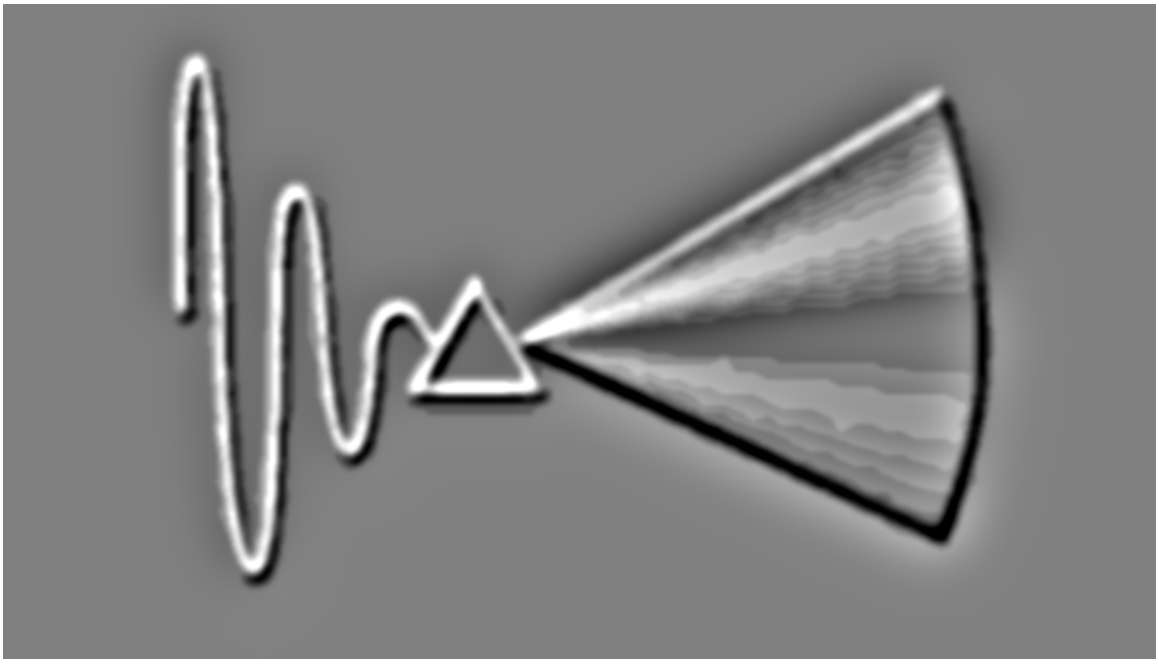
Quick Start

IPLab 6D

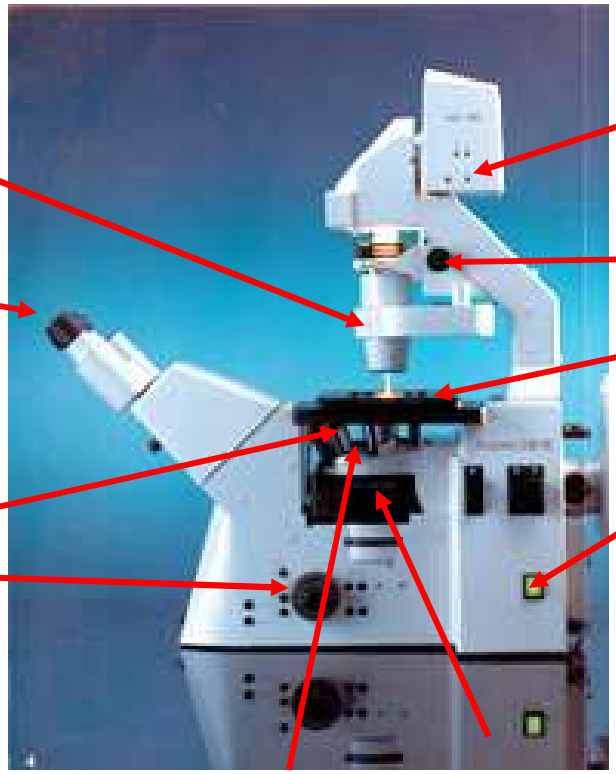


Wright Cell Imaging Facility
Toronto Western Research Institute
University Health Network

Switch on Hardware

1. At least 1 hr before start environment controller if required (switch at back).
2. Arc-lamp power supply (under table) if required.
3. Switch on ‘scope.
4. Switch on Uniblitz shutter (switch at back).
5. Switch on camera.
6. Switch on stage controller (switch at back).
7. Start PC.
8. Start IPLab 4.

