Zeiss Axiovert 135 Live Imaging Microscope Operating Procedures

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Access to the microscope

- How to book online: https://ppms.eu/ucl/?ICHFCI
- How to get training: contact Dale d.moulding@ucl.ac.uk
- Login: Axiovert135 / Password: Axiovert135

Turn it on

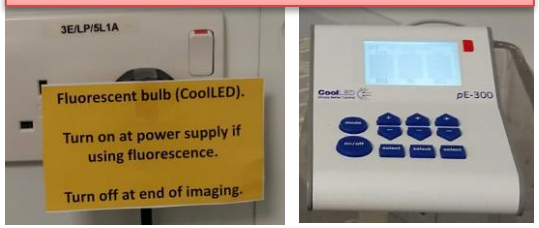
1. Turn on Mac controller
2. Turn on scope brightfield light (back right green switch) if doing phase contrast.
3. Temperature to 37 (if needed). At least 2 hours before starting
4. Turn on Fluorescence lamp (if needed).
5. Turn on the camera box

LEP MAC5000 controller

Halogen Lamp

Hamamatsu Camera controller

Fluorescence power and display panel



Incubator Temperature controller

Hold to set temperature

6. Start velocity software

Turn it on – CO2

CO2 enrichment controller

Increase / decrease speed so you get about 2 bubbles per second,
Or a new bubble forms just as the previous hits the surface.

Bottles live in the chamber.

Gas humidifier bottles

Environmental chamber

- Both bottle caps must closed when in use.
- Open the cap of bottle B when not in use.

Bottle A = filled with clean distilled water up to the 50 mL mark
Bottle B = must be empty

Live imaging Check-list

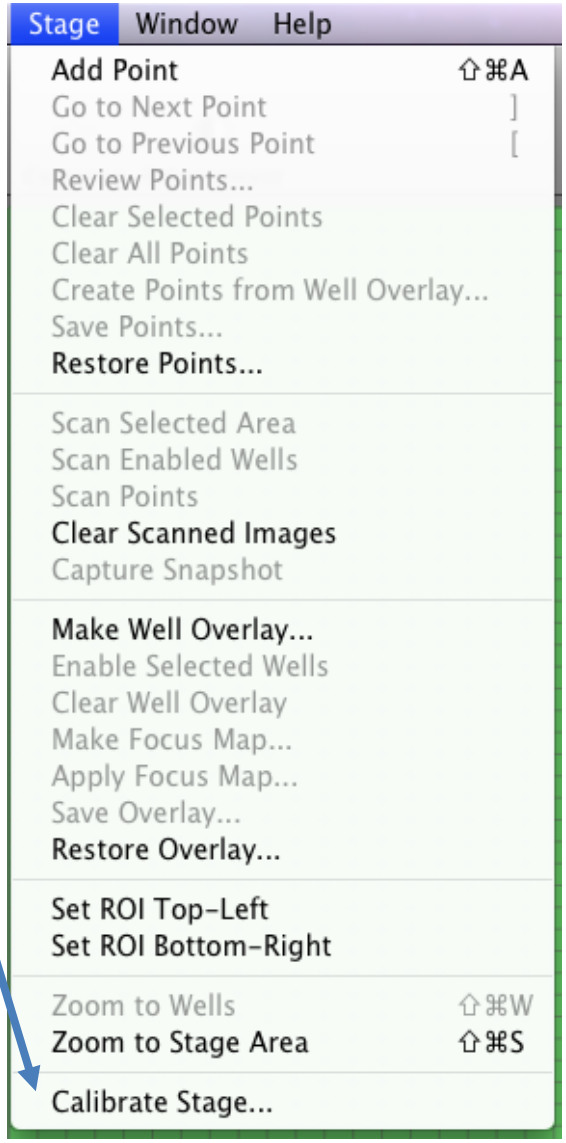
1. Environmental chamber temperature set and stable (at least 2 hours)
2. The CO2 cylinder contains enough gas for the whole length of the experiment
3. Microscope booked online for the duration of the experiment @ **PPMS**
4. Hard Drive less than 60% full
5. Calibrate the stage before putting the sample on the scope.
6. Leave the plate on the scope for about 20 minutes before setting final focus positions.

