

## System Start-UP

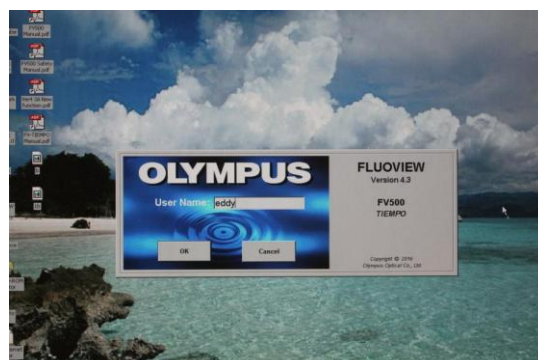
1. Turn ON main switch of Conrad Power- Manager .



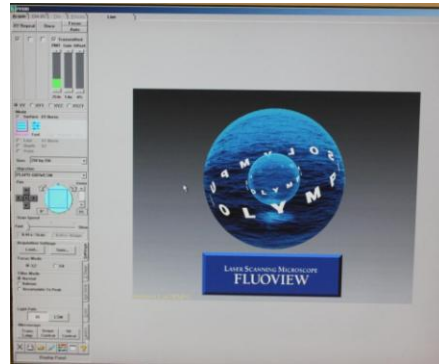
2. Press Power button on PC box.



3. Wait 3 min for microscope system to initialize, and then double click on FLUOVIEW icon. The password is: **eddy** .

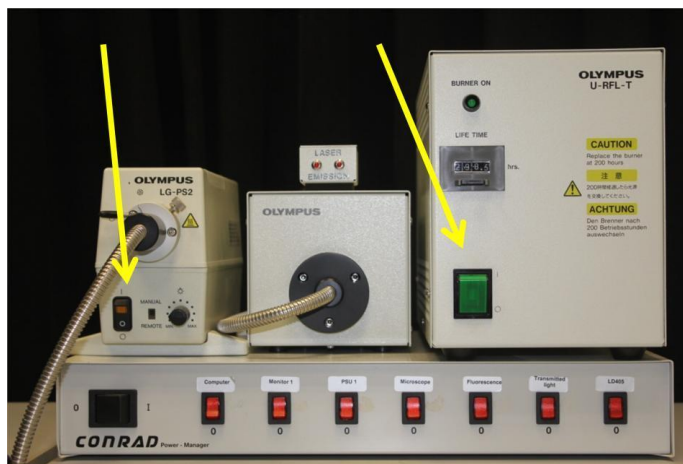


Wait about 1min till software window will appears.



Remove cover from microscope.

4. Turn ON transmitted light and fluorescence lamp (if necessary!!!!).

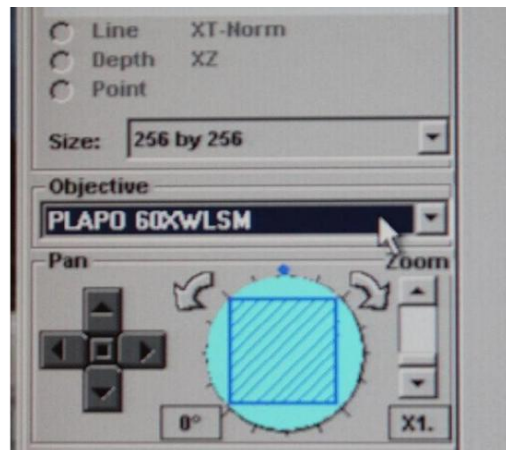


Wait 2 min. Be sure what fluorescence lamp is working.

5. Select appropriate objective using Manual control box, or via Fluoview software



6. If you will use x40 or x60 water immersion objectives , do not forget drop water from universal vial ( one drop !!!).

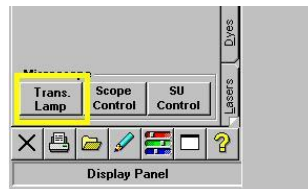


If x60 oil immersion – oil .

Put objective in the middle of the microscope plate circle.