Microscope parts



Uniblitz shutter control box

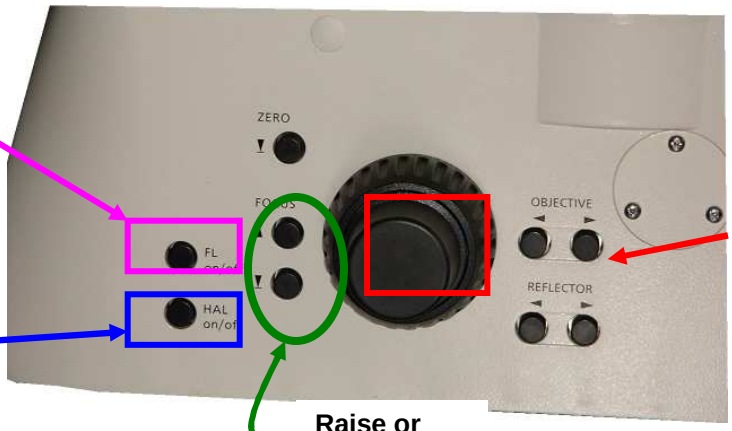
Front

Back

Power switch

Manual shutter open
Should be "NC" for PC control

Right hand side of microscope



***FL* - Excitation light shutter**

HAL - Halogen light power

Raise or lower stage

Reflector carousel control – selection appears in *Condenser assembly* display

Condenser assembly

Field iris diaphragm (metal lever)

Condenser aperture control

Condenser centering screws

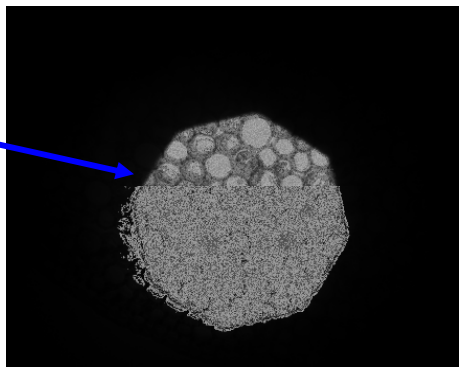
Condenser selector

Display showing current condenser

Set up Köhler Illumination *(if required)*

Köhler illumination was first introduced in 1893 by August Köhler of the Carl Zeiss corporation as a method of providing the optimum specimen illumination. Köhler illumination is not important for fluorescence or confocal, however if a transmitted light image is required, the microscope should be set up with Kohler illumination.

1. Mount slide.
2. Switch to 10× objective.
3. Focus on sample (maybe use fluorescence channel if possible then turn to brightfield).
4. Close silver lever ***Field Iris Diaphragm*.**
5. Raise condenser close to sample using ***Condenser Focus Wheel***
6. Turn condenser to **I/H** for bright field using the ***Condenser Selector*** buttons on the condenser turret.
7. Focus condenser using ***Condenser Focus Wheel*** until ***iris-diaphragm*** (NOT THE SPECIMEN!) is in sharp focus.
8. Re-centre condenser if necessary using silver, ***Condenser centring screws***.



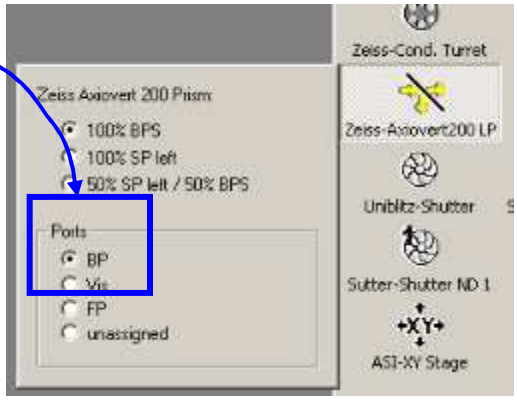
9. Open the ***Field Iris Diaphragm*** until it just fills the field of view as observed through the eye pieces

Remove one eyepiece and close/open the ***Condenser Aperture*** until it just disappears from the ocular. Put eyepiece back!

