



Olympus Fluoview 1000S Spectral Confocal Microscope

Introduction to the
NRI-MCDB Microscopy Facility
Spectral Confocal Microscope

The NEW

- Improved Optics
- More Lasers
 - 405 diode
 - 440 diode
 - 488 Argon
 - 515 Argon
 - 559 diode pumped
 - 635 diode
- ZDC
- Spectral Scan
- Motorized Prior Stage

Contents

- Start-up
- Preparing for Imaging
 - Part I – General
 - Part II – Fluorescence
 - Part III – Transmitted with Fluorescence
- Shut-down
- Image Analysis
 - Fiji
 - Imaris

Start-up: Sign-in

Record the following:

- Date:
- Your name:
- Your Project Code (i.e. Index Code):
- Your Principal Investigator (PI):
- Hg On (hour listed on the Mercury Burner):
- Hg Off (fill-in when you finish):
- Time-in (the time you arrived):
- Time-off (the time you left):
- Comments (any notes on system condition):



Start-up: Remove Dust Covers

- If you are the first user of the day, the Dust Covers should be on.
 - Please note in the log if they are not
- Fold and place on the counter.



Start-up



Start-up: Turn on the Instrument

Computer should be on

Prior Stage Controller should be on.

(No LEDs? Check the switch on the back)

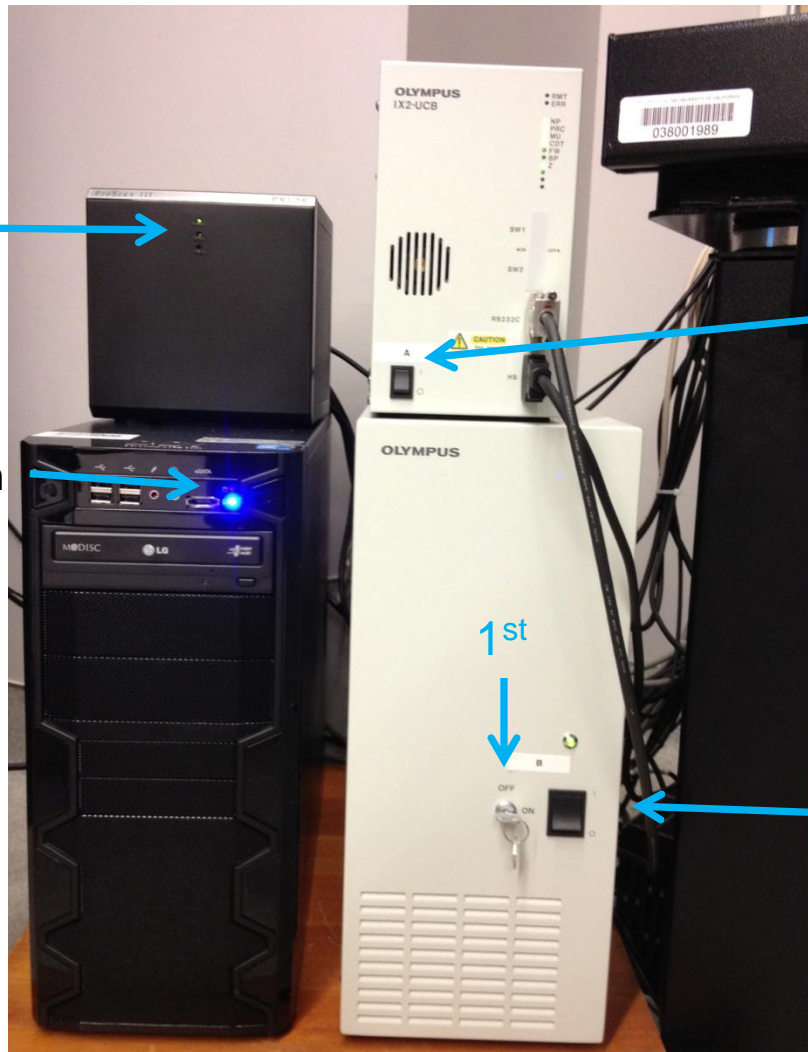
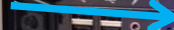
- A) Turn on microscope controller (Switch)
 - B) Turn on scan head control (Key on and then switch)
 - C) Turn on the mercury burner (Switch)
 - D) Turn on the 559 laser switch (only switch – NOT KEY)
 - E) Turn on the Argon laser (key and then switch)
 - F) Turn on the Diode laser bank (Switch)
-
- D) Once the Temp light is stable, turn the key for the 559 laser.

