Zeiss Imaging Microscope

12/3/03vr

Instructions for turning on LCI microscope are posted on the Nelson Lab website.

1/2hour before using microscope, check scope to see if objective that you plan to use is warmed to 37^{o}C. Change objectives, if necessary, and put heater unit onto objective. Oil objectives need objective heater. 40X dry does not need objective heater.

If you are doing overnight live cell imaging, you will need to set up the spooling program. Follow instructions listed on "Spool Function" page from So. (So-can you post this instruction page onwebsite?)

Mounting slide for live cell imaging

Assemble mounting chamber-

•Have mounting brackets on slide mounting chamber screwed down slightly before mounting coverslip with cells. Mounting brackets have curved edge facing inward, towards opening.

•Apply vacuum grease (in syringe) to opening at the bottom of mounting chamber.

•Lightly grease the inside edge of etched circle on clear rectangular plastic plate where RED rubber gasket is inserted. Insert gasket.

•Prepare DMEM phenol red free media +HEPES:

Add 1M Hepes (sterile stock from Gibco in common t/c bin) 1:40 dilution to DMEM phenol red free media to final conc=25mM. Keep at 37^{o}C.

•Wash cells 3 times with DMEM/Hepes media. Use 3 separate dishes for each wash.

•Wipe the bottom side of coverslip with kimwipe.

•Mount coverslip (1.5mm thick) with cells facing up onto the bottom of mounting chamber, over circular opening. Center coverslip and push down on edges of coverslip using forceps, so that coverslip adheres to holder.

•Put a little bit of media onto cells to keep wet using a fine transfer pipet.

•Place clear rectangular plate and gasket with gasket side facing down onto coverslip. Be sure circle is centered over coverslip with cells. While holding center of clear plate with 2 fingers, one finger on each side of circular opening, put one clamp in place and tighten screws until just tight. Don't clamp down too tightly! Put other clamp in place, while still holding clear holder, and tighten screws in place.

•Add media to circular opening so that it is curved over the top. No bubbles!

•Generously grease a 1.5mm coverslip along edge and mount coverslip on top with grease side down to seal coverslip in chamber. Apply pressure on edges of coverslip using forceps to seal coverslip. Remove extra media.

•Wipe bottom with water and kimwipes. Check for leaks.
•Score bottom of coverslip with diamond pen. Find center of coverslip and mark with X.
•Put unit into 37^{o}C incubator to let settle down for 5 minutes or keep at 37^{o}C if not using right away. Otherwise, mount onto LCI scope.

Microscope

Lift back top part of scope to expose objectives and stage. Remove cardboard box.
Wipe objective with lens paper.
Switch objectives manually and move heater unit, if necessary.
Check stage to be sure that it is seated in place. (spring on bottom left side).
Put oil onto objective sparingly, if using oil immersion objective. Bring objective up to slide using coarse focus adjustment until the objective touches oil. Continue moving objective UP slowly with fine focus adjustment until oil stops moving outward on objective.
If using the 40X dry objective, bring objective UP to slide using coarse focus adjustment, until it touches slide.
Slide chamber is placed onto stage between 2 black sliders such that the dot or markings on chamber are on the left side. Move 2 black sliders to fit against slide chamber. Do not clamp holder onto stage at this point.
Center slide over objective.

TO move objective-
UP turn focus adjustment towards DOOR
DOWN turn focus adjustment towards USER.

Put cardboard box cover and heater hose in place on stage.

Bring top of scope down and Zero X, Y on stage controller.

On scope -turn on Halogen light source - bottom right switch.
On computer go to FOCUS window, choose OPEN BRIGHT and DIC. You should see 3.0v on upper microscope LCD display.
Intensity of Halogen light can be controled by long oval black togel switch with white arrowhead on bottom front of scope.

Turn on Fluorescent light source
On scope-lower right button. You should see FL upper microscope LCD display, when the Fluor light is on. If you don't see FL, go to **FOCUS window, choose OPEN FLUOR and FITCvi or GFP.**

To view cells with the binnocular eyepiece, the binnoculars need to be in Binnocular position-Adjust this using **MIDDLE button on left side of scope.** Press until scope screen shows "Binnocular 100%".

To send view to camera, use with **MIDDLE button on left side of scope** and push one time and "LEFT 100%" will be displayed on scope screen. View of cells can be viewed on moniter screen.

View cells with binnoculars and find cells in DIC. Check DIC and adjust to correct DIC filter using middle? button right side on upper part of scope until correct DIC is displayed on scope screen. Use DIC III for 63Xoil and 100Xoil objective and DIC II for 40X dry objective.

Find focal plane for viewing cells. Clamp slide chamber onto stage using 2 small black clamps. Find cells again and then Zero "Z" by holding in the Z zero button until it beeps. Z=0.0 will be displayed on upper microscope LCD display .

Move objective with fine focus adjustment to –170 in "Z" to find crosshairs junction, "X", etched in bottom of coverslip. Focus onto etched X and zero X,Y button (far right button) on stage controller box. X, Y display is on stage controller.
(For 40X dry objective- "Z"is =-120→ -135)

Find cells using DIC or FLUOR and set multiple points. Stage is moved using the joystick on stage controller box. Stay in the XY region of –3→ +3 mm. This information is displayed on stage controller box.

To collect multiple sites for imaging, find cells, center cells in field, go to Focus window, "X, Y" tab, and choose "set point". The X, Y and Z co-ordinates are recorded on the list. To delete a point choose "delete point" and to reset "Z" coordinate, choose reset "Z".

After all images have been set you will need to set-
1) Auto focus settings
2) Capture preferences
3) setting exposure for DIC and Fluorescence
4) Settings on Capture window

1) Auto focus window
Method-**Spectal 2D template**
Total search range= **10**um
Peak delta threshold=**0.8**
Select **Auto focus** button on window.

2) Capture Preferences window
FOCUS tab-
√ Auto focus during timelapse/multipoint captures.
1 update focus every ____ image captures.
DIC channel

10 total search range (select 15 if not all points are in focus)
0 post focus offset
0.8 peak delta threshold

Spool tab-
√ spool to spool files (if doing overnight imaging)
OK

3) Set exposure time for DIC and for GFP (FITC)
Find the lowest expressing cell point and adjust GFP (FITC) with this point.
Capture window-
•2X2
√ timelapse
1 #timepoints (if you want to check all set points set to # of points)
100 interval (m)
• current point
GFP tab or DIC
√ expose: **choose exposure time (ms)**
TEST

Check graph on this window to see if exposure needs to be increased or decreased based on what you see with all points.

Check DIC exposure as well, same as above only choose DIC tab

4) settings on Capture window for overnight timelapse imaging
Capture window
Image extent and binning factor
• 2X2 bin

Multiplane capture
√ Timelapse
150 #Timepoints
10 interval
m units (minutes)

Multiple Point [X,Y] capture
• entire list
Check GFP and DIC exposure settings-GFP and DIC tab, which you set up above.
OK

Retrieving files from Spool file after run is finished.

Slidebook should be open.
IMPORT
Slidebook spool

Get OPEN window
Select spool file.

Spool files are located in
Local Disk (C:) drive
Program files folder
Intellegent Imaging Innovations, Inc folder
Slidebook folder
Spool files are listed as spool-0.spl, spool-1.spl, etc

Select spool file
OPEN

Get spool file window
LOAD

The spool file will be loaded into your open slidebook file. The file will be named "capture 1", 2, etc.
Take note of the size of each spool file and load only 3-4 spool files into each slidebook file (~650MB total). You may need several slidebook files for each overnight run.

To rename your spoolfiles (Capture 1). Open the image and do Control I and rename image.