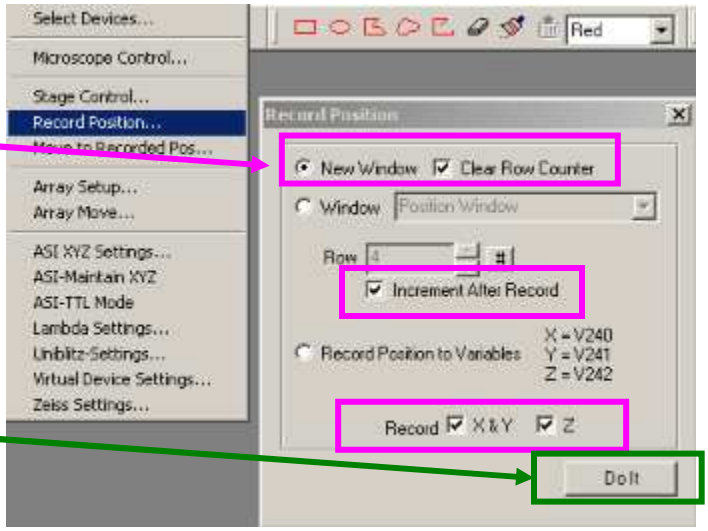
Mark positions

In IPLab, activate the Live-preview window by:

1. Turn on camera with menu command "***Camera/Acquire".***
2. Send light to ***base port*** using the vertical side-toolbar.
3. Open transmitted light ***(Uniblitz) shutter*** on the side tool-bar.



4. Activate the "Record positions dialog" using the menu command "***Control/Record Position***"
5. In the ***Record Position Dialog*** select:
   a. New Window
   b. Increment after record
   c. Record X, Y and Z.
6. Use the Joystick to local a field of view of interest.
7. Focus using the microscope's focus wheel.
8. Record the position with the "***Do It***" button in the "Record Positions" dialog.
9. Repeat Steps 4 to 7 for all desired positions/wells.



Save the ***Positions window*** (Select window then menu command "***File/Save***") to a new experiment folder and name it give it a relevant name.

Close the ***Record positions*** dialog.

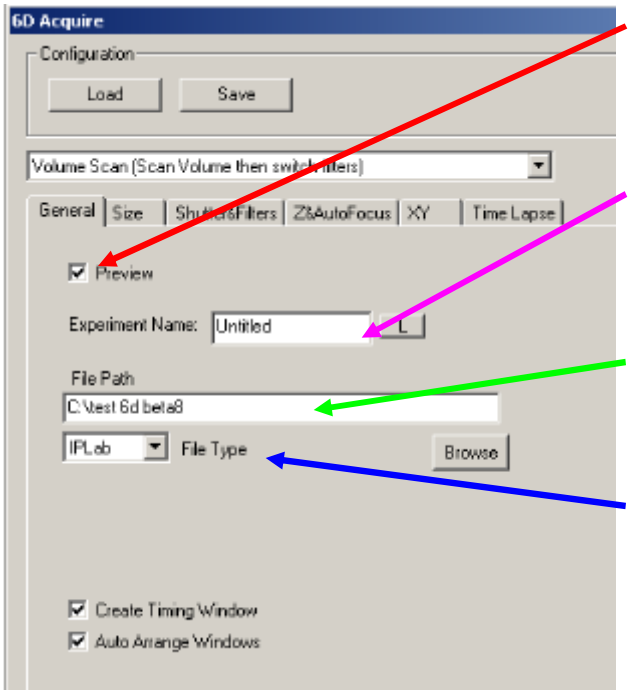
Cancel ***Acquire preview***.

Set up Acquisition

Activate the Acquire 6D dialog with the menu command "**Camera/Acquire 6D**".

This is a multi-tabbed dialog.

General Tab



Activate ***Preview*** to set exposures for each channel just before starting acquisition.

Provide an ***Experiment name*** which will be the folder in which the image files will be stored.