A & B

Prior Stage controller
should stay on



Computer should stay on

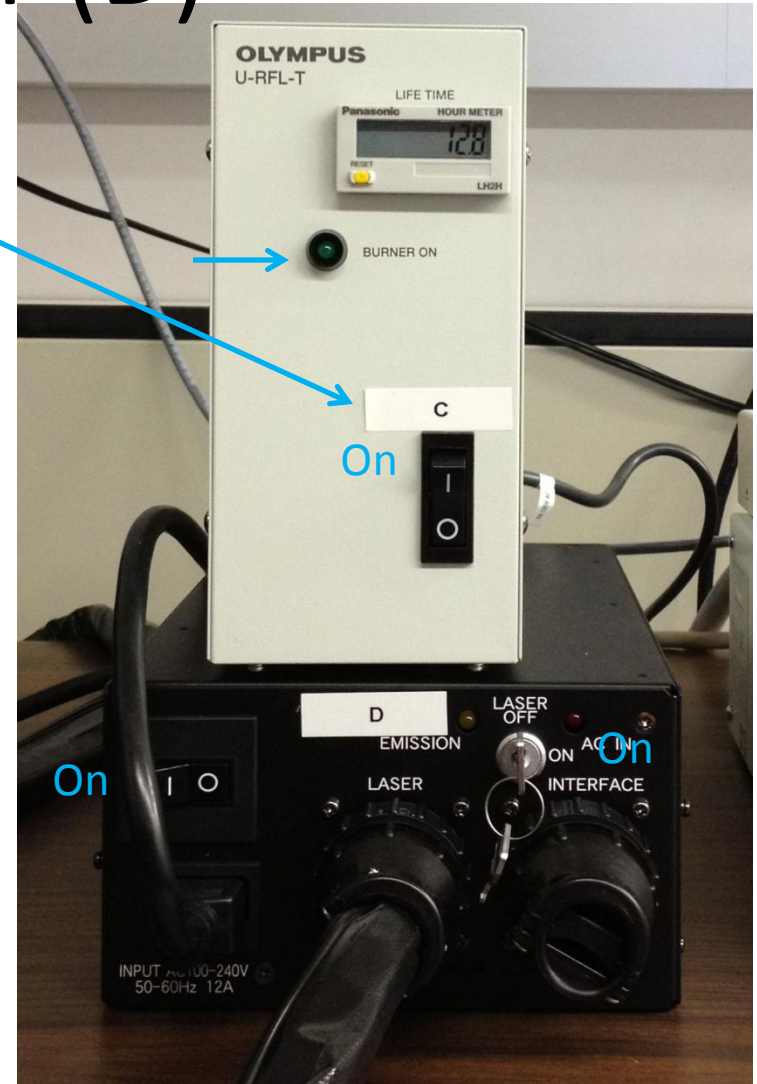


A – Switch ON

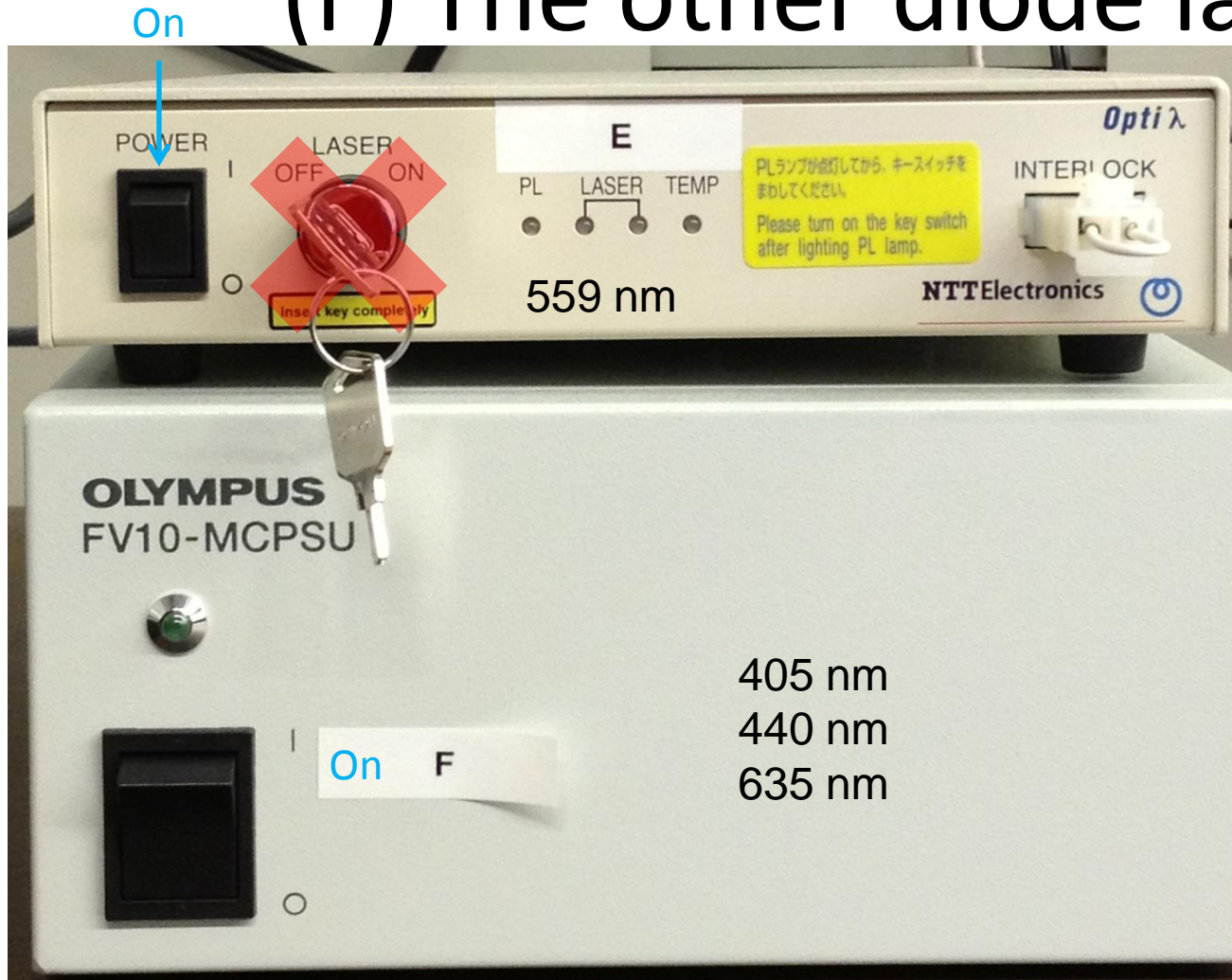
B – Turn Key to ON 1st
Then Switch to ON

Start-up: Mercury Burner (C) and Argon Laser (D)

- (C) Turn-on the burner.
 - Confirm that the burner ignites (i.e. you see a glowing BURNER ON indicator light).
 - Leave the burner on for at least 30 minutes before shutting it down.
- (D) Turn on the Argon Laser
 - Switch the Argon Laser On
 - Turn the Key for the Argon Laser



(E) the 559 laser and (F) The other diode lasers



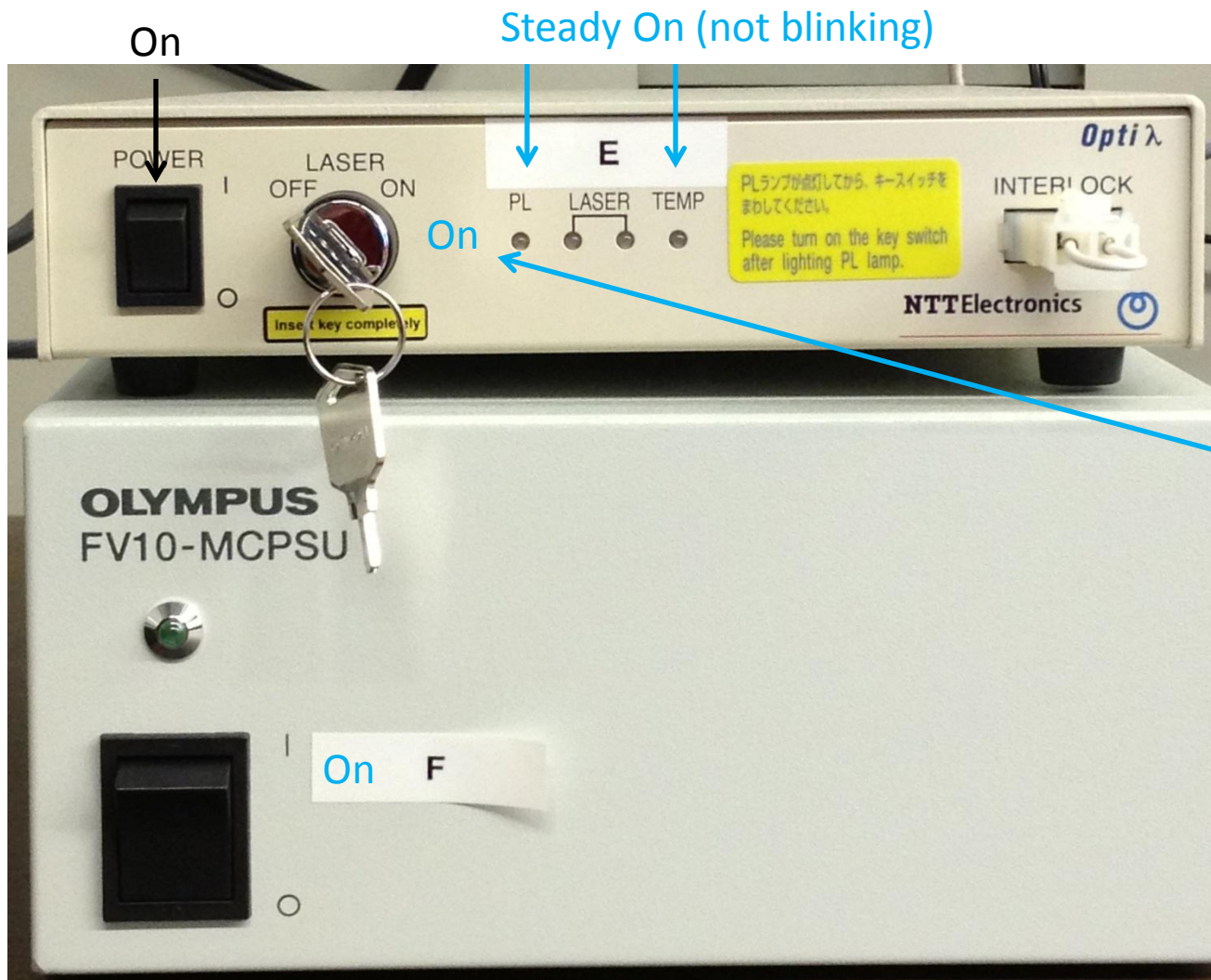
E – Switch ON

Not Key!!!