7. Our microscope is inverted, so turn to the other side you prepared slide before landing on microscope plate.

8. Use TSHT button in manual control box to ON-OFF usual light or turn on the transmitted light button (*Trans. Lamp*) within the software in the bottom left hand corner of the screen.



Adjust focus via focus knobs. Turn transmitted light OFF.

9. If you use fluorescence dyes , select appropriate fluorescence filter cube:



WU – for UV observation ( DAPI, calcofluor, etc.) NB – blue excitation ( eGFP, CY2, FITC, etc.)

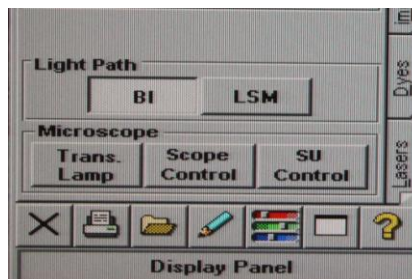
WG – green excitation ( CY3, RFP, PI, etc)

CFP-YFP – special double-bands filter cube for CFP-YFP observation.

Use RSHT button in manual control box to ON-OFF fluorescence light. Find an interesting object.

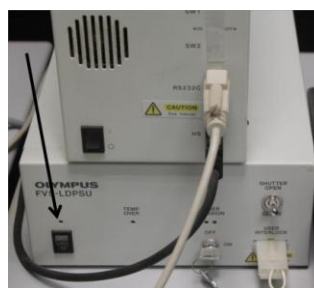
10. Switch OFF fluorescence light via RSHT button.

Using FluoView select light pass to LSM.



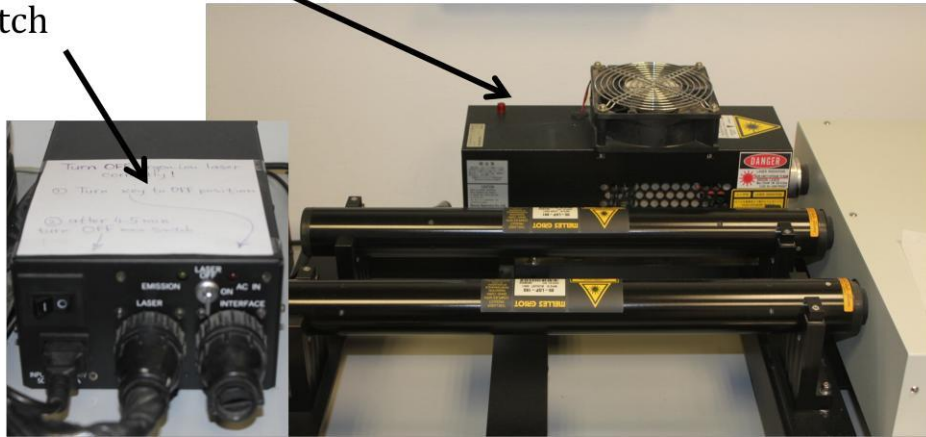
11. In accordance with fluorescence stain switch ON lasers . **ONLY**