XY stage calibration

1. The motorised XY stage needs to be calibrated before placing the sample on the stage
2. Manually bring the objectives 'turret to its lowest position by disconnecting the focus motor (red button on the right hand side focus knob), engage 5x objective.
3. Start the Calibrate stage routine in Volocity **Stage>Calibrate Stage**
4. Place the sample on the stage
5. Focus onto the sample
6. Check the Heidenhain length gauge is moving freely

NOTE: also check 'Dummy Hardware on software matches your objective magnification

Heidenhain Length gauge



Ocular/Camera light path switch

XYZ & shutters/filters control

LEP MAC5000 controller

Only ever use the large manual focus wheel.
You MUST disengage the motor before using manual focus.
As soon as you use the software or Joystick to focus the motor is re-engaged.
Press the red button once each time before manual focus.

Large manual focus wheel

LEP XY stage joystick

XY movement

Z Focus

Shutters/Filters selector

1 = Fluo shutter
2 = Brightfield shutter

1 = DAPI
2 = FITC
3 = TxRd

Heidenhain Length gauge

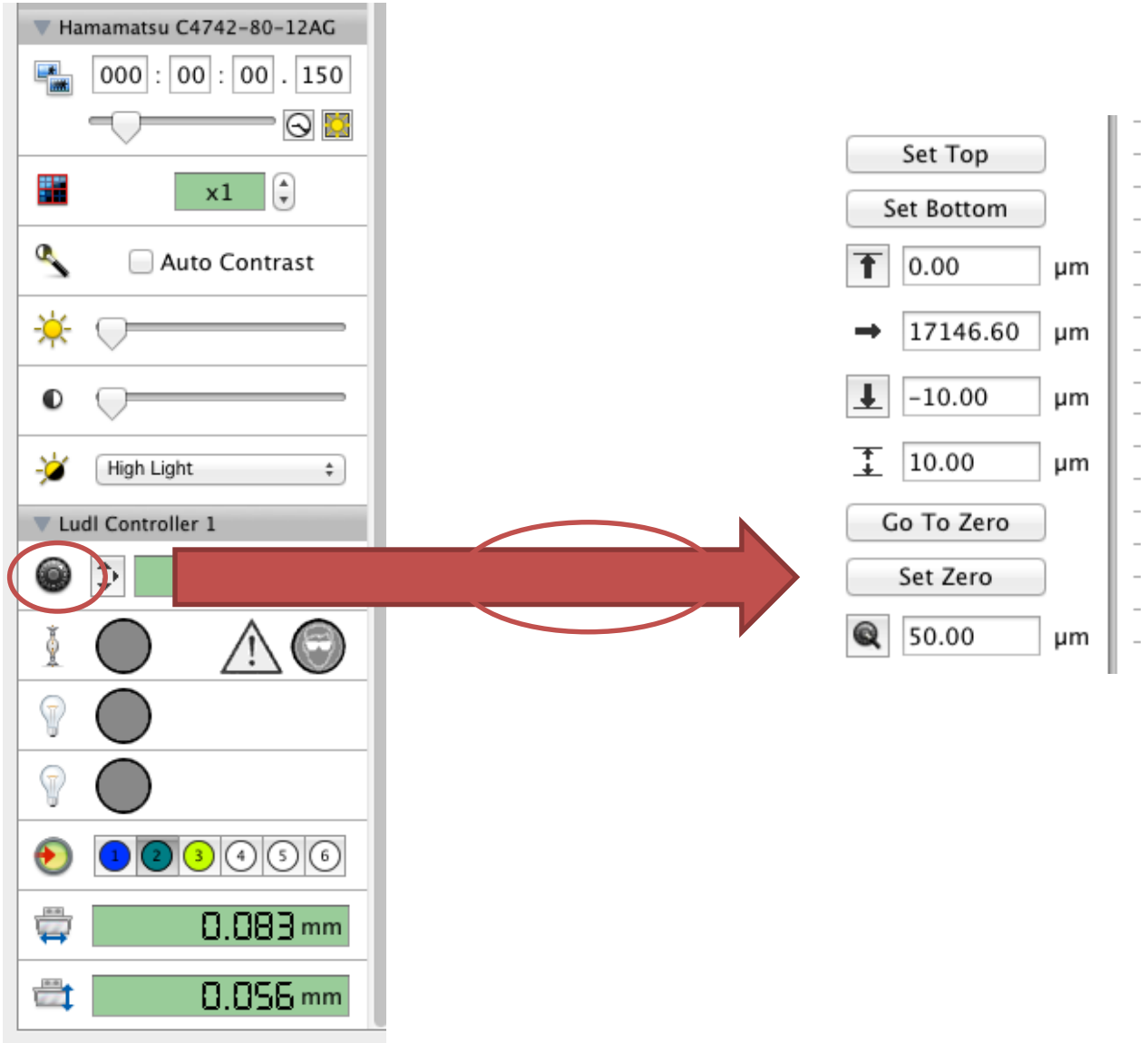
Length gauge to prevent Z drift

Motorised focus

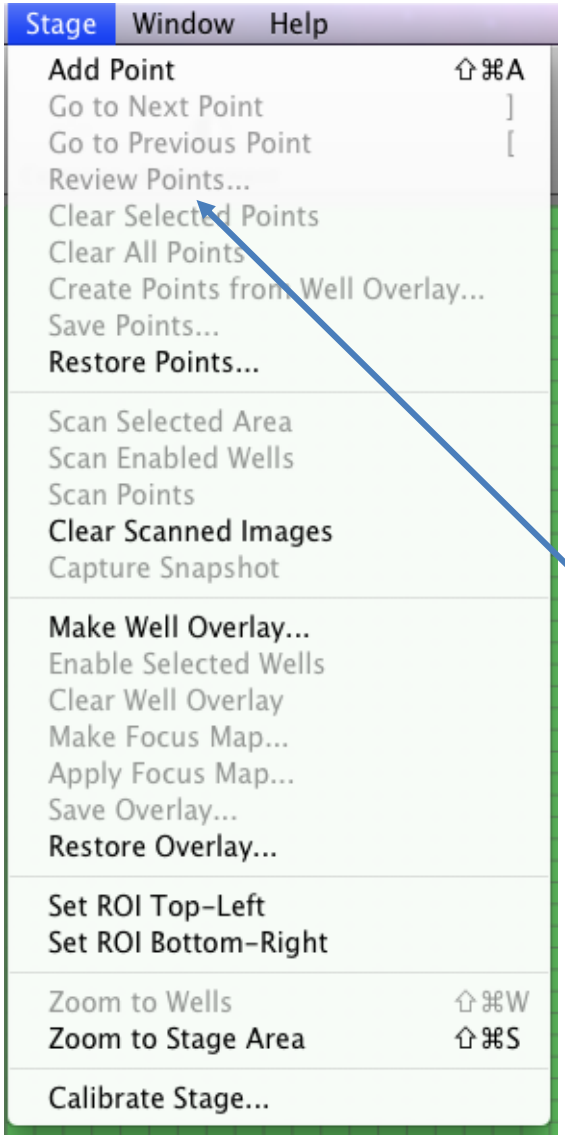
Focus Motor on/off

Reset the Z coordinate

Focus on your specimen,
Then set focus to zero

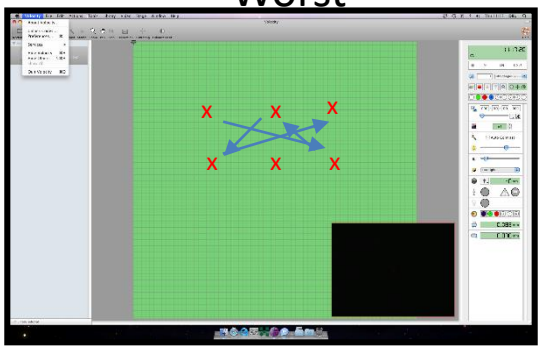


Selecting the XYZ points for imaging

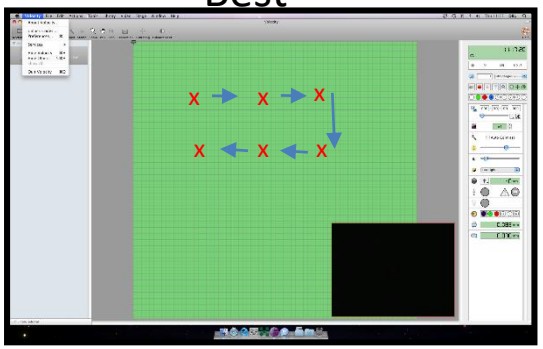


1. Delete previous XYZ positions
2. Draw a map of your sample
3. Determine the shortest travelling distance to visit all the XYZ points within the shortest time interval
4. Find the first XYZ point
5. Set the Z coordinate to zero
6. Add the first point to the list with **Stage>Add Point**
7. Move to the next point(s) until all points have been added.
8. (Optional) Save all the points **Stage>Save Points …**
9. Review the points and adjust focus if necessary