F –Switch to ON

405 nm
440 nm
635 nm

E – The Key



Once the PL and Temp lights stop blinking (i.e. steady on)

Turn the Key to On

Step 5: Set-up the computer

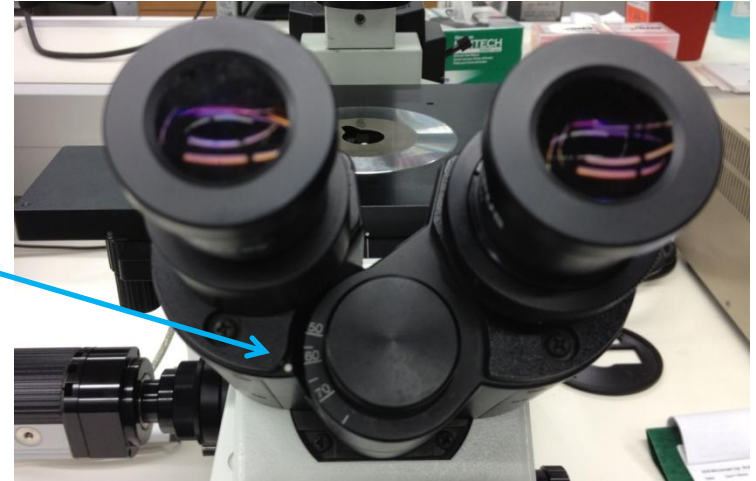
- Turn-on the computer if necessary
- Log-in as
 - User:microscopy-user
 - Password:m1cr0sc0py
- Select the Microscopy-Nas-1 shortcut
 - Login:ADS\username
 - Password: your ADS password
- Start the Fluoview Software
- Login using your Fluoview Username

Preparing for Viewing and Imaging

- Part I – General Preparation
- Part II – Fluorescence Applications
- Part III – Fluorescence with Transmitted Light Recorded

General Prep - Adjust the Eyepieces

- Adjust the spacing to accommodate your interpupillary distance.
- For those with vision corrected to normal, position the diopter to 0. To correct the system for your eyes adjust the diopter.



Confirm that no extra optical components are in the lightpath

- When not imaging DIC
 - Manually remove the DIC Slider



Preparing for Fluorescence Imaging

- Place the proper oil on the objective you will be using
 - **Blue Bottle** of Type F refractive index 1.518 for the Oil Immersion Objective
 - **Green Bottle** of Silicone Immersion Oil 1.4 for the Silicon Objectives (**red collar**)



If you use the wrong oil for your objective, you will have spherical aberration. This causes a loss of signal and resolution.

Had an Oops? See cleaning the objectives in the shut-down procedure.

Standard Objective Options

- 10x dry objective (no oil of any kind)
 - NA 0.40 WD 3.1mm
- 20x dry objective (no oil of any kind) ZDC compatible
 - NA 0.75 WD 0.60 mm
- 40x Oil Immersion Objective
 - NA 1.30 WD 0.20 mm
- 60x Oil Immersion Objective ZDC compatible
 - NA 1.40 WD 0.12 mm
- 30x Silicone Oil Immersion Objective (correction collar) ZDC compatible
 - NA 1.05 WD 0.80 mm
- 60x Silicone Oil Immersion Objective (correction collar) ZDC compatible
 - NA 1.30 WD 0.3 mm

The PLAPON60XO SC

- The PLAPON60XO SC Serial 12A00415

Chromatic Aberration DATA

	405 nm	480 nm	540 nm	630 nm
Lateral (X,Y) microns	-0.01	0	0	0.01
Longitudinal (Z) microns	0.02	0	-0.02	-0.01

High tolerance 0.170mm Coverglass is important for best results

Warner Instruments – US

<http://www.warneronline.com>



Start Software



Welcome to "FV10-ASW"

OLYMPUS

FV10-ASW

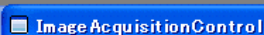
User ID: Administrator

Password:

OK Cancel

Your User ID
Is on the pull-down list

Unless you specified
otherwise, your password
is identical (i.e. is
Your User ID)



LambdaScan

Start	500	nm	End	520	nm
StepSize	5	nm	Num	3	
			Band Width	10	nm

Microscope

UPLANapo 10x/0.40

BX

Start Set 0.10 um Go

10406.83 15.05um Go

End Set 30.00 um Go



0.00um Set 0

StepSize 14.95 um Op.

Slices 3

Focus Handle On Escape

X:300.000um/pix Y:300.000um/pix

TimeScan
Interval 00:00:05.9 Num 3  

The screenshot displays the MicroManager 2.10.0 GUI in the Acquisition tab. The top toolbar includes icons for Focus x2, Focus x4, XY Repeat, XY, LZt, Stop, SIM, Bleach, and Auto Contrast. Below these are buttons for Lambda, Depth, and Time. The main area contains five channel control panels (CHS1, CHS2, CH3, CH4, TD1) and two output panels (SU, TD). Each channel panel has settings for HV, Gain, Offset, and Laser. The SU panel has C.A. and Lamp settings. The TD panel has a Lamp setting. A status bar at the bottom shows '100%' and 'Completed all initialize'.

OlympusMicro

- DP70
- DP-BSW
- FV10-ASW
 - Init
 - Language
 - 1033
 - 1041
 - 2057
 - Macro

Ch Num: 4	User Comment:
Mode: XYZ Image Size: 214*247 File Size: 6.61 MB	

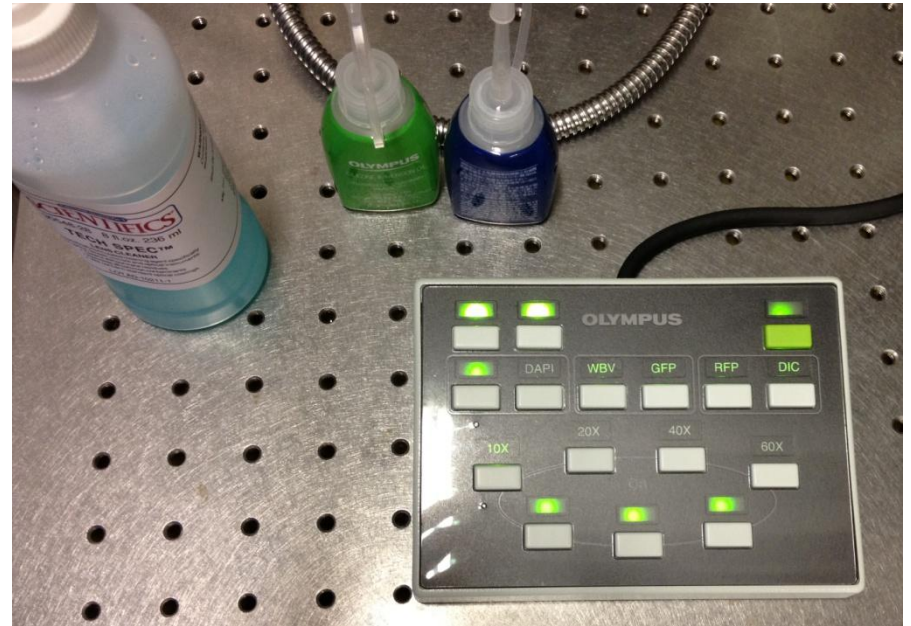
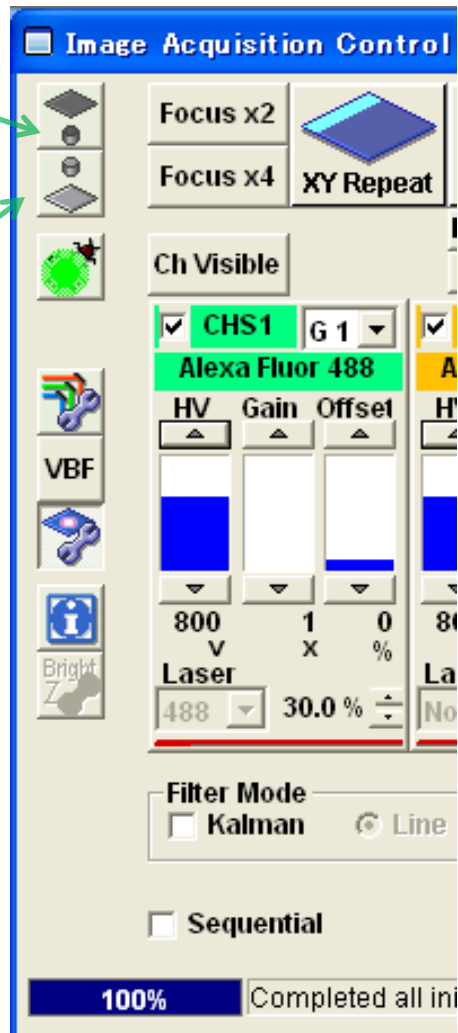
MultiChZSeries.tif