**NECESSARY LASERS!!!!!!** 405 nm diod laser for DAPI, calcofluor etc .

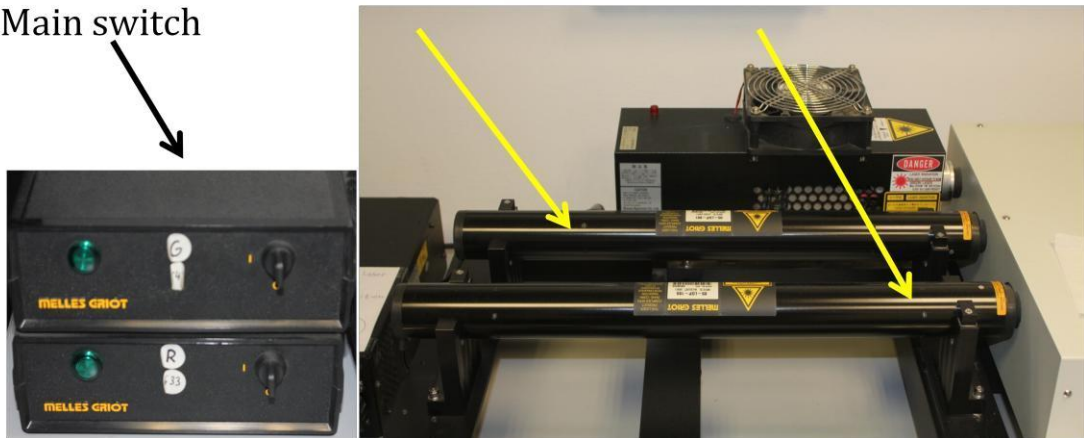


**Multiline Argon laser ( 458,488 and 515 nm) for CFP, GFP, YFP, CY2,FITC, etc.**

Main switch



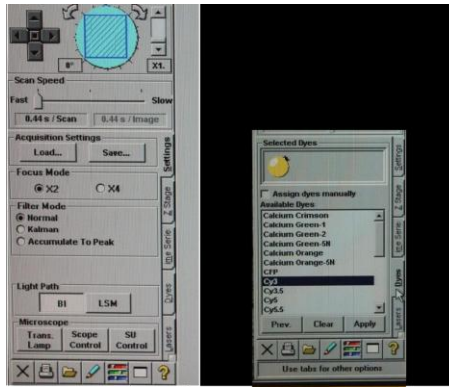
**Helium-Neon 543 nm for CY3, RFP, DsRed, PI, etc. Helium-Neon 633 nm for CY5 etc.** Main switch



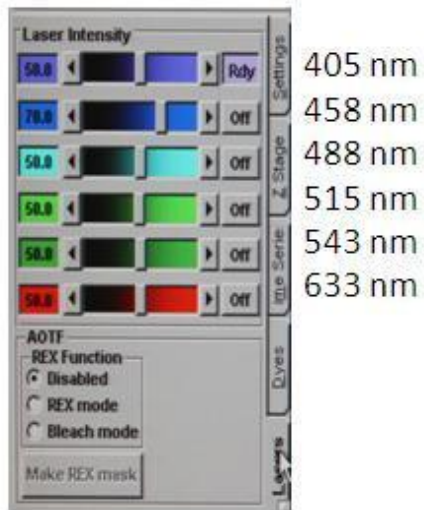
When you use Argon laser, first you switch main power knob and after turn key from OFF to ON position. Wait 2-3 minutes.

**If you will be use 405 nm (DAPI) laser, close Fluoview500 software, switch ON 405 nm laser and after this ON FluoView .**

**11.** From the page tabs on bottom right of the Acquire panel, select Dyes subpanel . Clear all previous dyes from selected and from available dyes list box select specimen suitable dye (one, two, tree...) by double click and then click Apply button.



12. Under Dyes subpanel find Lasers subpanel



This is your choose, but recommend for you following laser power :

**405 nm – 3-5%**

**458 nm – 70%**

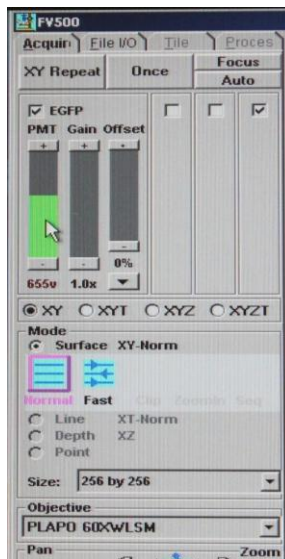
**488 nm – 20-35%**

**515 nm – 5-10%**

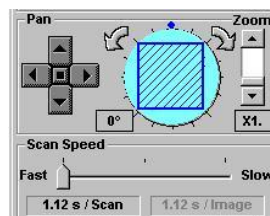
**543 nm – 50%**

**633 nm – 50%**

If you use only one fluorescence dye

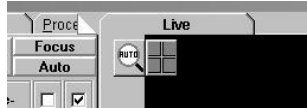


- Select XY and Normal Mode ( upper left side of menu )
- Be sure what necessary channel is activated
- Check PMT (for first time 600-650v),Gain (1.0) and Offset (0)
- Switch ON Transmitted light channel if necessary, adjust PMT-200v, gain 1.0 and offset 0.
- Select lower resolution by 256x256 pixels.
- Work your way up the control panel by next making sure **Scan Speed** is set on Fast.
- The **Pan** section should be set to 1x (field should be largest as possible).