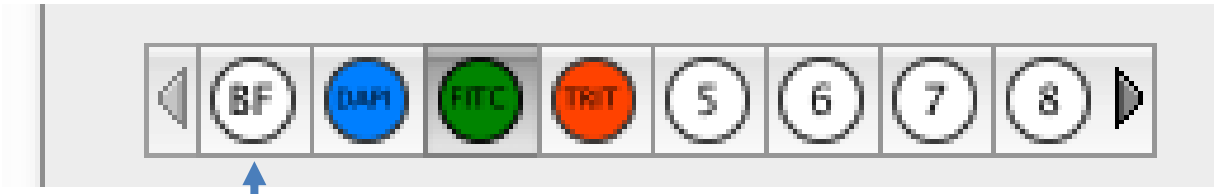
Worst



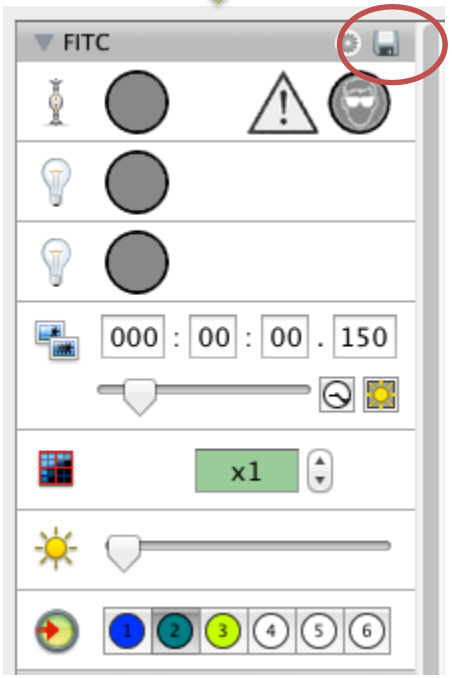
Best



Channel Set-up



Phase Contrast



Save channel parameters:

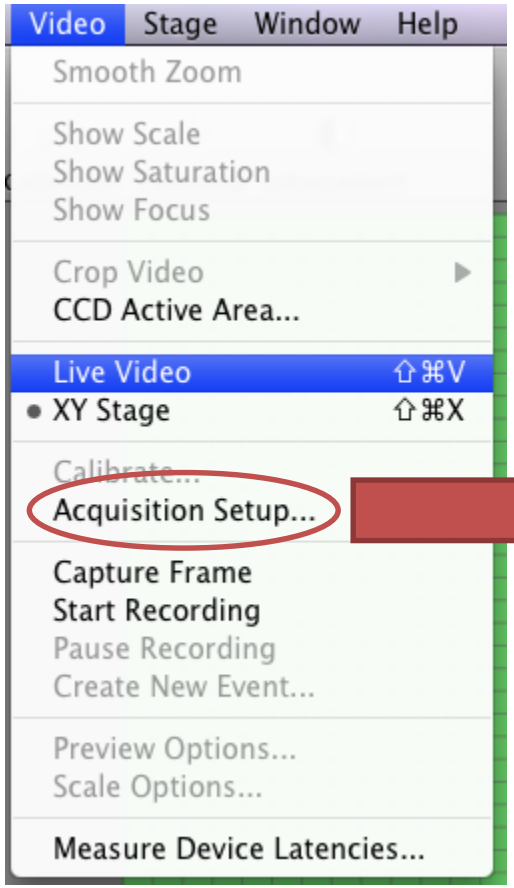
- exposure
- camera binning
- Camera gain

