stop flashing).*
    b. Release the button and WAIT until the laser reaches the designated power *(5.00 W).*
    c. Allow the laser to equilibrate for approximately 15 minutes before proceeding.

13. Turn on the OSCILLOSCOPE (L) *(button on front panel).*

14. Turn on the REESE METER (M) located on top of the oscilloscope *(button on back panel – ALSO, press the* TRIG button.

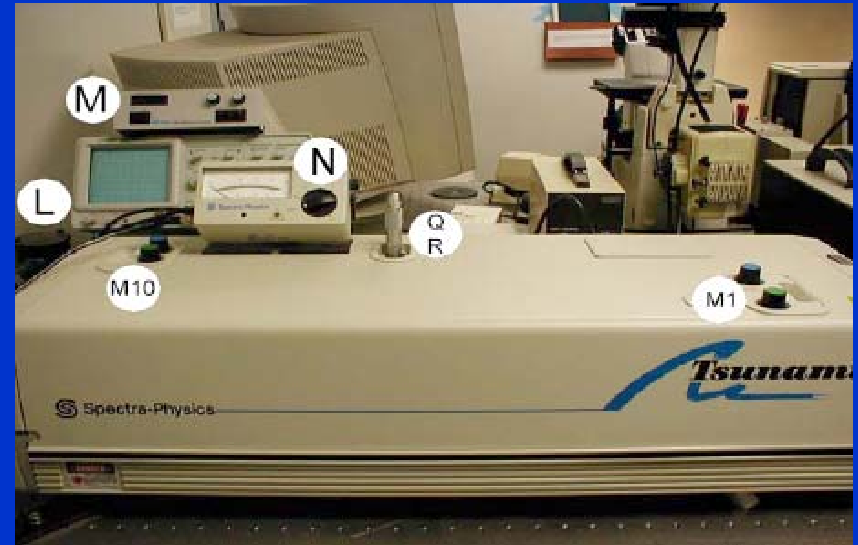
15. Turn the LASER SPECTRUM ANALYZER (N) to "1", if turned off.



Starting Up The 2 Photon System

16. Align the system using Mirror 1 (M1) and Mirror 10 (M10) knobs.

17. IMPORTANT: Gently tweak the knobs until the highest reading is established on the LASER SPECTRUM ANALYZER (N) in the following order:



  a. Adjust M1 *(blue knobs)*

  b. Adjust M10 *(green knobs)*

  c. If there is no reading or there is difficulty in reaching a substantial reading, ask for help.

  d. NEVER TWIST OR TURN KNOBS TO ANY GREAT EXTENT!

Starting Up The 2 Photon System

18. Acquiring Mode Lock:

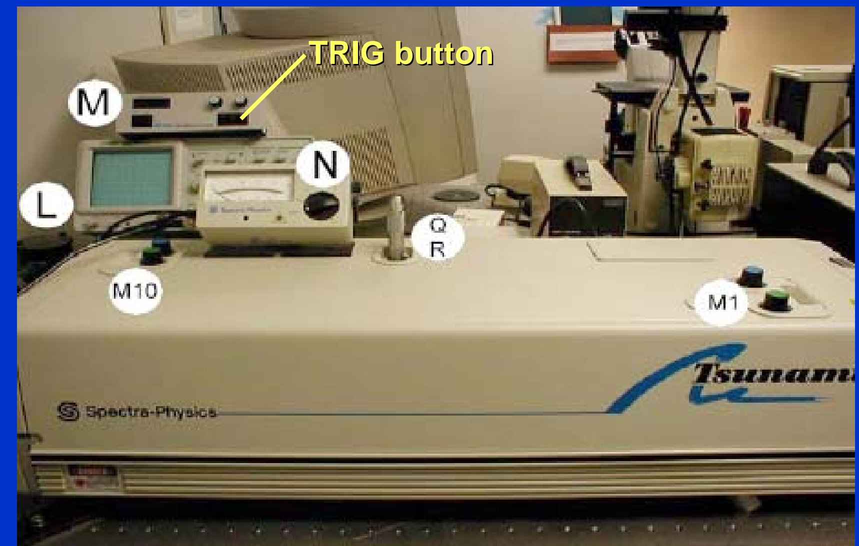
    a. Set the REESE METER (M) to 800nm.

    b. Press the TRIG button on the Reese Meter.

    c. Gently adjust the SLIT POSITION (Q) and the PRISM COMPENSATION (R).

19. If you are unable to see a peak or a bell-shaped curve, look at the chart below to help adjust the slit and compensation settings:

nM	SLIT	Prism
740	8.62	8.14
760	10.34	10.29
780	10.5	11.45
800	11.30	12.40
820	11.82	13.32
840	12.34	14.88
860	12.87	15.05
880	13.34	16.08



Acquiring Images

Preparing Your Specimen To Image

1. Setting up the microscope:
   a. Choose an objective lens *(see chart below)*:
   b. Place appropriate fluid on objective.