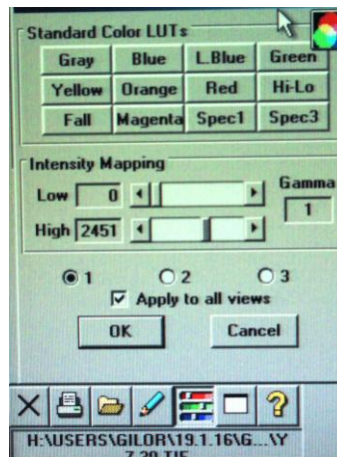


- Make ONCE

- For larger displays of each channel go to the top left of the image window and you will see 2 tabs, one tab will say *1:1* (in a magnify glass), click on this button and choose *Auto* from the drop down list. The other tab will show you the display of images on the screen. When you click on this button you will have a few choices of the display you would like to use. For example choose one single image represented by a single square or any number of multiple images represented by 2-5 squares.



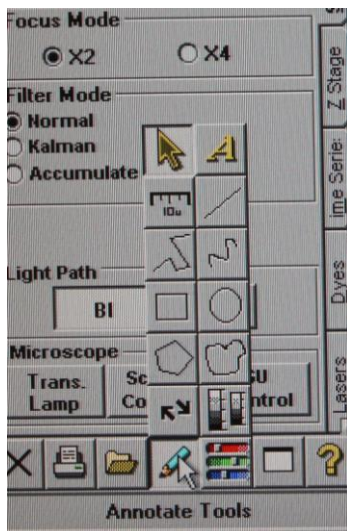
- In addition the channels in each window can be added or deleted by highlighting the corresponding numbers in the bottom right hand corner of each image.
- Go to Coloring Tools (bottom left side), choose colour for your channels, e.g. GFP-green, transmitted light image usually grey. For beginning adjust image parameters with Low-0 , High-2500 and Gamma-1



- Back to Main panel and switch XY REPEAT to adjust PMT level and correct focus. STOP SCAN!



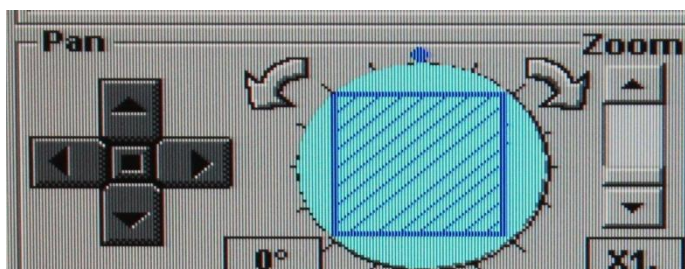
- Select high resolution by 1024x1024 pixels.
- Make ONCE.
- Correct image with Coloring Tools function.
- Put scale



- Right click with mouse on the image and Save Experiment( Multi Tiff format-automatically) in your folder(choose from local disk).
- Again right click and save display as 24-bit tiff format (change from 8 to 24-bit once time)
- Change resolution to lower (256x256) and find new field of interest.

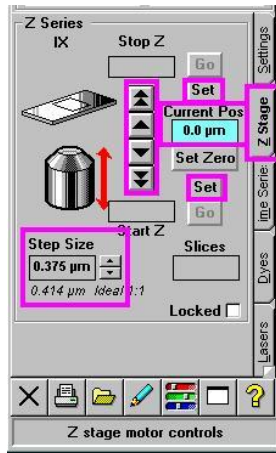
Additional function :

- Rotation of scanning field
- Zoom
- Pan (only when zoom more then 1)



For Z-Stack imaging:

- a. In the **Aquire** tab, click on the **Z-Stack** tab in the control panel.



- b. Click on the *XY Repeat* button and start scanning your image. Rotate focus knob by itself to move the objective to down position until you find the bottom of your sample focus. Click **Set Zero** and after this click **Set**. Rotate focus knob in itself to opposite direction until you find the top of your sample, then click the **upper Set button** in that direction. **Stop** your scan.  
Change Step Size of Z-resolution in accordance with the software.
- c. Change the scan from XY to XYZ, then click on the *XYZ* upper button at the top to start the lasers.