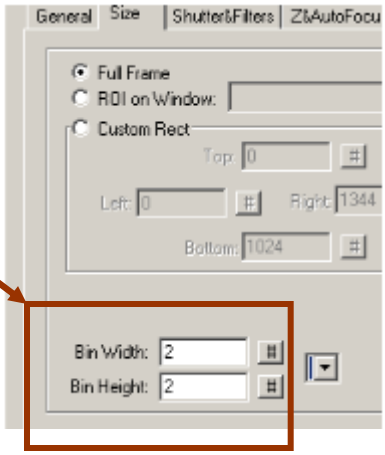
Set the location of this folder with the "***File Path***" option. This should be the same as the location of your positions window.

Keep as IPLab ***File type*** to maintain metadata.

Size Tab

This sets some parameters for the camera but many can be adjusted during the pre-acquisition preview.

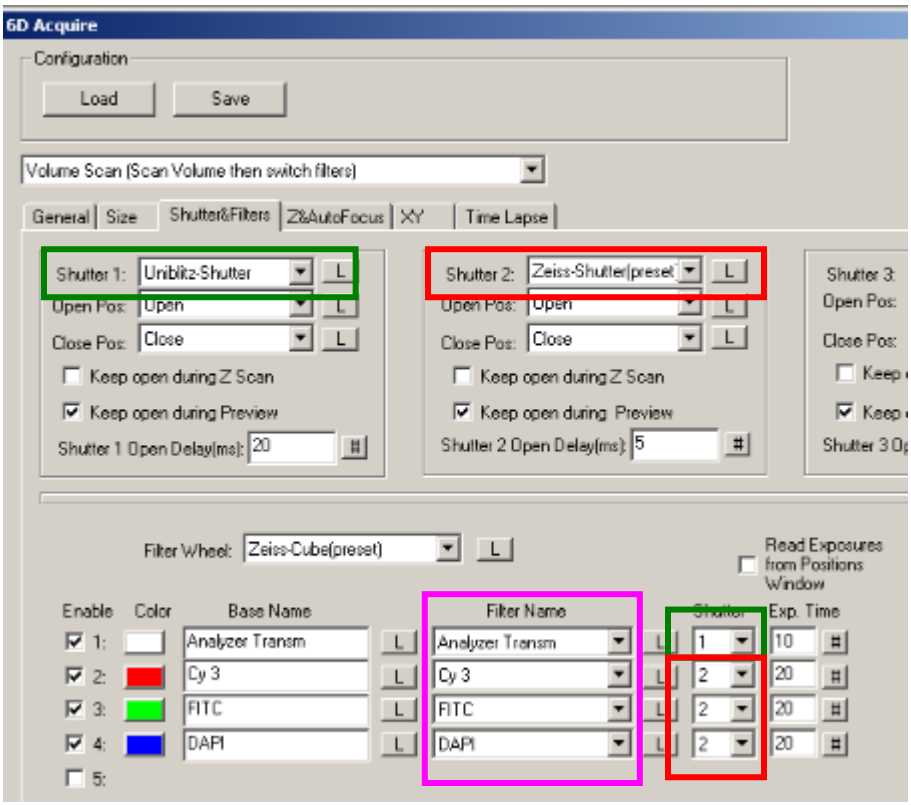
Typically you would set it to be full frame and ***Bin Width*** and ***Height*** as 2.



Shutters and Filters

Here you set the channels you wish to acquire. For bright field (phase or DIC) you need to use the Analyzer Filter and the Uniblitz shutter (which should be set as ***Shutter 1***).

For Fluorescence channels, the shutter is the Zeiss microscopes shutter (***Shutter 2***). The filter cube can be selected from the "***Filter name***" drop down box.



Select the required channels.