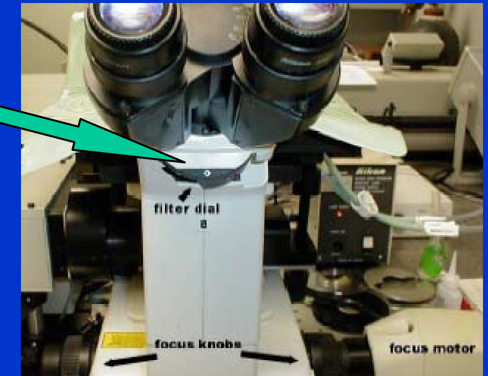
Lens Type	Immersion Media	NA/WD(mm)	Coverslip Thickness (mm)	DIC	Brightfield	Phase	Fluor
20X Fluor	Water/Glycerol/Oil	0.75/0.35	0 – 0.17	YES	YES	NO	YES
60X Apo	Water	1.2/0.29	0.15 – 0.18	YES	YES	NO	YES
63X Apo	Oil	1.4/0.17	0.17	YES	YES	NO	YES
100X Apo	Oil	1.4/0.13	0.17	YES	YES	NO	YES

*NA = Numerical Aperture

WD = Working Distance

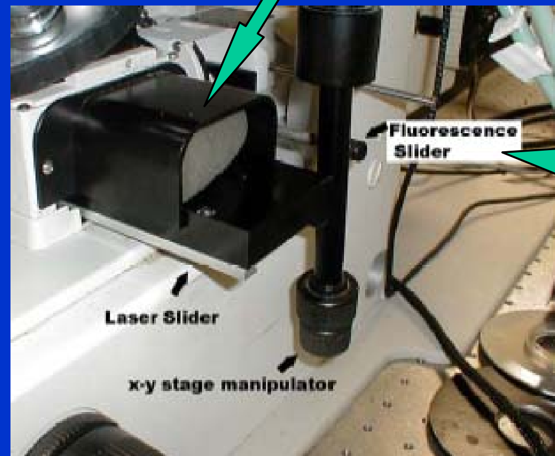
Preparing Your Specimen To Image

2. Turn the filter selector dial (just below the oculars) to the **"O" position.**



To View Specimen Using Transmitted Light:

3. Push the **LASER SLIDER IN** on the right side of the microscope below the stage.
4. Press the **WHITE BUTTON** on lower left side of microscope.



To View Specimen Using Epifluorescent Light:

5. Push the **LASER SLIDER IN.**
6. Push the **EPIFLUORESCENCE SLIDER IN.**

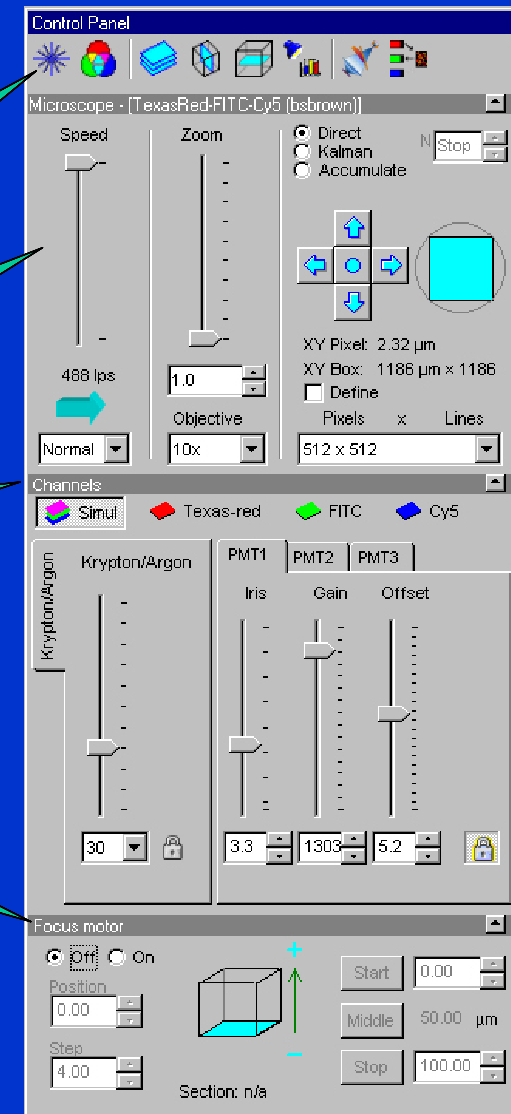
Be careful not to bleach the specimen with excessive mercury light!

Using the LaserSharp 2000 Software

Using the LaserSharp 2000 Software

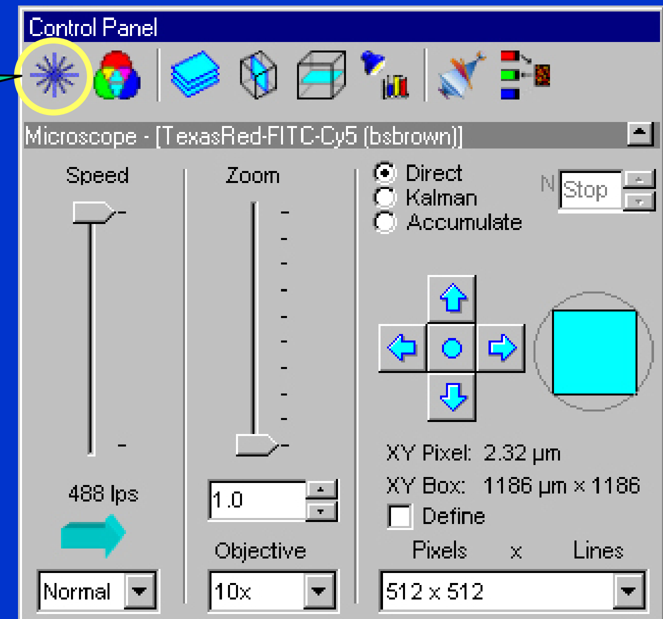
1. Click on METHODS from the top menu bar to select which method is needed.