**Do not touch to any details of microscope until XYZ scanning is stop!!!!**

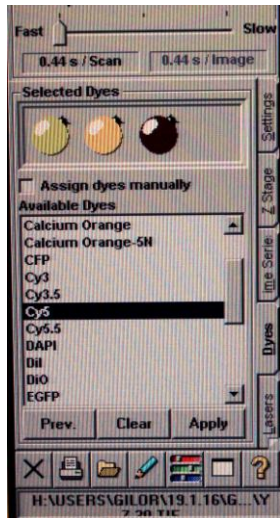
When the Z-Stack has finished click on the **Series Done** button at the top of the control panel and save as an experiment.

Select XY Mode and start new field.

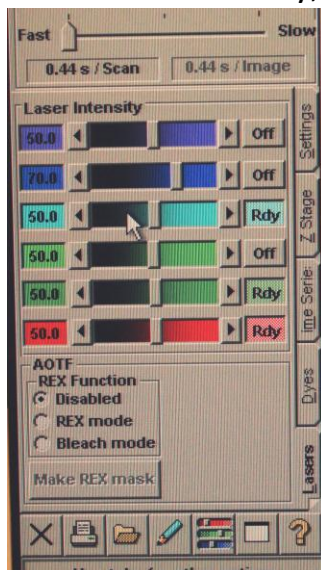
## Sequence Mode

When you use more than one fluorescence dyes following next:

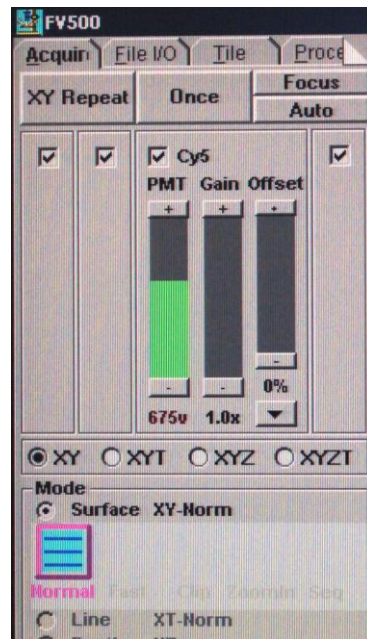
- From the page tabs on bottom right of the Acquire panel, select Dyes subpanel. Clear all previous dyes from selected and from available dyes list box select specimen suitable dye (one, two, three...) by double click and then click Apply button.



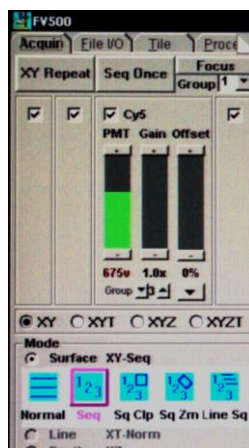
- Set lasers intensity,



- Select XY and Normal Mode ( upper left side of menu )
- Be sure what necessary channel is activated



- Check PMT (for first time 600-650v), Gain (1.0) and Offset (0) for every channel.
- Switch ON Transmitted light channel if necessary, adjust PMT-200v, gain 1.0 and offset 0.
- Select lower resolution by 256x256 pixels.
- Make ONCE.
- Go to Coloring Tools (bottom left side), choose colour for your channels, e.g. GFP-green, transmitted light image usually grey. For beginning adjust image parameters with Low-0, High-2500 and Gamma-1.
- In Mode panel select **Seq**.