[illegible]

Viewing your Sample with the Eyepieces

Transmitted
Illumination

Fluorescent
Illumination



Control the filter Cubes with the Keypad

Filters are

DAPI

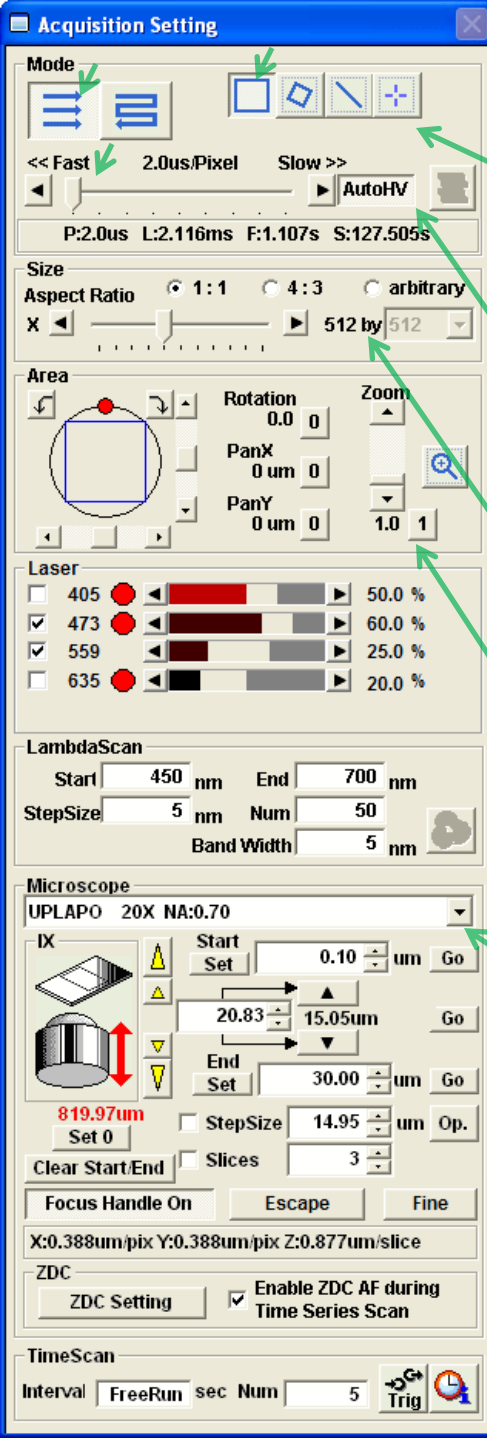
Wide-band violet (CFP)

GFP (FITC, Alexa 488)

RFP (TRITC, Cy3)

DIC

Acquisition Setting



Mode  (Normal)

Set scanning speed - On [Acquisition Setting] window, by dragging (Speed) the slider to the desired acquisition speed.

Select AutoHV for Focus Mode Setup

On [Acquisition Setting] window, set zoom magnification of 1-fold clicking (Zoom1) button of [Area].

Set zoom magnification

Set magnification of objective lens

Image Acquisition Control

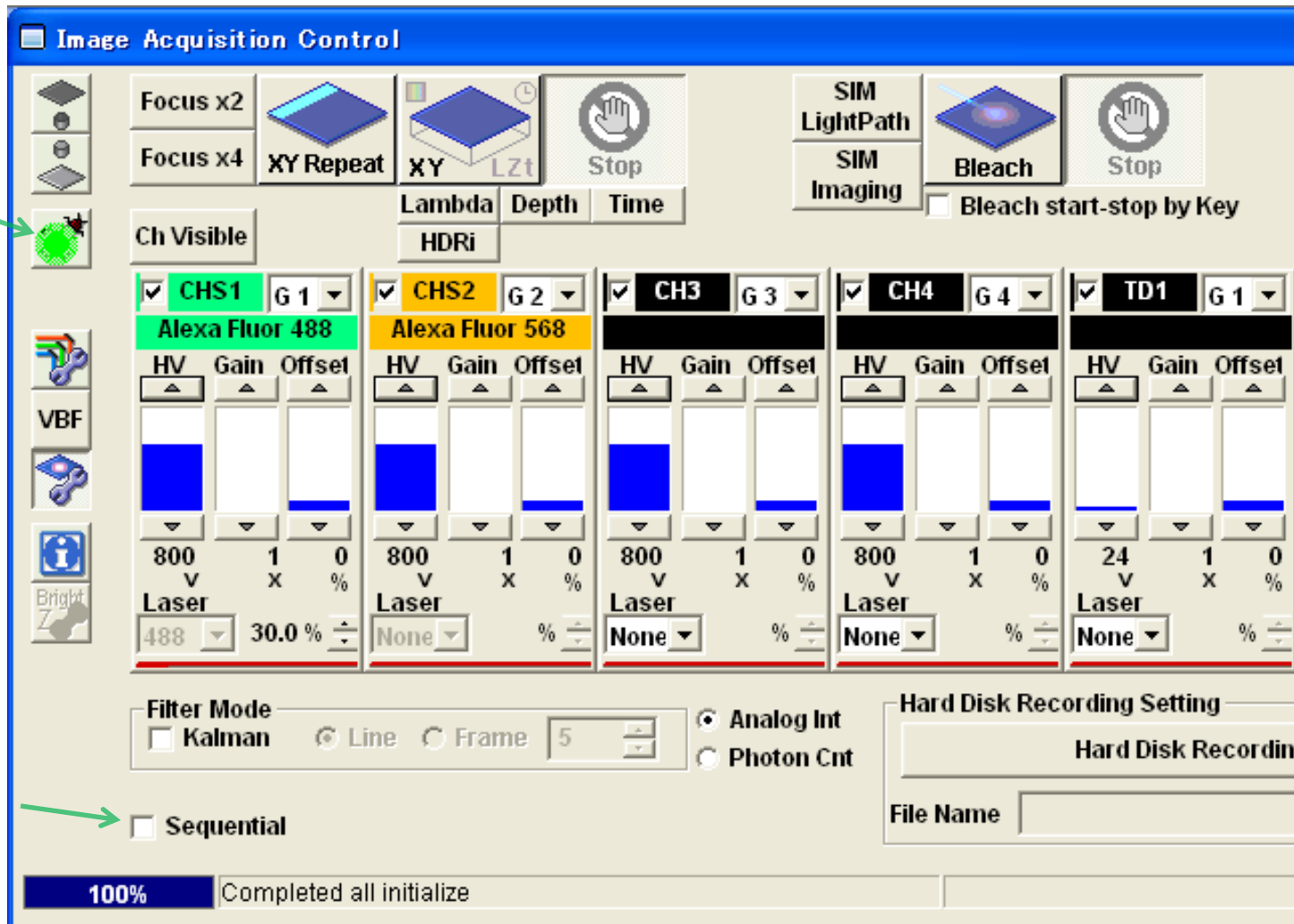
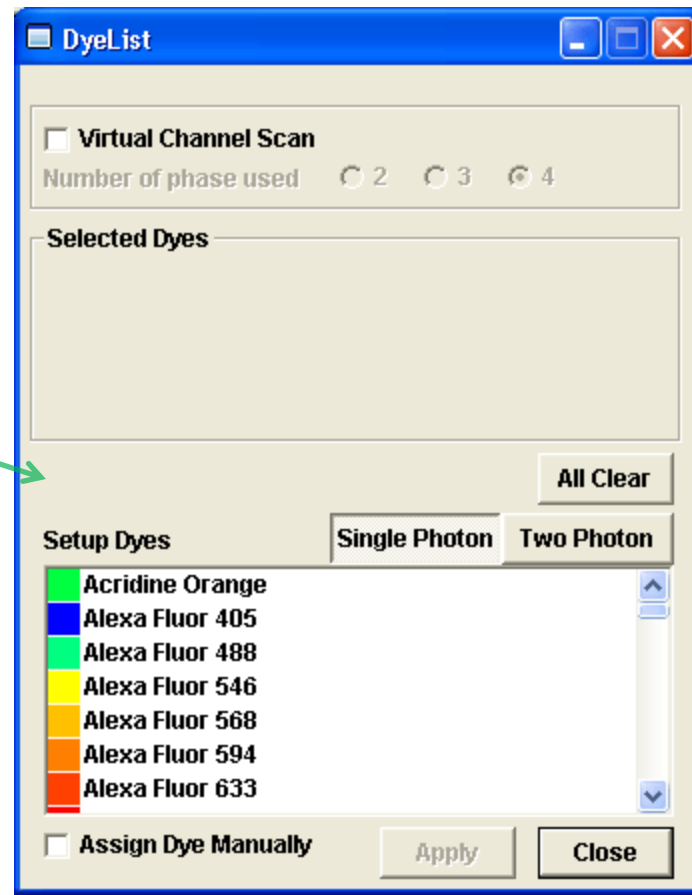
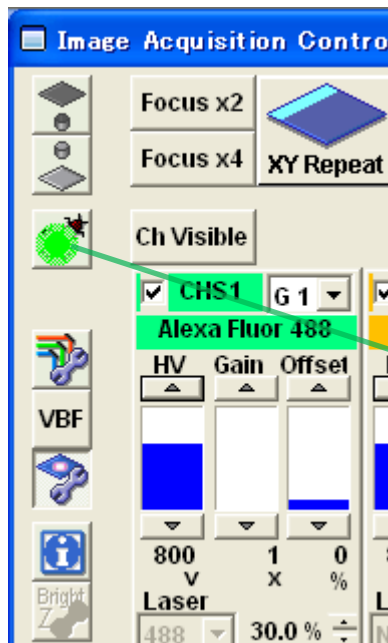