2. The Control Panel Toolbox is subdivided into the following sections:
   a. Menu Bar
   b. Microscope
   c. Channels
   d. Focus Motor (Z steps)



Using the LaserSharp 2000 Software

The **MENU BAR** consists of the following options:



1. **Live Scan:**
   - Scans an individually selected channel.
   - Scans multiple channels simultaneously.
2. **Sequential Live Scan:**
   - Scans multiple channels one at a time.
3. **Z-Stack Collection**
4. **X-Z Stack Collection**
5. **X-T Line Scan**
6. **Optic:**
   - Gives user the ability to change the lasers and filters within a method.
7. **Mix:**
   a. PMT Mode:
      - Assigns which PMTs are visible.
      - PMT 1 = red / PMT 2 = green / PMT 3 = blue *(2-Photon = DAPI; Confocal = Cy5)*

   b. Transmission Mode (TLD):
      - Mostly used for making complicated DIC photos.

Using the LaserSharp 2000 Software

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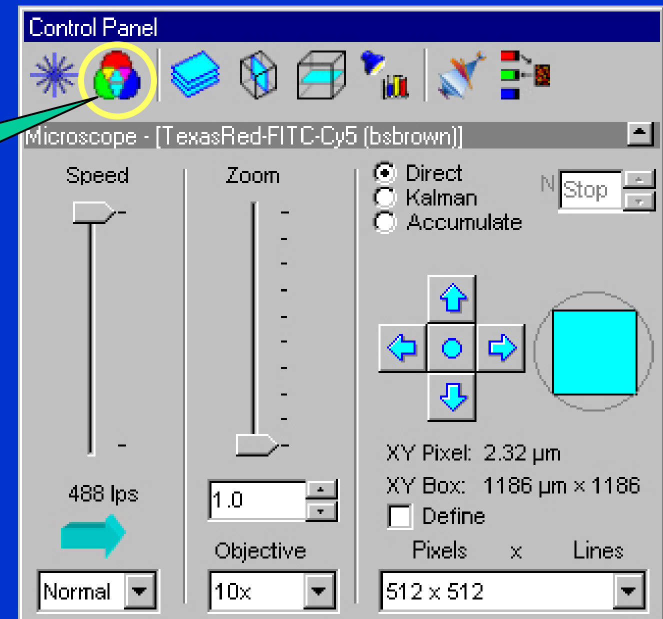

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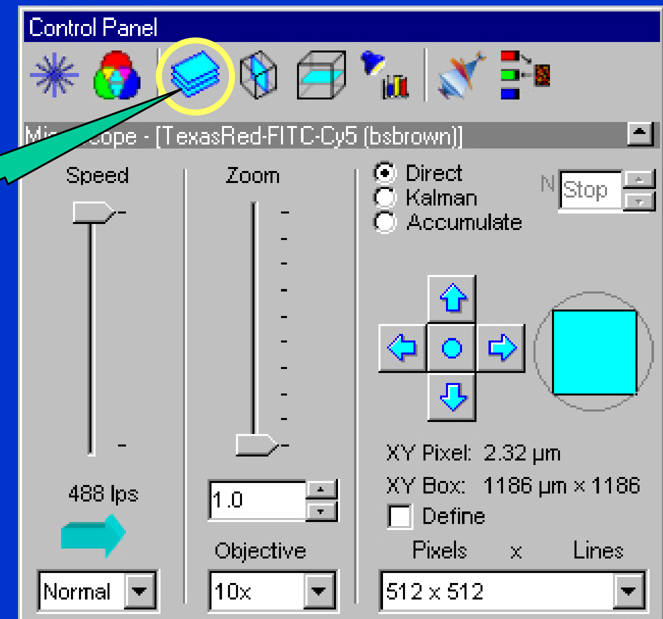

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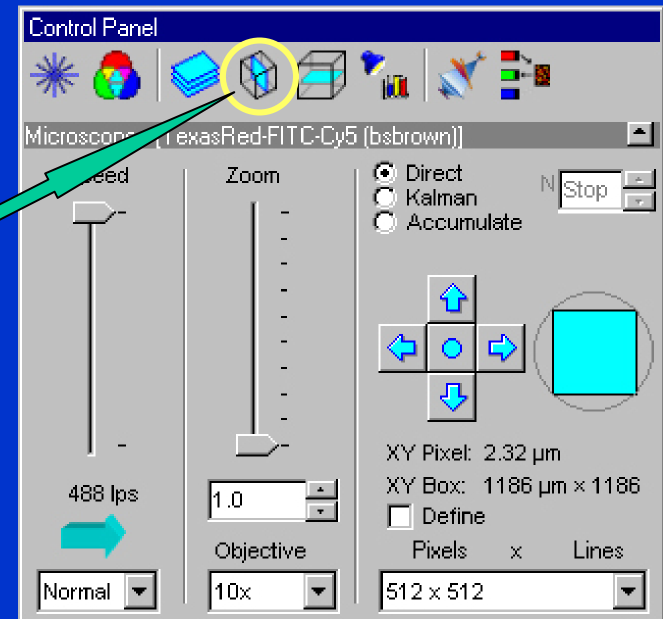

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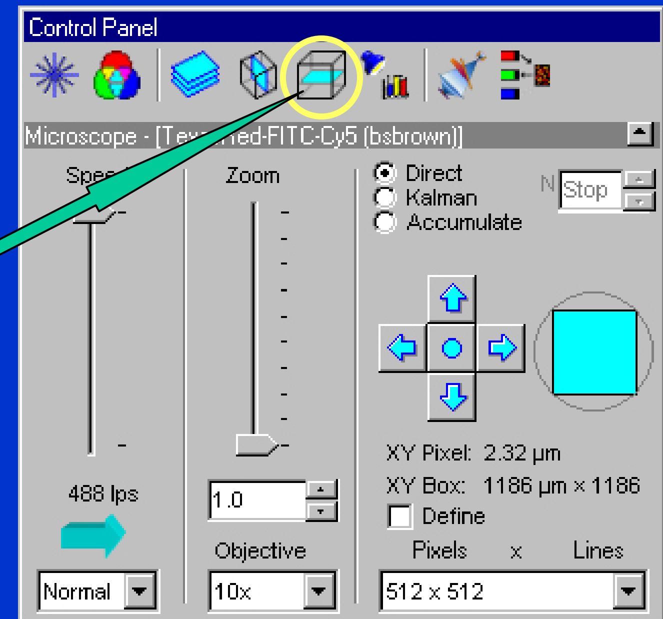

