

6D

Do NOT use this microscope unless you have been properly trained by Kurt or Sebastian - nic.ucsf.edu

Start Up Procedure -

1. Turn on arc lamp underneath microscope table
 - There are **two switches**: first turn on the LAMP and then MAIN
2. Turn on main power switch
3. Start up NIS Elements software
 - Login: imaging workstation
 - Password: blank
 - Login To: Computer



Shut Down Procedure -

1. If no one is signed up 2 hours after your session is over:
 - Lower objectives to bottom
 - Wipe off oil with lens paper
 - Turn off main power switch
 - Turn off arc lamp (MAIN and then LAMP)
2. If someone is signed up after you:
 - Turn off NIS Elements
 - Leave everything else on

Overview

The 6D microscope is a highly automated widefield fluorescence/brightfield microscope on an inverted Ti-E automated microscope. The 6D stands for 6 Dimensions – X, Y, Z, multiple wavelengths, time, and multi-positions, and image stitching. It has fluorescence capabilities utilizing a Sutter DG-4 arc lamp and excitation filter switcher, a Sutter emission filter wheel and a Photometrics Coolsnap HQ² camera with a -30°C cooled CCD detector, 1392 x 1040 imaging array with 6.45 x 6.45 μm pixels, and 14 bit images. It can also easily be binned and capture regions of interest for higher speed.

It can do bright-field imaging to a color CCD for colorimetric dyes as well as to the black and white camera and use DIC and phase contrast.

Objectives

#	Objective	Resolution limit (nm)	Pixel Size (nm/px)	Depth of field (um)	Suggested step size (um)	transmitted light	Comments
E1	4x/0.13 air Plan Fluor	2350	1635	76.9	31	n/a	
E2	10x/0.45 air Plan Apo	678	654	6.42	2.6	DIC N1/10x	
E3	20x/0.75 air Plan Apo	407	455	2.31	0.92	Ph2	
E4	40x/0.95 air Plan Apo	321	163.5	1.44	0.58	DIC N2/40x I	
E5	60x/1.40 oil Plan Apo VC	218	109	0.66	0.26	DIC N2/60x I	
E6	100x/1.40 oil Plan Apo VC	218	65.4	0.66	0.26	DIC N2/100x I	
E6a	2x/0.10 air Plan Apo	3050	3270	130	52	n/a	
E2a	10x/0.30 air Plan Fluor	102	654	14.4	5.8	Ph1	Fura-2
E4a	40x/1.30 oil S Fluor	235	163.5	0.77	0.31	DIC N2/40x II	Fura-2
E6b	100x/0.5-1.3 oil S Fluor	610 - 235	65.4	5.2 - 0.77	2.1 - 0.31	n/a	Fura-2

The first 6 objectives are always on the turret. If you want to use the 2x, or the objectives for fura-2 imaging please switch them out in the corresponding location and replace them when you are finished.

Note: you need to change the optical calibration when switching objectives with different magnification to keep the scaling correct.

<http://microscopyu.com/articles/optics/objectiveintro.html>

Brightfield Contrast Methods

Köhler Illumination – How to focus the condenser to get uniform illumination

1. Open the EPI illumination shutter
2. Focus your sample in brightfield
3. Close down the field diaphragm (knob near the top of the condenser)
4. Focus the condenser until you see a small hexagon of light on your sample
5. Center the condenser using the two centering screws
6. Open the field diaphragm until the hexagon of light just fills the field of view
7. Adjust the pupil diaphragm for contrast

You may need to tweak the illumination when switching objectives

<http://microscopyu.com/tutorials/java/kohler/index.html>

DIC – Differential Interference Contrast Microscopy

For DIC you need 2 Wallaston prisms and 2 polarizing filters, the light path is as follows:

1. *Lamp* – Turn it on
2. *EPI Shutter* – Open it (using NIS Elements)
3. *1st Polarizing Filter* – Slide the polarizer in and twist it to modify contrast

4. *1st Wallaston Prism* – Turn condenser wheel to either N1 or N2 depending on the objective
5. *Condenser Lens* – Focused using Köhler Illumination
6. *Sample* – Make sure you are looking at something
7. *Objective Lens* – Focused on your sample
8. *2nd Wallaston Prism* – Sliders located below objective in turret (slide them in only when doing DIC)
9. *2nd Polarizing Filter* – Also called Analyzer (electronically controlled by NIS Elements in the TE2000 pad window)

<http://microscopyu.com/articles/dic/>

Phase Contrast Microscopy

For Phase you need a **phase condenser** and a **phase objective**

1. Focus on your sample
2. Perform Köhler Illumination
3. Turn to a phase contrast objective
4. Choose the corresponding phase condenser position (either Ph1, Ph2, or Ph3)
5. Align the phase rings using the Bertrand lens
6. Take pictures of your sample

<http://microscopyu.com/articles/phasecontrast/>

Fluorescence Microscopy

Filters			
Fluorophore	Excitation	Dichroic	Emission
Brightfield	halogen	1. Empty	2. open
GFP		2. GFP LP	
Fura-2	1. D340xv2	3. Fura-2	2. open
Fura-2	2. D380xv2		2. open
CFP	2. ET430/24x	4. CFP/YFP	9. ET470/24m
YFP	1. ET500/20x		10. ET535/30m
GFP	4. S470/30x	5. GFP/mRFP1	7. S510/30m
mRFP1	3. S565/50x		8. S650/75m
DAPI	4. D350/50x	6. Sedat Quad	3. ET455/50m
FITC	3. ET490/20x		4. ET525/36m
Cy3	2. ET555/25x		5. ET605/52m
Cy5	1. ET645/30x		6. ET705/72m

The Fluorescence set up on the 6D uses Chroma interference filters, a Sutter DG-4 arc lamp/excitation filter switcher and a Sutter emission filter wheel.

<http://microscopyu.com/articles/fluorescence/fluorescenceintro.html>

Troubleshooting

- If the computer cannot connect to the camera, the microscope, the stage or the filter wheels make sure that everything is powered on and plugged in and restart the software (and computer if necessary) and power cycle the main power switch
- If your magnification is not correct check to see if the 1.5x magnifier is in to the right location
- If you can't see any light using **brightfield** follow this checklist:
 - ✓ You have the optical configuration "BF" selected in NIS Elements
 - ✓ Light is going to your sample:
 - The halogen lamp is on and the brightness turned up
 - The DIA shutter is open
 - ✓ Light is going to your eyes/camera:
 - The light path is set correctly (eyes – up, Coolsnap B&W camera – left, Nikon color camera – right)
 - ✓ You have set Köhler Illumination (see above)
- If you can't see **fluorescent** light follow this checklist:
 - ✓ You have the correct optical configuration for your fluorophore selected in NIS Elements
 - ✓ You are focused on your sample
 - ✓ Light is going to your sample:
 - The correct filters are in the correct place in the DG-4 illuminator
 - The correct location of the filters are selected in NIS Elements
 - The manual shutter is open and the neutral density filters are not in
 - ✓ Light is getting to the camera:
 - The Light path is set correctly
 - Your exposure times are set correctly
 - You have the scaling set correctly in the LUT(look up table) – usually auto-scaling works best for finding your sample