Time Lapse

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
2. Turn on main power switch
3. Turn on perfect focus system
4. If you are using $C0_2$ turn the small black knob located on the
5. Start up NIS Elements software
   - Login: imaging workstation
   - Password: blank
   - Login to: Computer

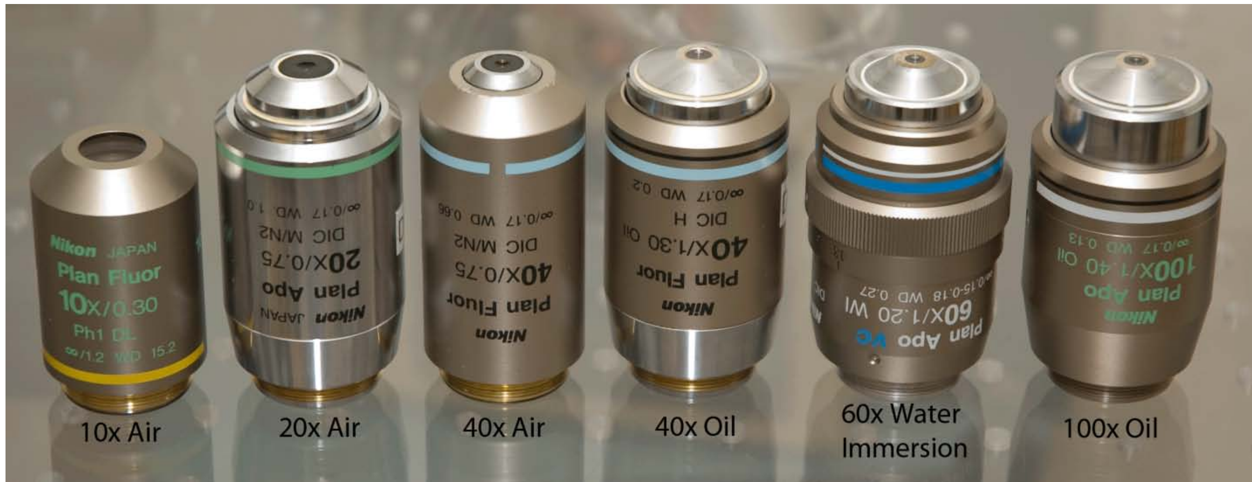
Shut Down Procedure:

1. If no one is signed up 2 hours after your session is over:
   - Lower objectives to bottom
   - Wipe off oil or water with lens paper
   - Turn off perfect focus system
   - Turn off main power switch
   - Turn off arc lamp
2. If someone is signed up after you:
   - Turn off software
   - Leave everything else on

Overview

The Time Lapse is an automated widefield fluorescence/brightfield microscope on an inverted TE2000-PFS automated microscope. It has a built in Perfect Focus System that uses an infra-red LED to detect the surface of the coverslip and constantly correct for the focus to keep your sample in focus indefinitely. It has an enclosure that is kept at 37°C with an optional 5% CO_2 attachment for imaging of live cells.

Objectives



#	Objective	Resolution limit (nm)	Pixel size (nm/px)	Depth of field (um)	Suggested step size (um)	Transmitted light
D1	10x/0.30 na air Plan Fluor	1017	654	14.4	5.76	Phase 1
D2	20x/0.75 na air Plan Apo	678	327	6.42	2.57	DIC N2/20x
D3	40x/0.75 na air Plan Fluor	407	164	2.31	0.924	DIC N2/40x I
D4	40x/1.30 na oil Plan Fluor	235	164	0.77	0.576	DIC N2/40x II
D5	60x/1.20 na WI Plan Apo VC	254	109	0.9	0.248	DIC N2/60x II
D6	100x/1.40 na oil Plan Apo	218	65.4	65.4	0.264	Phase 3

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 (the lever located on the top of the microscope)
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

Note: 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)

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

Fluorescence Microscopy

Filters			
Fluorophore	**Excitation**	**Dichroic**	**Emission**
DAPI	2. D350/50x	1. Sedat Quad	2. ET455/50m
FITC	3. ET490/20x		3. ET525/36m
Cy3	4. ET555/25x		4. ET605/52m
Cy5	5. ET645/30x		5. ET705/72m
GFP	6. S470/30x	2. GFP/mRFP1	6. S510/30m
mRFP1	7. S565/50x		7. S650/75m
CFP	8. ET430/24x	3. CFP/YFP	8. ET470/24m
YFP	9. ET500/20x		9. ET535/30m
roGFP	0. 390/22x	4. roGFP (505LP)	0. 535/30m
roGFP	1. 485/15x		0. 535/30m
D/F/R eyes		5. D/F/R	
		6. Empty	

- Fluorescence uses a Sutter Lambda LS xenon arc lamp with Sutter Lambda 10-3 filter wheels for quick changing of wavelengths.
- Sensitive Photometrics Coolsnap HQ2 camera.
- Fluorescence is only to the camera except the D/F/R cube can be used to focus on your specimen using your eyes.

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
    - If the computer still cannot connect to camera try power cycling it and restarting the computer and NIS Elements.
- If your magnification is not correct check to see if the 1.5x magnifier is clicked in to the right location (forward – 1.5x, rear – 1.0x)
- 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 selected in NIS Elements
        - The manual shutter is open and the neutral density filters are not in
    - Light is getting to the camera: (you can only use fluorescence with the CoolSnap)
        - The Light path is set correctly (CoolSnap camera – left)
        - Your exposure times are set correctly
        - You have the scaling set correctly in the LUT(look up table) – usually autoscaling works best for finding your sample