Z &AutoFocus

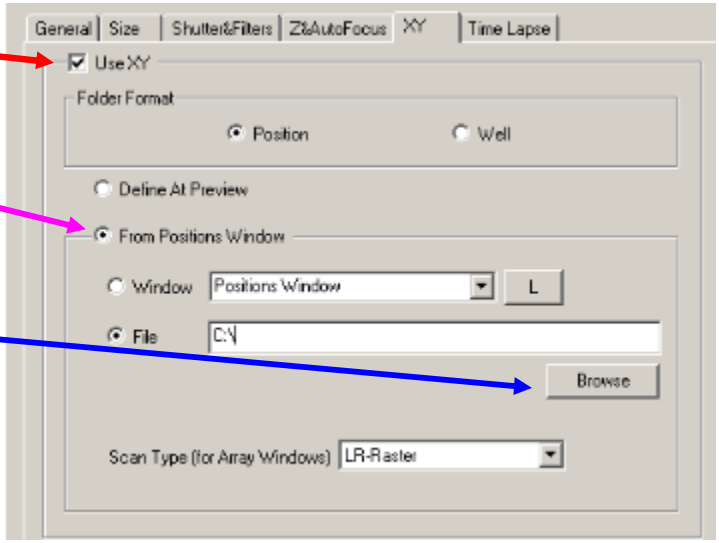
Turn on AutoFocus at each XY position.

XY

Activate "***Use XY***".

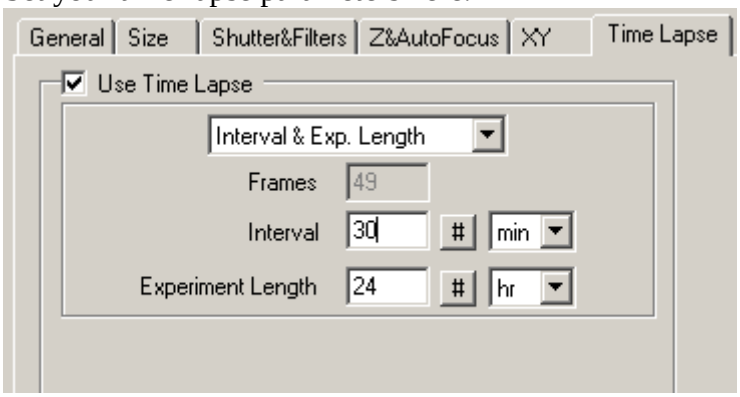
Select the option "***From Positions Window***".

Use ***Browse*** to locate your saved positions window.



Time Lapse

Set your time-lapse parameters here.



Save Settings

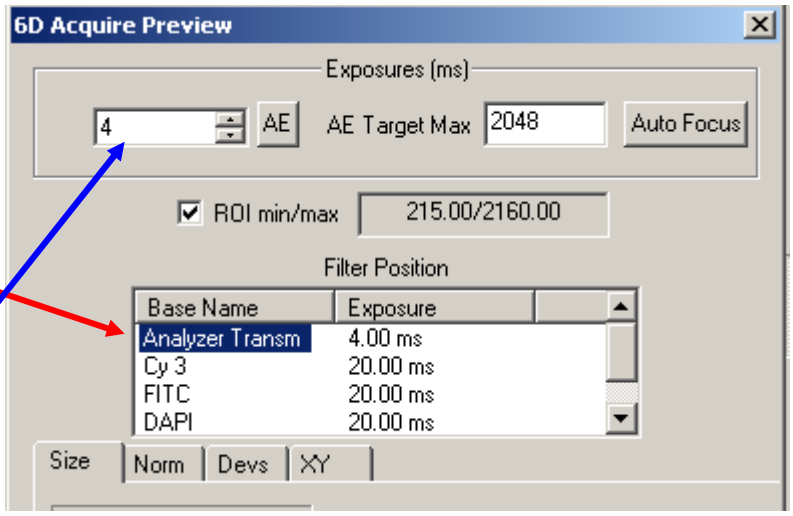
This experimental configuration can be saved with the "***Save***" button at the top of the dialog box and used later with the "***Load***" button. Save the configuration to your experimental folder.

Activate Preview Mode

Clicking OK in the Acquire 6D dialog activates “Preview Mode”.

Here you can switch between each channel by clicking on it in the “***Filter Position***” table.

Set the exposure for each channel by switching between them and changing the ***exposure***.



Click OK in the 6D Acquire Preview dialog.

Another dialog will open telling to click OK to start acquisition.

Open Series in ImageJ

The timepoints of each position will be automatically saved to a subfolder in the experimental folder as a series of IPLab files.

Run ImageJ and open the time course series with the menu command “***File/Import/Image Sequence***” and select the position’s subfolder.