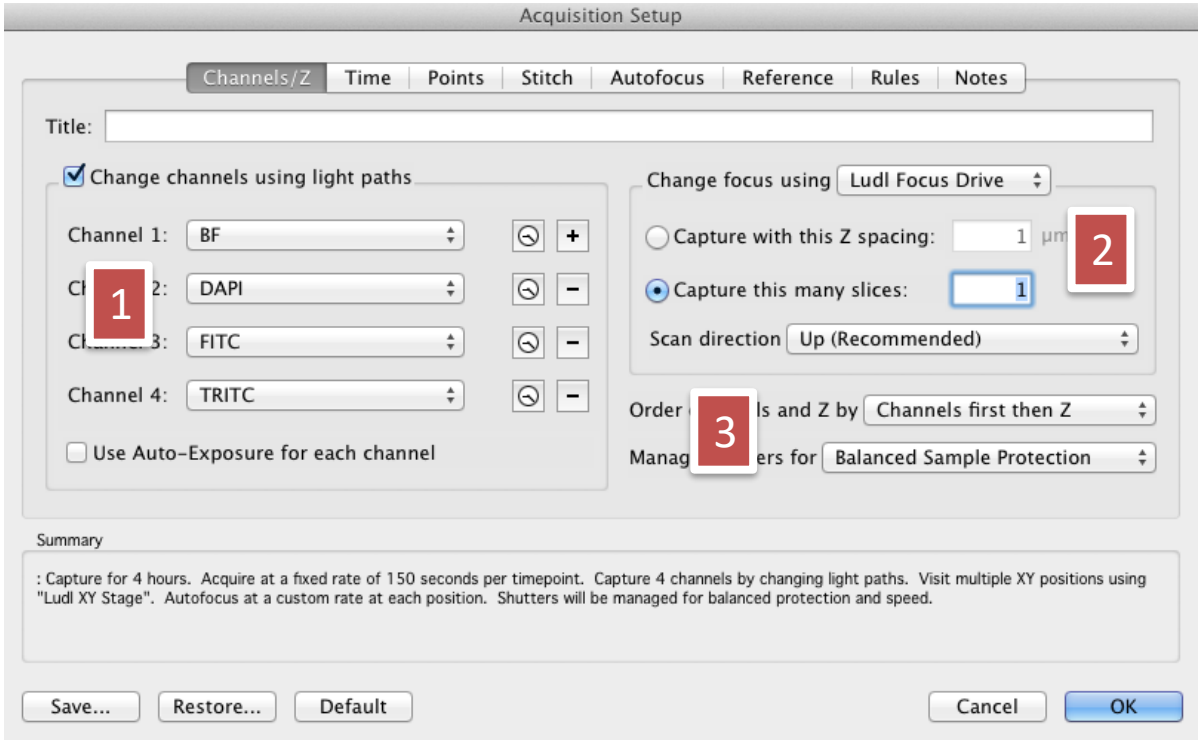
Exposure time

Camera binning (set default @ 1)

Camera Gain (set default @ 0)
More gain = brighter signal but more noise.
Balance Gain with Exposure time.