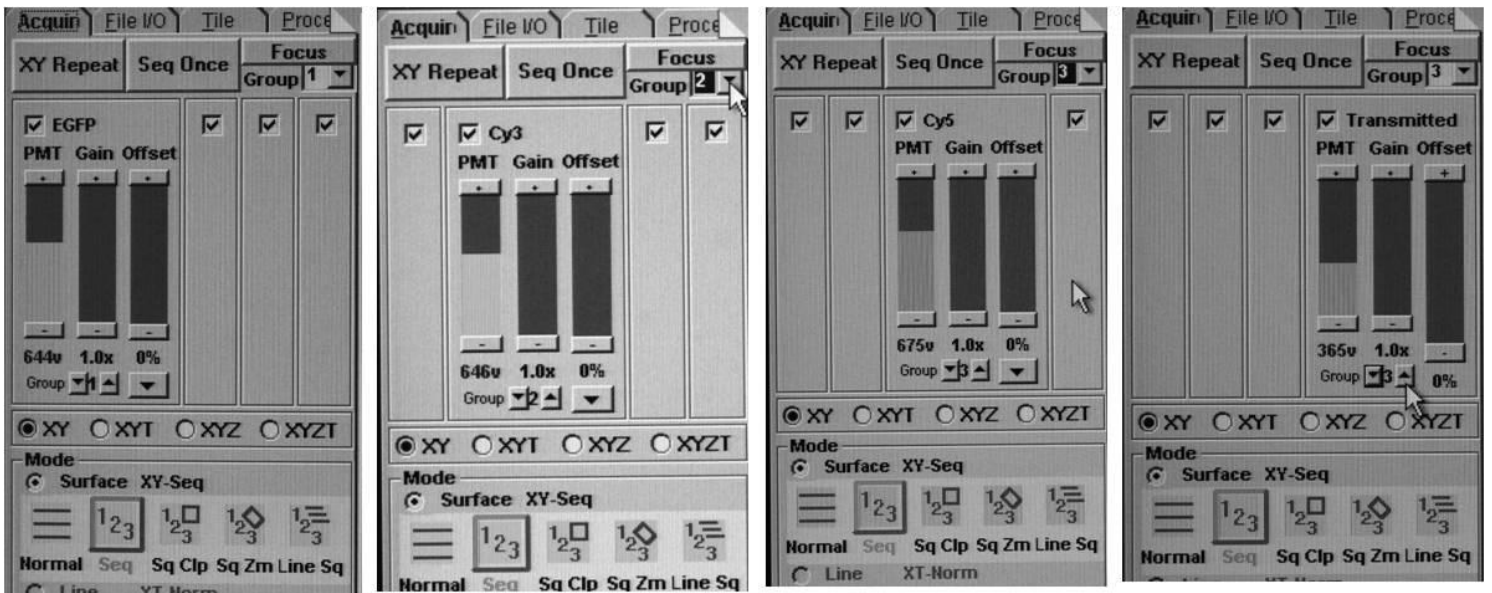
Now every channel separated to group with number under PMT, Gain and OFFSET buttons.

Channel 1- group1

Channel 2-group2

Channel3-group3

For transmitted light channel select laser from one of groups.



Make SEQ ONCE.

In right upper part of Acquire panel select from group one of them and switch XY REPEAT mode to adjust fine pictures. Stop Scan.

Check all groups.

- Select high resolution by 1024x1024 pixels.
- Make ONCE.
- Correct image with Coloring Tools function.
- Put scale
- Right click with mouse on the image and Save Experiment (Multi Tiff format-automatically) in your folder (choose from local disk).
- Again right click and save display as 24-bit tiff format(change from 8 to 24-bit one time)
- Change resolution to lower (256x256) and find new field of interest.
- XYZ imaging same with single-dye setting. For focusing and adjust Start and End parameters you select one of your channels.

## System Shutdown

1. Make sure objectives are clean of any residual water or oil using ***lens paper only***.
  2. Return microscope to BI position.
  3. Exit from the FV program. This may take awhile, however if you turn off the microscope before exiting, a system error will occur.
  4. Transfer your files to removable media( DVD, CD disc) or transfer your files via FTP.
  5. If someone is signed up after you, do not turn things off.  
Otherwise:
  6. Turn off Transmitted light unit.
  7. Turn off the Mercury Burner.
  8. Turn off Laser Power Supplies (405nm,Argon, Gr HeNe, Red HeNe).
- See special requirements for Argon laser safety shut down.**
9. Shut down the computer.
  10. Turn off Conrad Power-Manager.
- . Cover microscope and turn off lights, turn OFF air condition.