Image Acquisition Window

- On [\[Image Acquisition Control\] window](#), click (Dye List) button. [\[Dye List\] window](#) appears.



Dye List

- Double-click the fluorescent dyes in your experiment from [Setup Dyes] list box and click (Apply) button. Fluorescent dye is set.
- To cancel fluorescent dye setting, double-click Dye undesired over [Selected Dyes] and click <Apply> button.
- To cancel all Dyes from [Selected Dyes], click (All Clear) button.
- Click (Close) button and close [Dye List] window.

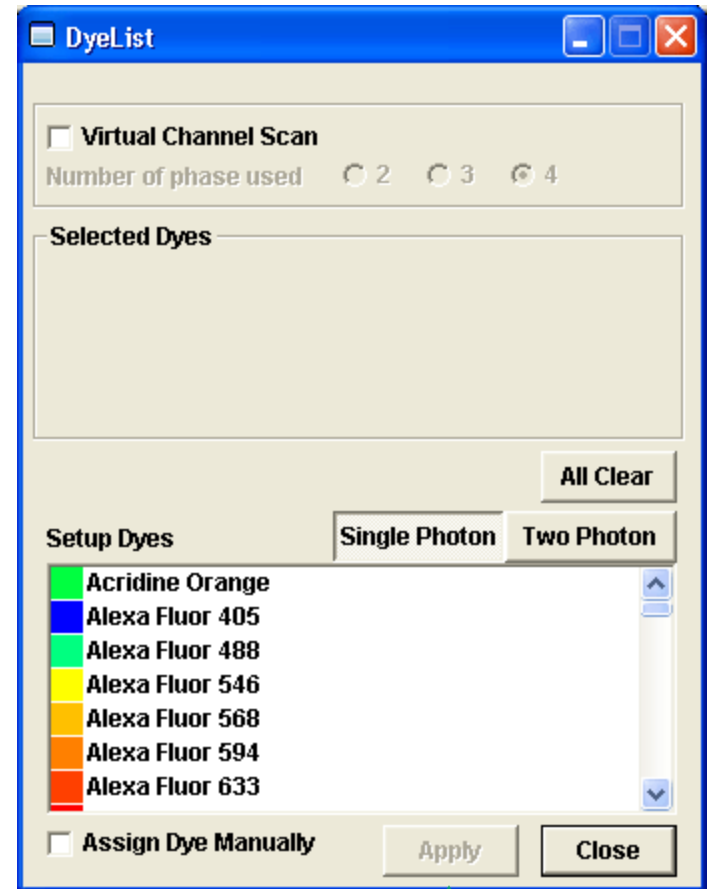
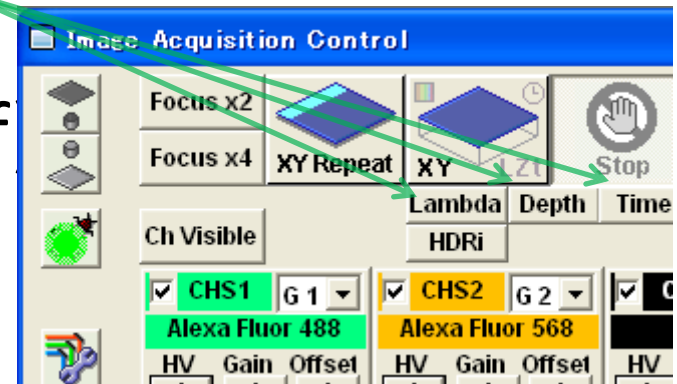
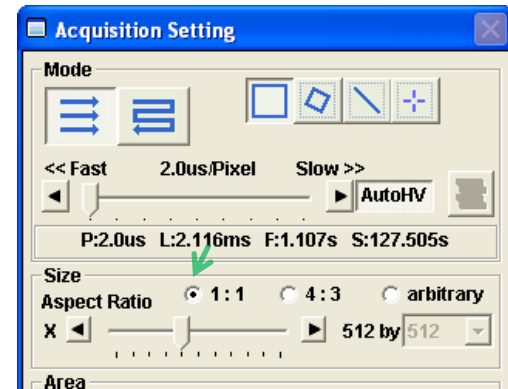


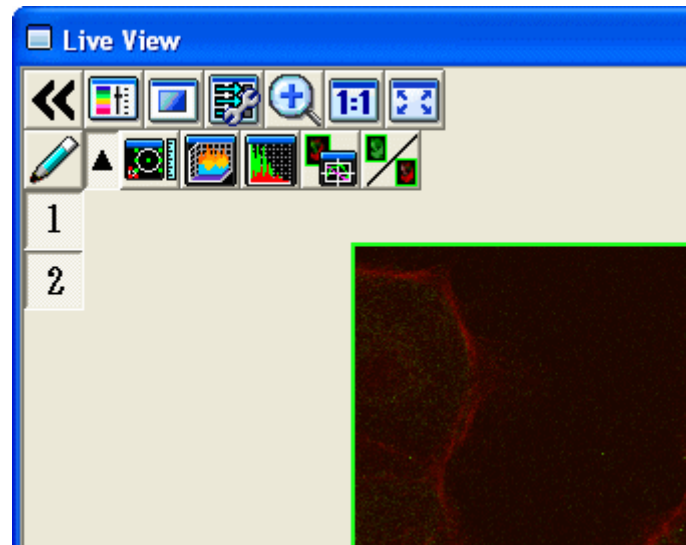
Image Acquisition Window

- On [\[Acquisition Setting window\]](#), select (Normal) button of [Mode].
- Select (1:1) option button of [Size] and set number of pixels to with the slider.
- On [\[Image Acquisition Control window\]](#), verify that (Series Image on/off buttons are all set to off.