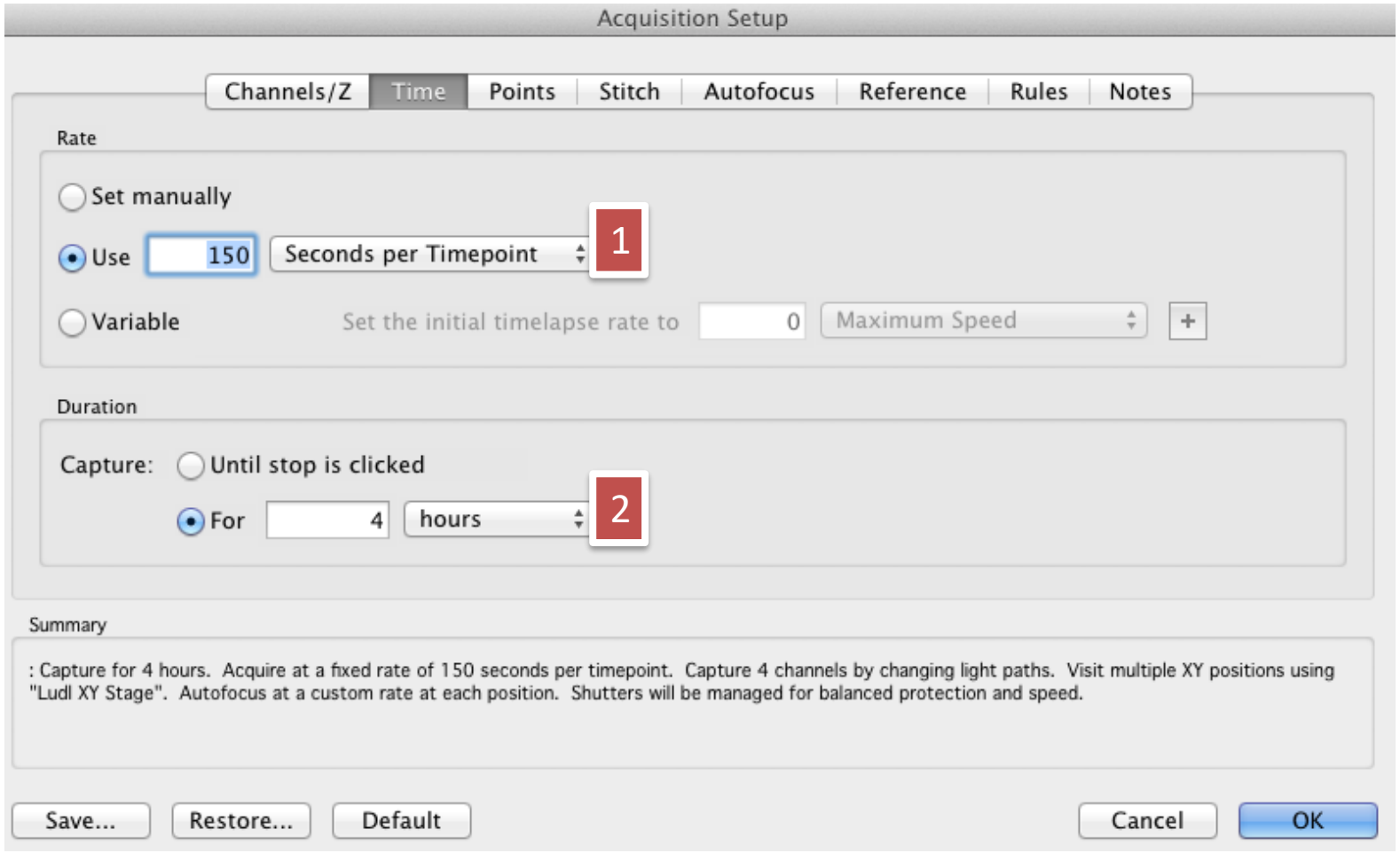
Acquisition Set-up: Channels/Z





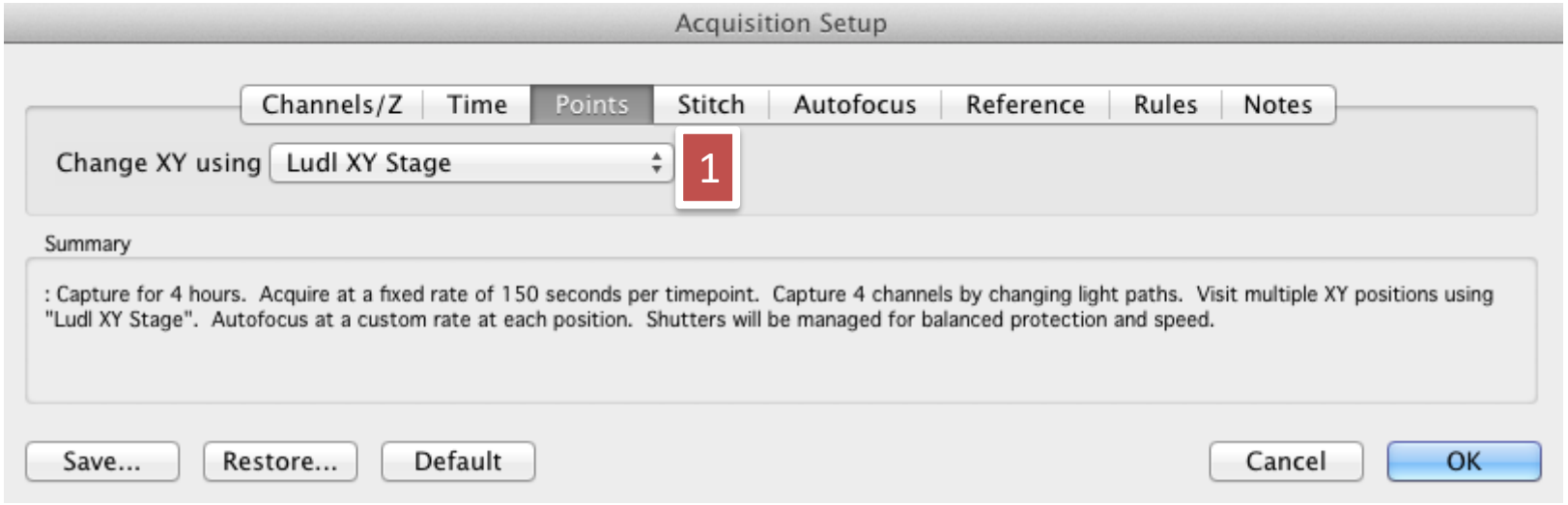
1. Add/remove channels
2. Select Focus option – set this to NONE unless doing z stacks
3. Select Order & Shutters management options – set as it is on the picture above – Channels first then Z, Balanced sample protection