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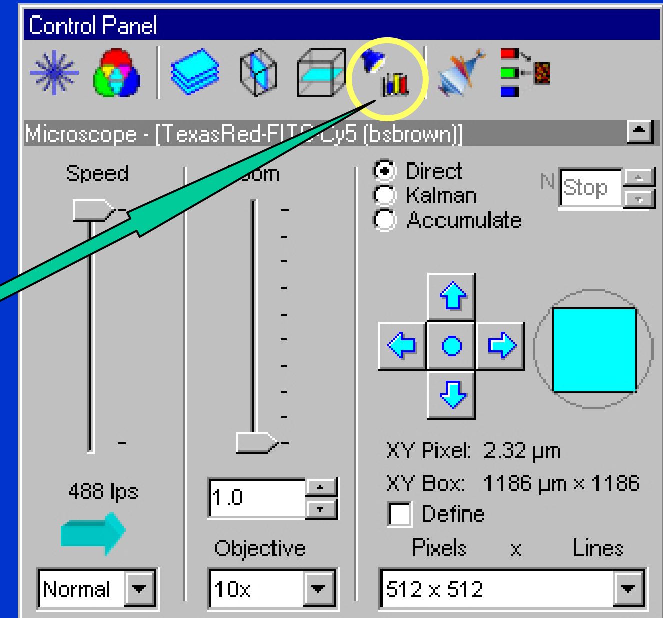

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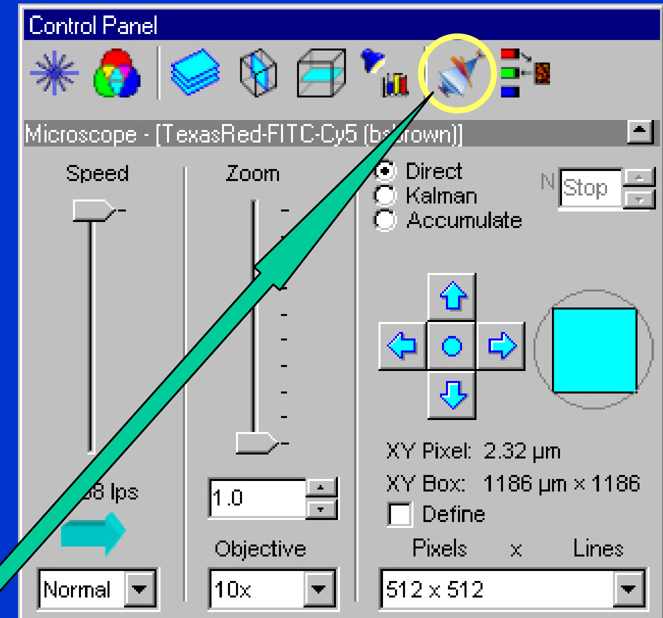

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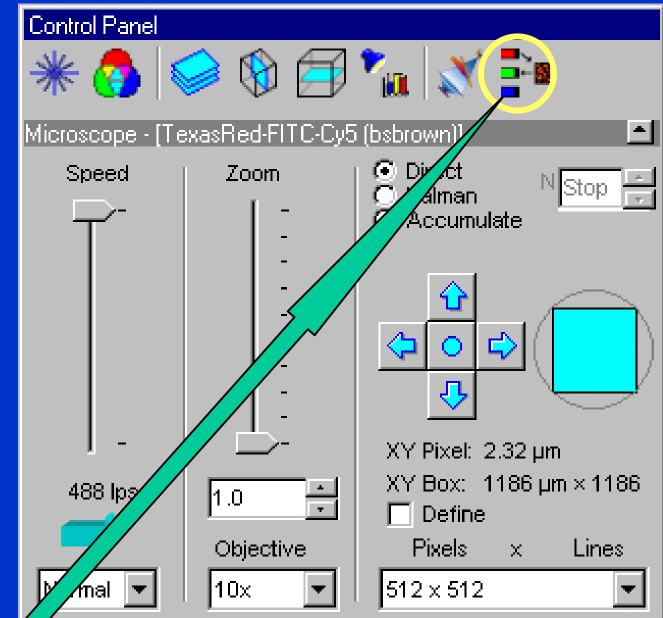

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3. Z-Stack Collection
4. X-Z Stack Collection
5. X-T Line Scan
6. Optic:
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7. Configure Mixers:
   a. PMT Mode:
      - Assigns which PMTs are visible.
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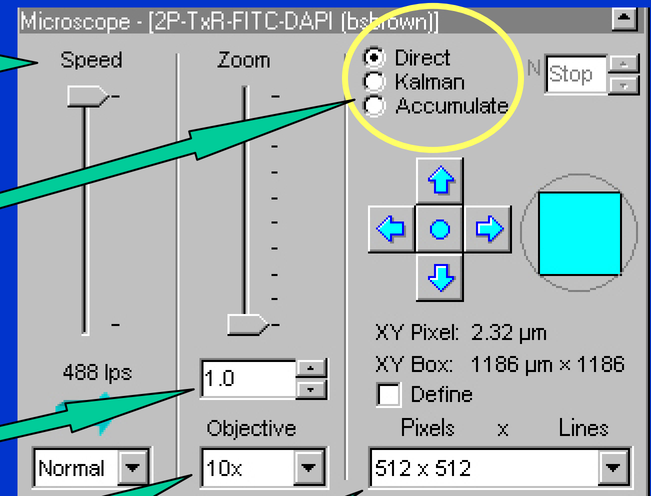
   b. Transmission Mode (TLD):
      - Mostly used for making complicated DIC photos.