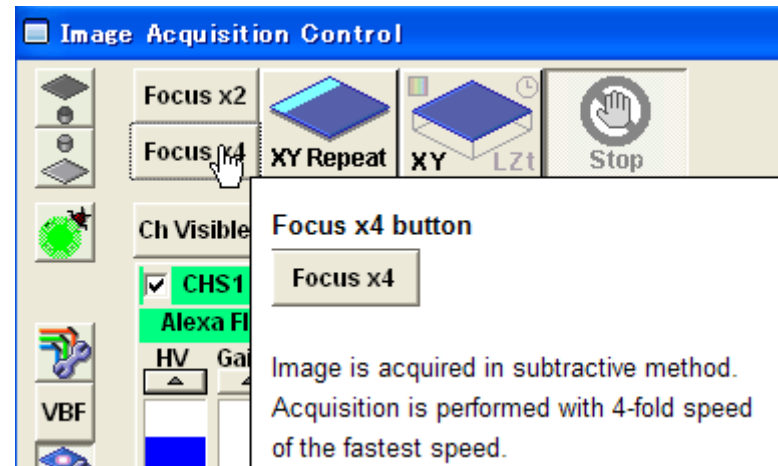
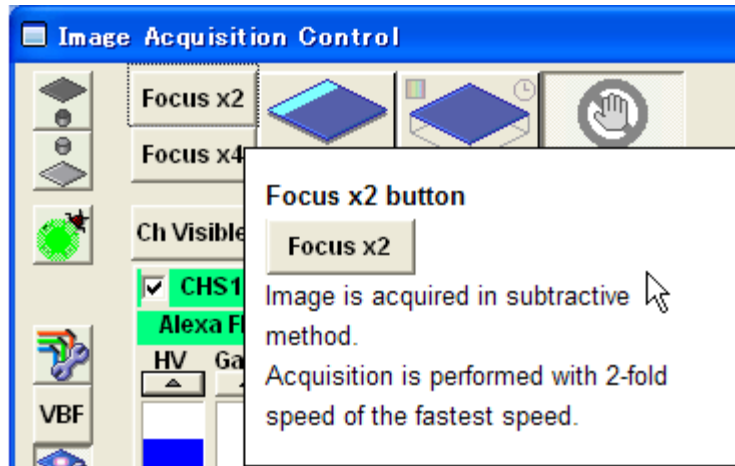


Preparing for Acquisition

- Execute scanning repeatedly
- On [\[Image Acquisition Control\] window](#), click (XY Repeat) button.  The image acquired is displayed in [\[Live View\] window](#).



Focus Mode



- Engage the AutoHV
- Use Focus x2 or X4 for faster updates
- Optimize Image

Optimize Image

- Adjust brightness of image
- Set cross-section (Z) to be observed
- Adjust confocal aperture.
- Adjust laser output.
- Make scanning speed slower
- Stop repeat operation

Tip

- Switch from a colorized image to Hi-Low by pressing Ctrl-H

Acquiring an Image

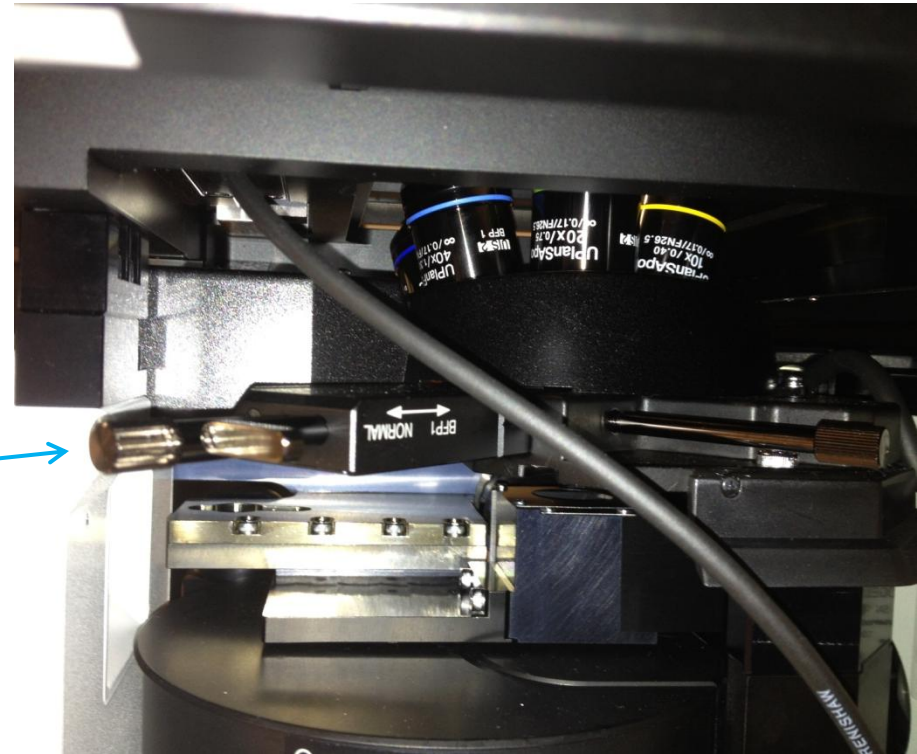
- [Set observation condition.](#)
- [Adjust live image.](#)
- On [[Image Acquisition Control](#)] window, click (Scan) button.



- When image acquisition is completed, Image will appear in [\[2D View\] window.](#)
- Save!

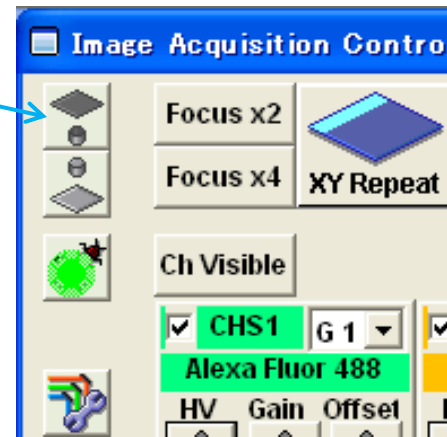
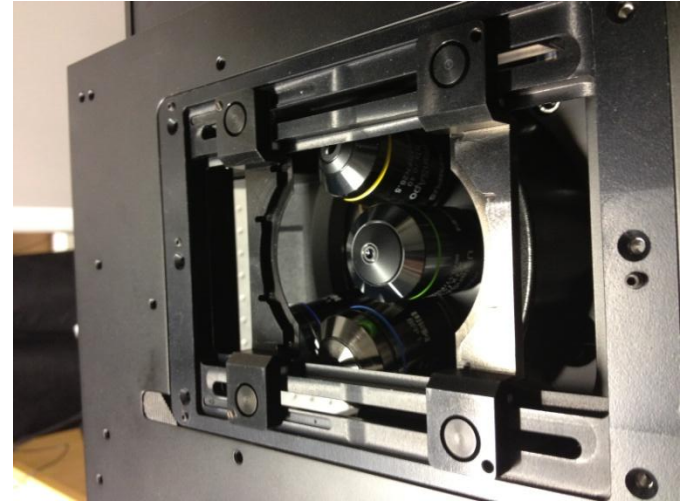
Part III: Preparing the microscope for transmitted imaging

- Confirm that no extra optical components are in the lightpath
 - Manually remove the DIC Slider