Using the LaserSharp 2000 Software

The MICROSCOPE panel consists of the following options:

1. **Speed:**
   - Fast or normal for live viewing.
   - Normal or slow for imaging.
2. **Filter:**
   - Direct for live viewing.
   - Kalman and number of scans to average for imaging.
3. **Zoom:**
   - Affects the dimensions of the image collected.
4. **Objective:**
   - Used to calculate the pixel size and stored with the image.
5. **Image Size:**
   - Used to set your image size in pixels.

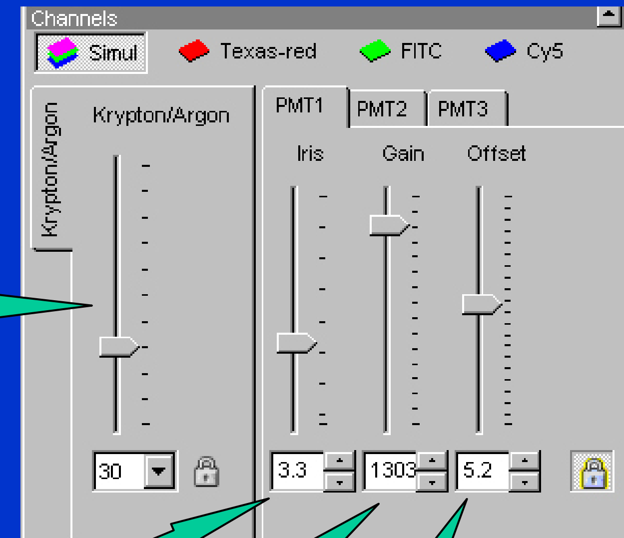


Using the LaserSharp 2000 Software

The CHANNELS panel consists of the following options:

1. **Channels:**
   - Allows the selection of all channels for simultaneous scanning or individual channels.

2. **Laser:**
   - Allows you to control the power output of the laser.



3. **PMT Mode (for Confocal ONLY):**
   - Iris - the pinhole diameter and should be the same on all channels.
   - Gain - used for adjusting brightness/saturation (red pixels).
   - Offset - used for black level/background darkness (green pixels).

Collecting A Series

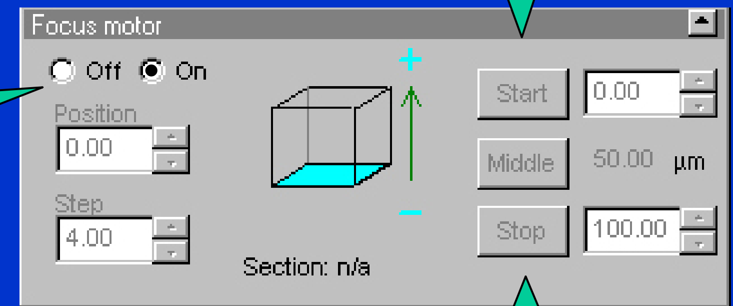
Collecting a Series

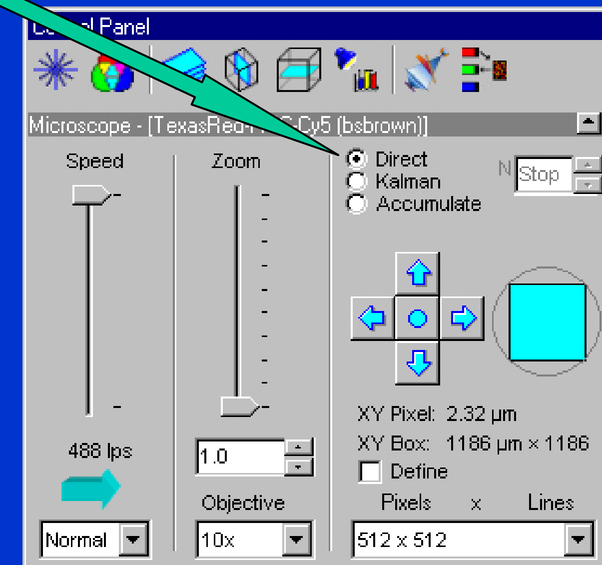
1. In the Control Panel under Channels, optimize each channel separately.

2. Under Focus Motor, click ON.

3. Using both hands, gently slide the Focus Motor into place.

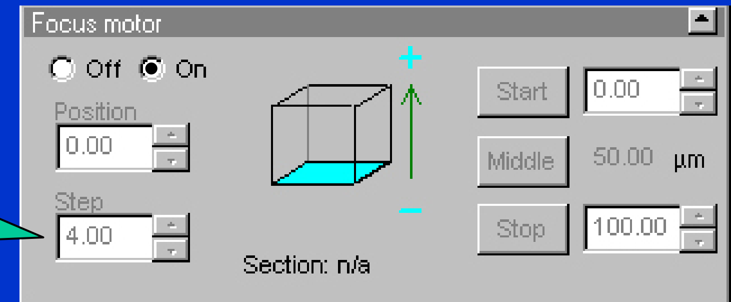
4. To set your START AND STOP POSITION, use DIRECT scanning.



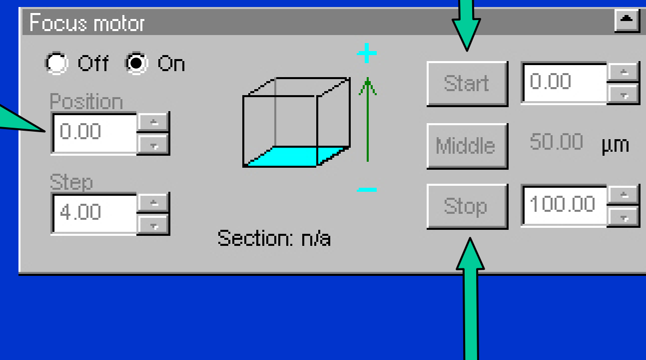


Collecting a Series

5. Select a STEP SIZE or Z-Step *(thickness of plane)* to the interval you want between sections *(i.e. 0.5 µm)*.



6. While scanning in DIRECT mode, use the DOWN ARROW beside the POSITION BOX, to focus to one side of the sample and click START *(bottom of stack)*.

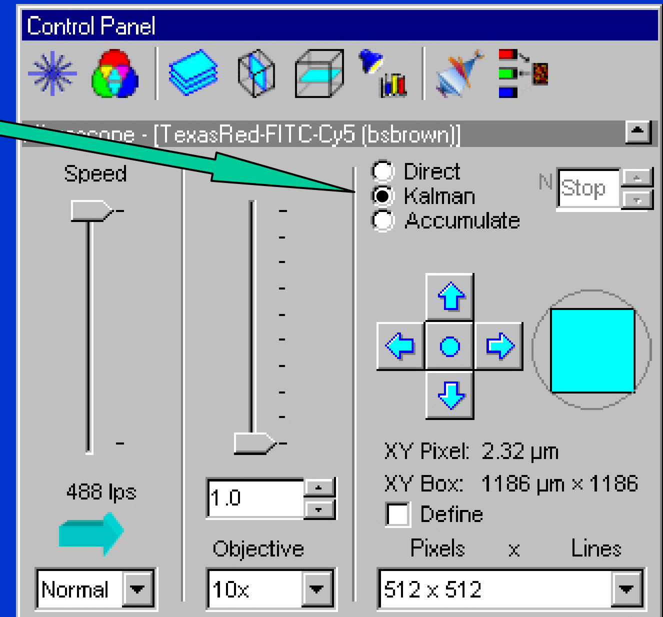


7. Next, select the UP ARROW to focus to the other side of the sample and click STOP *(top of stack)*.

Note:
For best results, collect the series from bottom to top.

Collecting a Series

7. To collect the series, select KALMAN mode in the control panel.

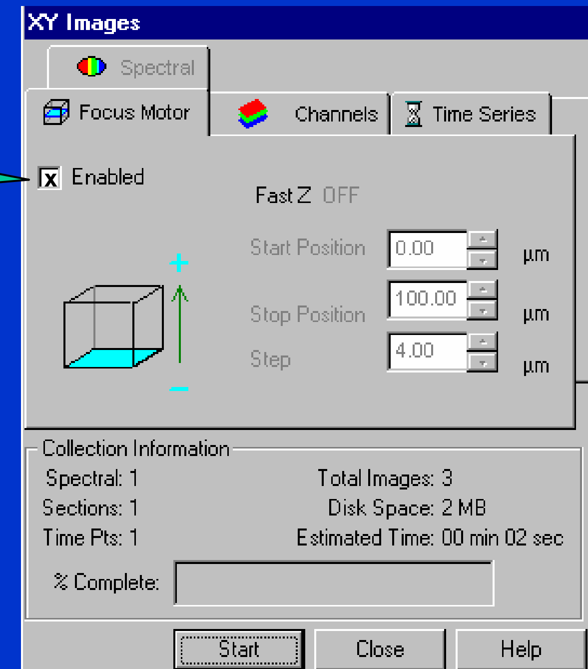


8. Click Z-Stack on the menu bar *(the XY Image panel will appear).*

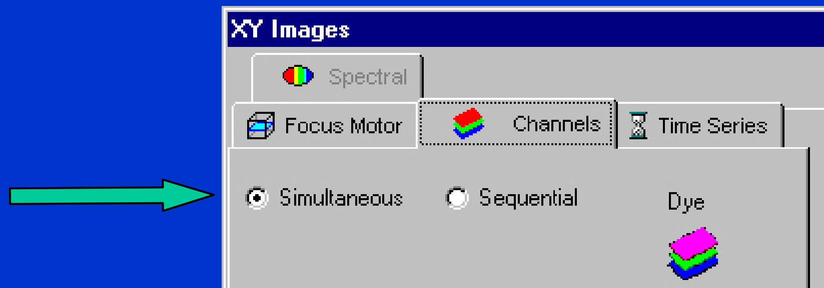
Collecting a Series

9. The XY IMAGE PANEL has three tabs:

a. **Focus Motor:**
Click ENABLED.

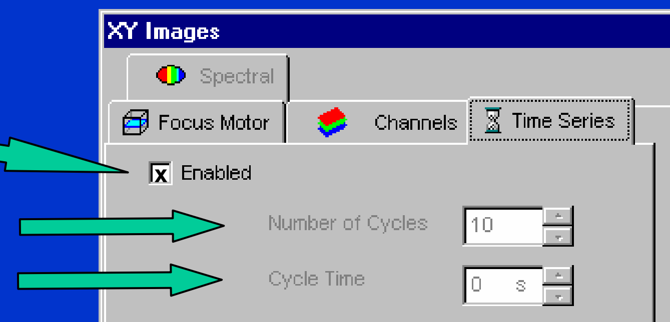


b. **Channels:**
Select SIMULTANEOUS for all 2 Photon and most Confocal imaging.



c. **Time Series:**
Click ENABLED.
Set the number of CYCLES.
Set the CYCLE TIME.