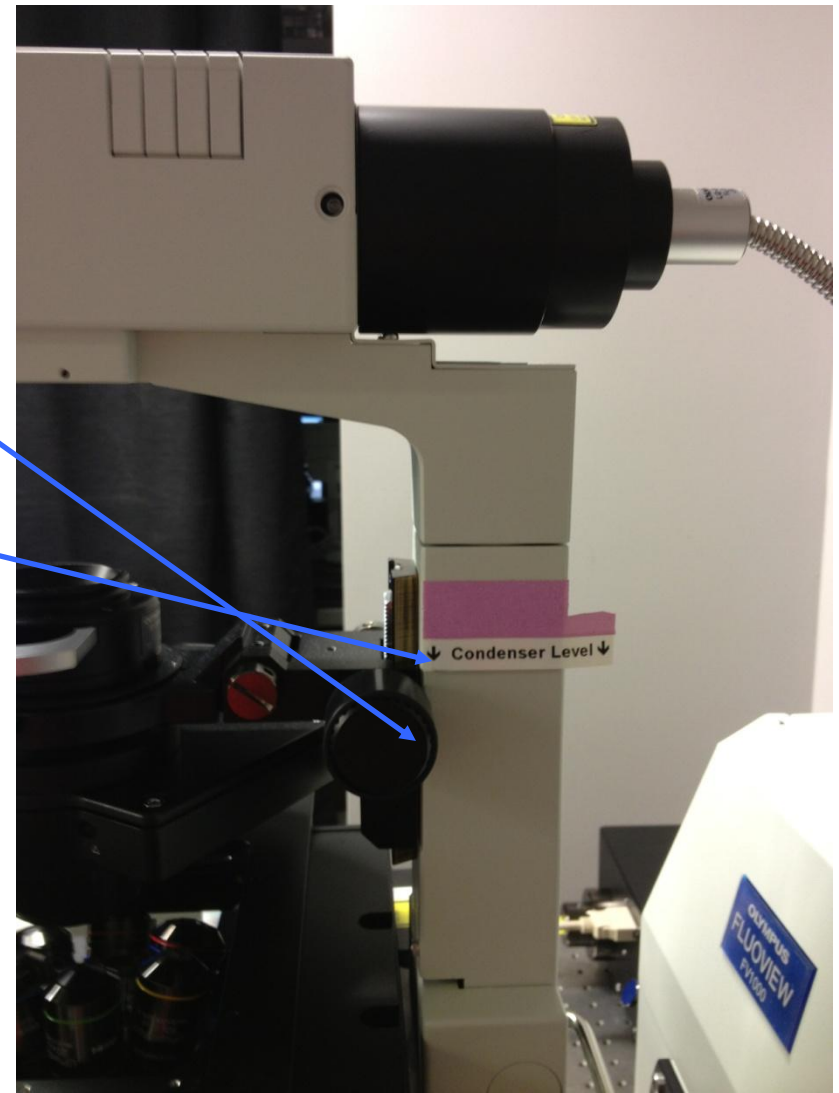
Part III: Establish Kohler Illumination

- Place a sample on the stage coverslip down.
- Using the Fluoview Software
 - Rotate the 10x objective into position.
 - Open the transmitted shutter



Part III : Establish Köhler Illumination continued

Use the condenser focus knob to position the top of the condenser rack near the mark.



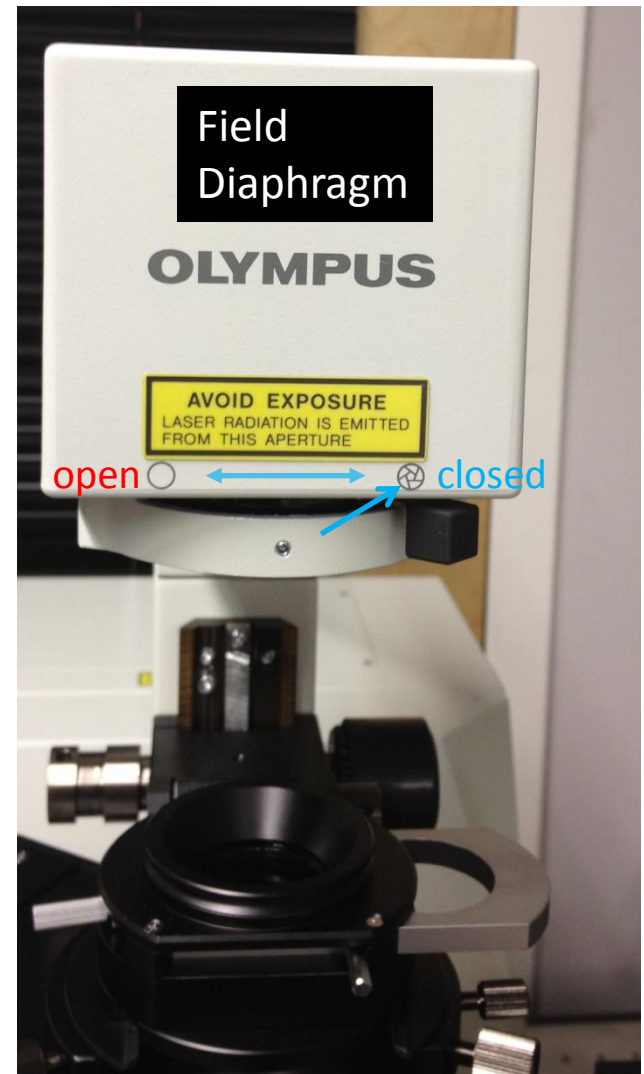
Part III : Establish Köhler Illumination continued

Use the microscope focus knob to bring the sample into crisp focus.



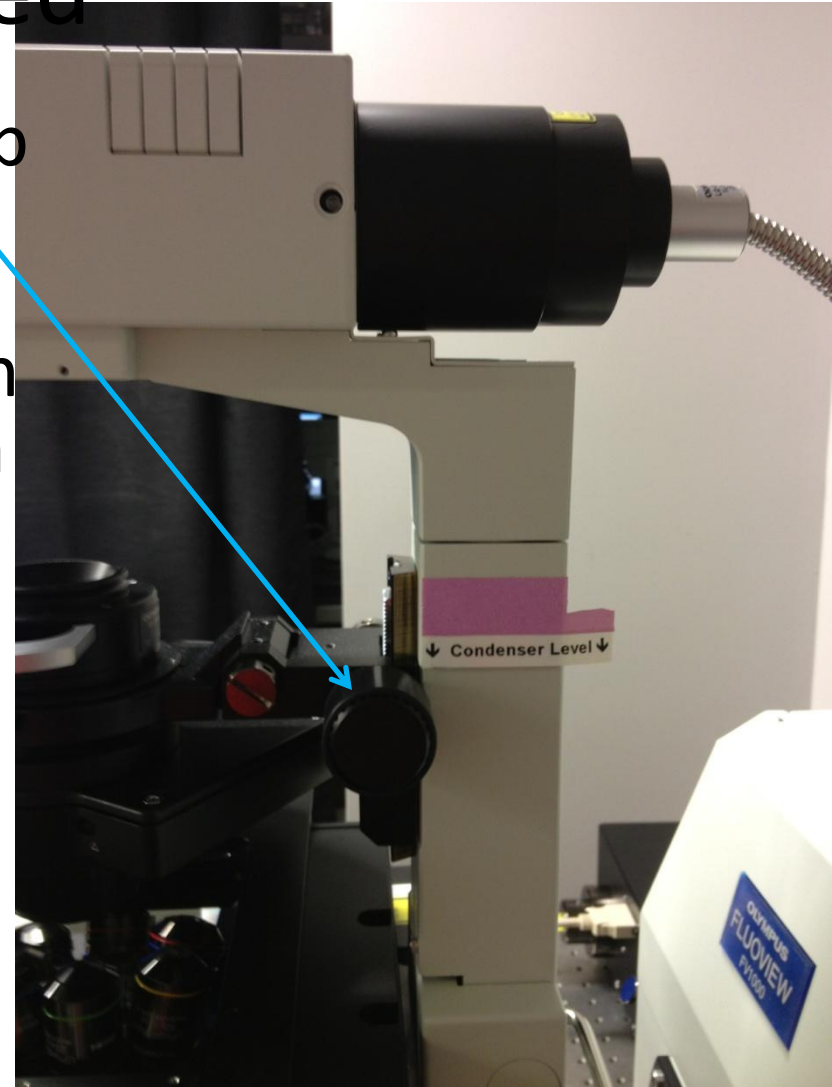
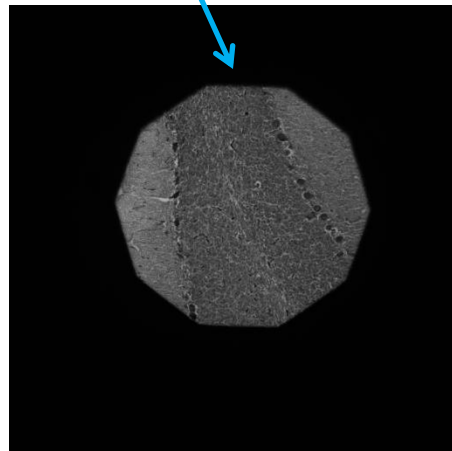
Part III : Establish Köhler Illumination continued

- Move the field diaphragm slider to the closed position.