Click START.
SAVE when Time Series is completed.



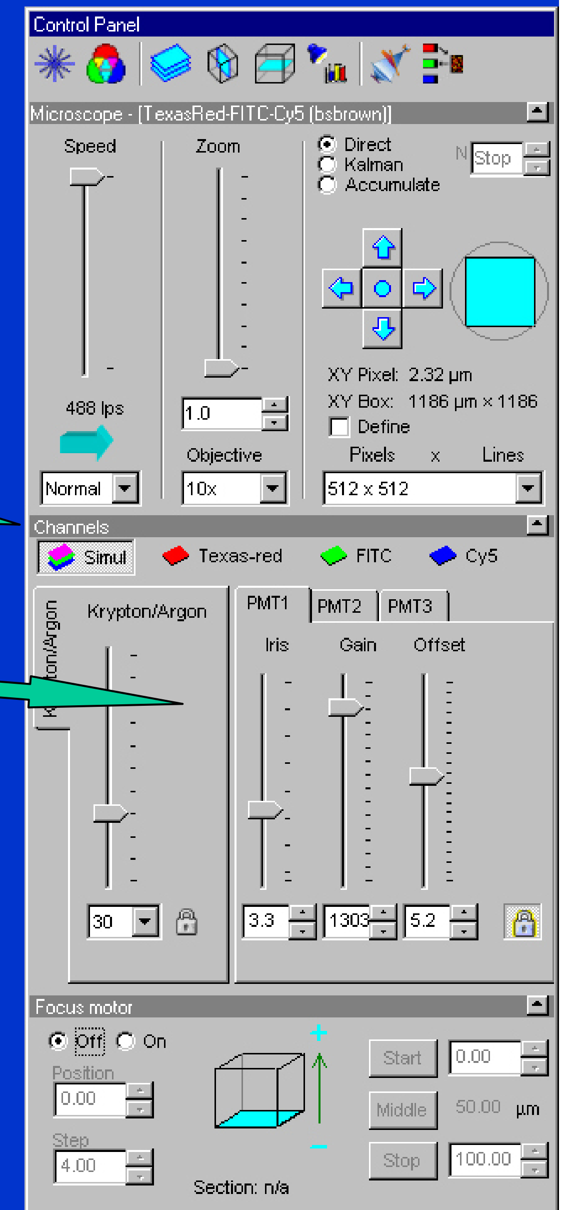
Collecting A Sequential Series

Collecting a Sequential Series

It is not recommended to use this microscope for sequential series. It is much more time consuming and the dyes are more likely to fade since the images must be collected frame by frame. Either Zeiss microscopes would serve better for this purpose.

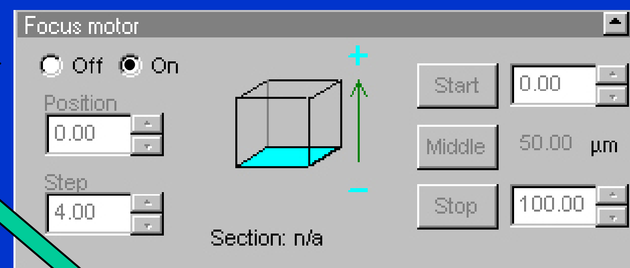
However, if you have subject matter that requires an inverted microscope, requires separate channel intensities or have a definitive problem with crosstalk, then refer to the following protocol:

1. In the Control Panel under **CHANNELS,** click the **RED.**
2. Set the laser, neutral density %, Iris, Gain and Offset for **PMT1.**
3. Click the **GREEN** and do the same for **PMT2.**
4. If necessary, click the **BLUE** and do the same for **PMT3.**



Collecting a Sequential Series

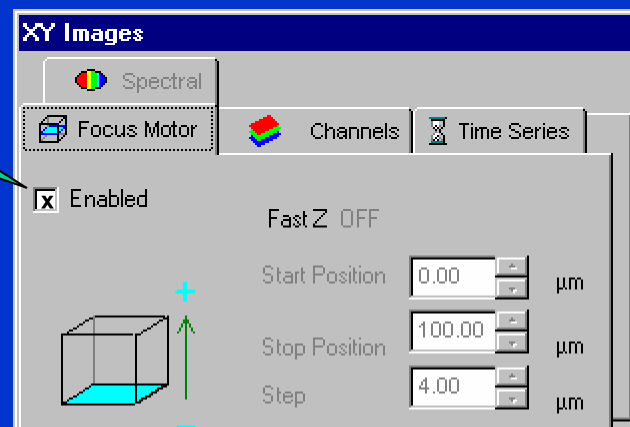
5. Go to FOCUS MOTOR and select ON.

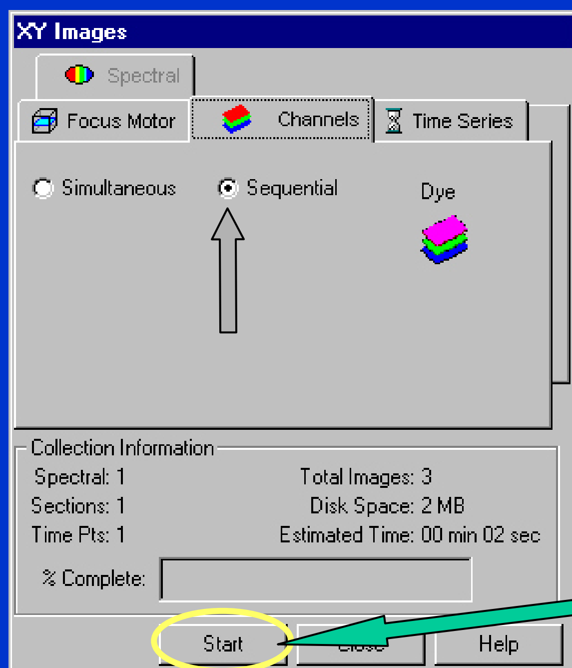


6. Select Z_STACK from the Control Panel menu bar.

7. In the XY IMAGE panel:

   a. Click on the FOCUS MOTOR tab.
      Select ENABLED.



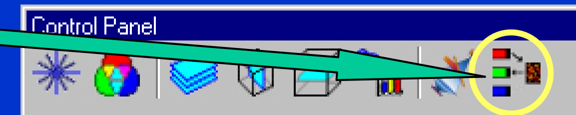


   b. Click on the CHANNELS tab.
      Select SEQUENTIAL.
      Click START.

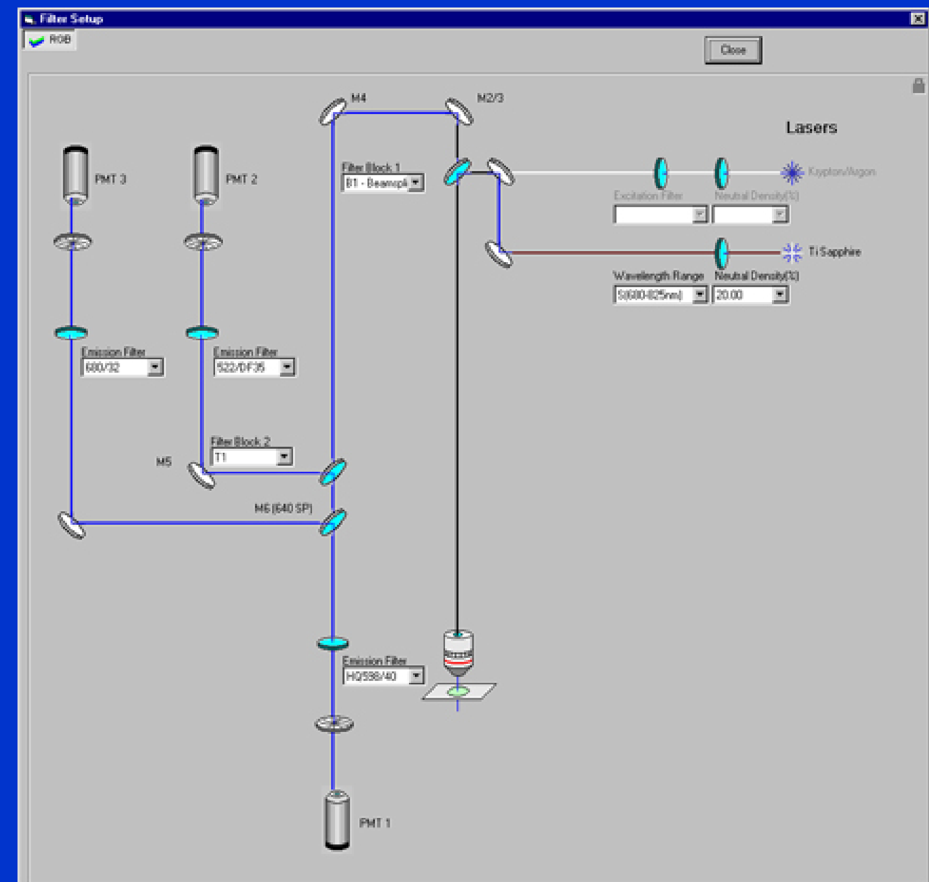
Collecting A Transmitted Light Image

Collecting a Transmitted Light Image

1. Bring the condenser very close to sample.
2. On the Control Panel Menu Bar, select Configure Mixers.
3. Select TLD on the Mixer C *(panel 3, blue channel, CY5).*



4. Select the 647nm (RED) filter *(use all lines to initially and then check to see which generates the best image).*
5. Click Transmission and use 100??? in the proper channel.
6. Now move the SHUTTER *(lever-like switch on the top of microscope)* just until the DIC picture appears on the monitor *(about half way in the range of movement).*



SHUTDOWN Procedure - *CONFOCAL*

1. QUIT the LaserSharp 2000 software.
2. TRANSFER all of your files to Imaging 4 or Imaging 5 and DELETE them from the BioRad computer (all files will be deleted if they are not removed!).
3. Select START -> SHUTDOWN.
4. After you see a message stating, *"It is now safe to turn off the computer,"* PRESS THE LEFT BUTTON on the front panel of the computer.
5. Turn off the MONITOR.
6. Turn off the BIORAD CONTROLLER BOX.
7. Turn off the TRANSMISSION LIGHT.
8. Turn off the MERCURY LAMP.
9. Turn off the Kr/Ar LASER by turning the key to the VERTICAL POSITION.
10. Remove your samples and clean the objective.

SHUTDOWN Procedure – *2 PHOTON*

1. QUIT the LaserSharp 2000 software.
2. TRANSFER all of your files to Imaging 4 or Imaging 5 and DELETE them from the BioRad computer *(all files will be deleted if they are not removed!).*
3. Select START -> SHUTDOWN.
4. After you see a message stating, *"It is now safe to turn off the computer,"* PRESS THE LEFT BUTTON on the front panel of the computer.
5. Turn off the MONITOR.
6. Turn off the TRANSMISSION LIGHT.
7. Turn off the MERCURY LAMP.
8. Turn off the BIORAD CONTROLLER BOX.
9. Turn off the PUMP LASER control *(press the power button once).*
10. Turn off the OSCILLOSCOPE.
11. Turn off the REESE METER.
12. Turn off the CHILLER.