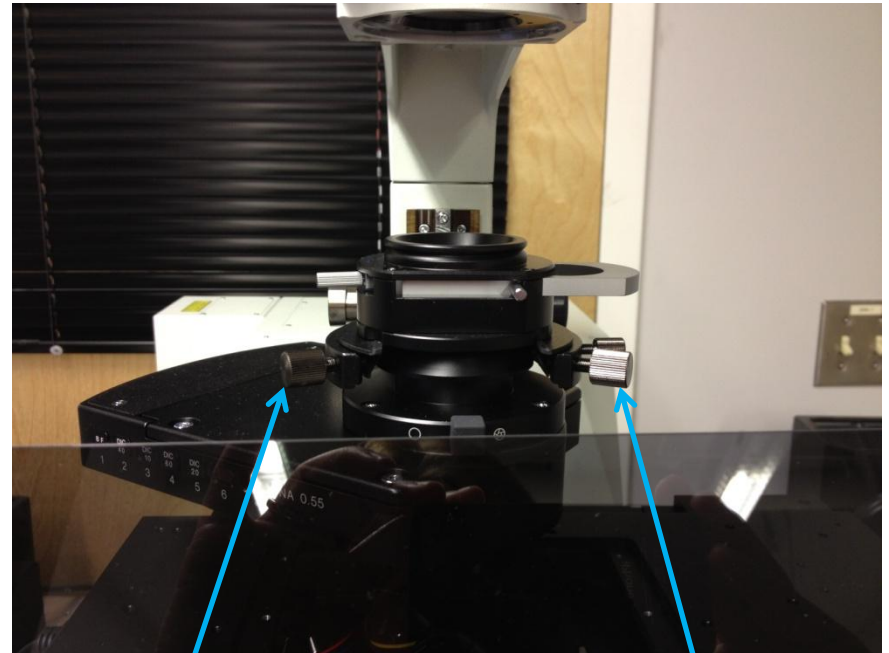
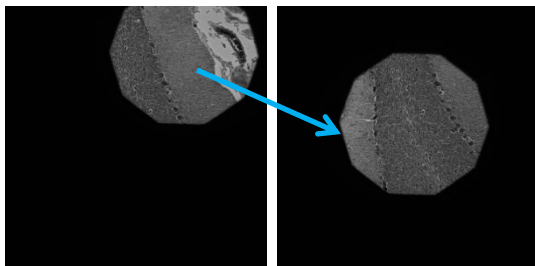
Part II: Establish Köhler Illumination continued

Use the condenser focus knob to further adjust the condenser height so that the outline of the field diaphragm appears crisply focused when viewed through the microscope.



Part III : Establish Köhler Illumination continued

- Use the centering knobs located on the left and right sides of the condenser to center the view of the field diaphragm.

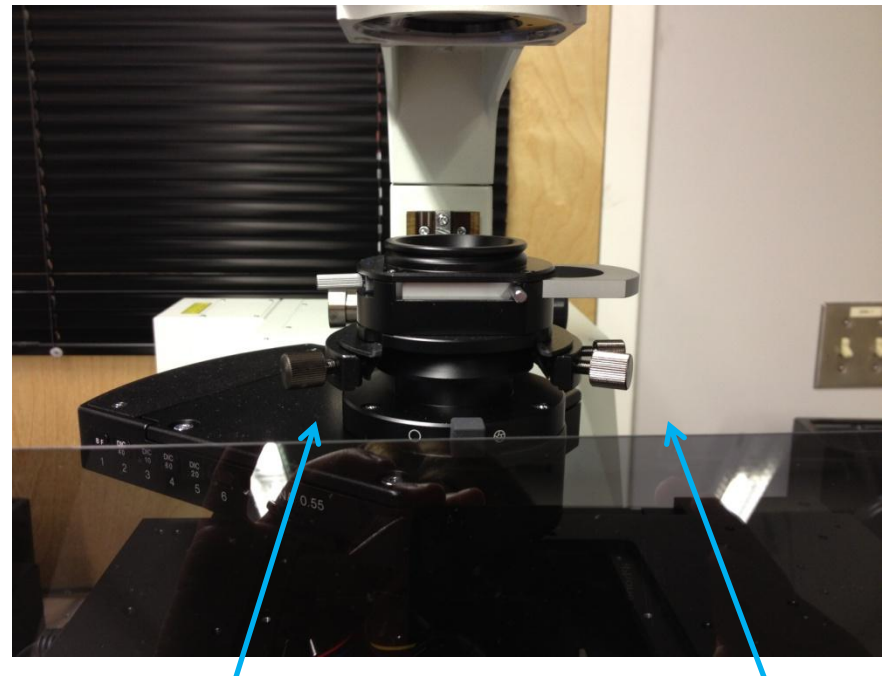
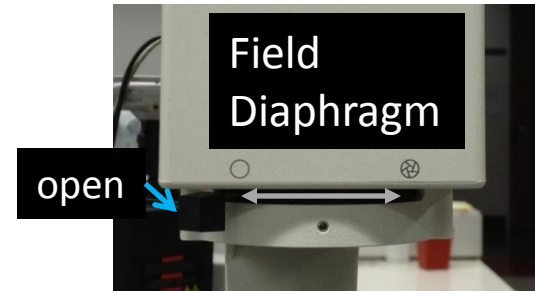


Centering Knob

Centering Knob

Part III : Establish Köhler Illumination continued

- Slide the field diaphragm toward the open position.
- As the view of the field diaphragm approaches the edge of the field of view, use the centering knobs on the condenser to fine tune the position of the light.
- Once the light is optimally centered, open the field diaphragm just beyond the field of view – no more.



Centering Knob

Centering Knob

Part III : Establish Köhler Illumination continued

- For brightfield images adjust the condenser diaphragm to optimize contrast
 - $\sim\frac{1}{2}$ or less for transparent specimens
 - Or to taste but the more closed the diaphragm the lower the NA (resolution) of the system



Part III: Reestablish Köhler Illumination

- Köhler illumination is objective specific
- Reestablish Köhler Illumination for each objective

Part III : DIC with the Fluoview 1000S

- After establishing Köhler Illumination
 - Manually place the DIC Slider into the optical path
 - The Fluoview Software will place the correct DIC Prism in the Condenser
 - For the 60x Oil BFP1 adjust the slider to the BFPI setting
 - Translate the DIC Slider to add negative or positive bias (contrast)
- Warning - Do not image for colocalization with DIC Optics

Shut-Down Procedure

- Check the online schedule
 - Shut-down if nobody is scheduled within the next hour
 - Leave the system on if somebody is using the system in the next hour but do the following.
 - Shut-down the Fluoview Software
 - Log-off the computer
 - Gently clean the objectives with a lens paper dampened with 100% EtOH
 - Return to the 10x objective
 - Sign-off in the log.
- Adjust your online reservation end-time if you finished early

Shut-Down Procedure Continued

- Turn the key on the Argon laser power supply (D)
- Shut-down the Fluoview Software.
 - Ignore the shutdown warning. Do not close the manual shutter. It isn't necessary
- Gently clean the objectives with a lens paper dampened with 100% EtOH
- Return to the 10x objective
- Turn off the lasers (F)(E)
- Shut down the mercury burner (C) (make certain it has been on for 30 minutes before shutting down)
- Turn off the scanhead (B) (switch then key)
- Turn off the microscope power supply (A) (switch)
- Turn off the Argon laser once the air is cool (D) Note the fan will not stop
- Sign-off and Complete the paper log by filling-in
 - Hg Off (the hours on the bulb)
 - Time you finished
 - Any comments
- Cover the microscope with the dust covers (avoid the burner)

Image Processing

- Fluoview Viewer 3.1
https://support.olympus.co.jp/cf_secure/en/lisg/bio/download/ga/fv10_asw/
 - Serial # 2A60535
- Fiji
 - <http://fiji.sc/wiki/index.php/Fiji>
 - Be sure to open files with
 - Plugins
 - LOCI
 - » Bioformats Importer
- Imaris – Available in Bio II rm5173