Acquisition Set-up: time points



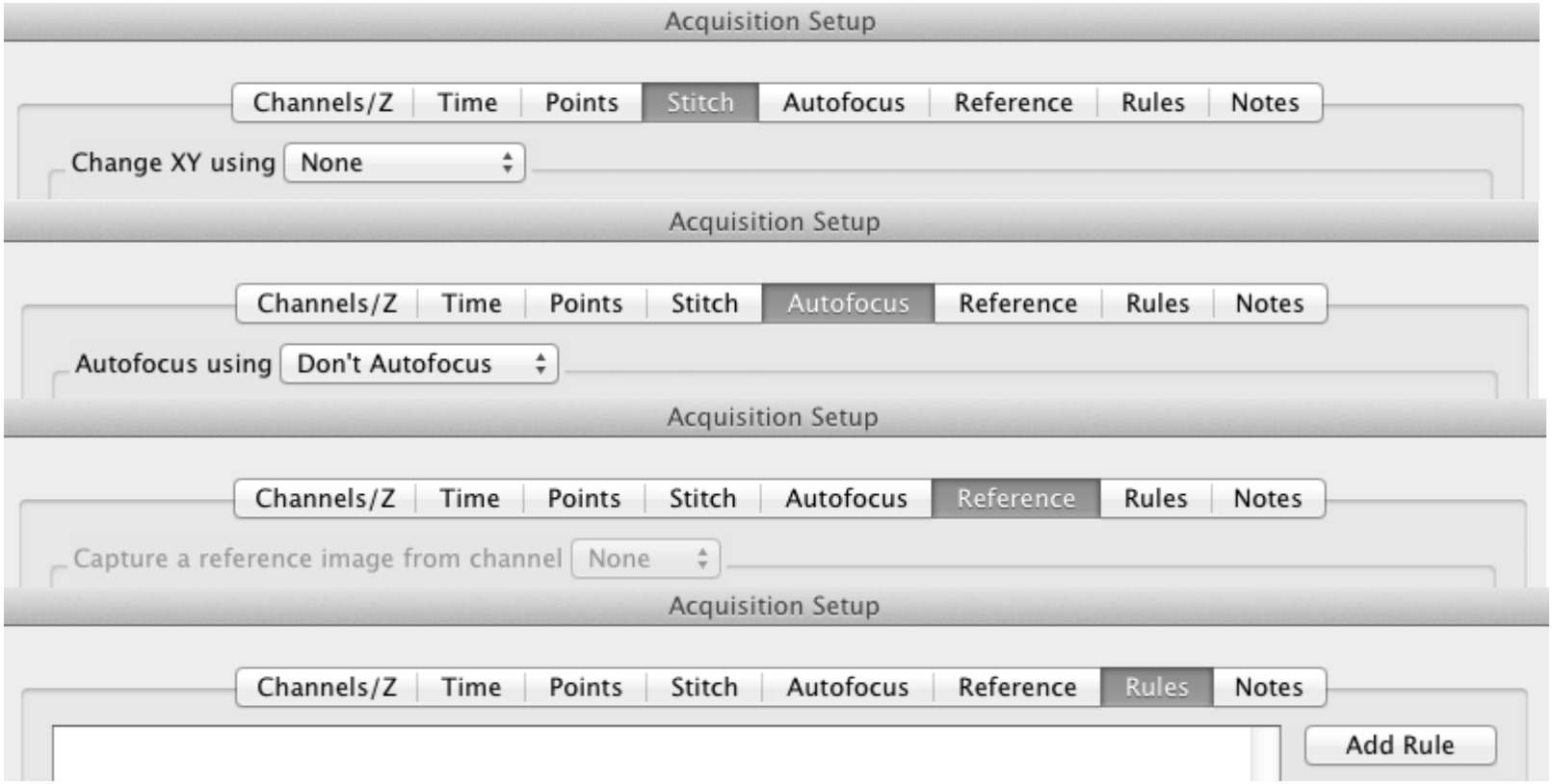
1. Choose time interval
2. Choose length of experiment

Acquisition Set-up: XY Points



1. Select Ludl XY Stage

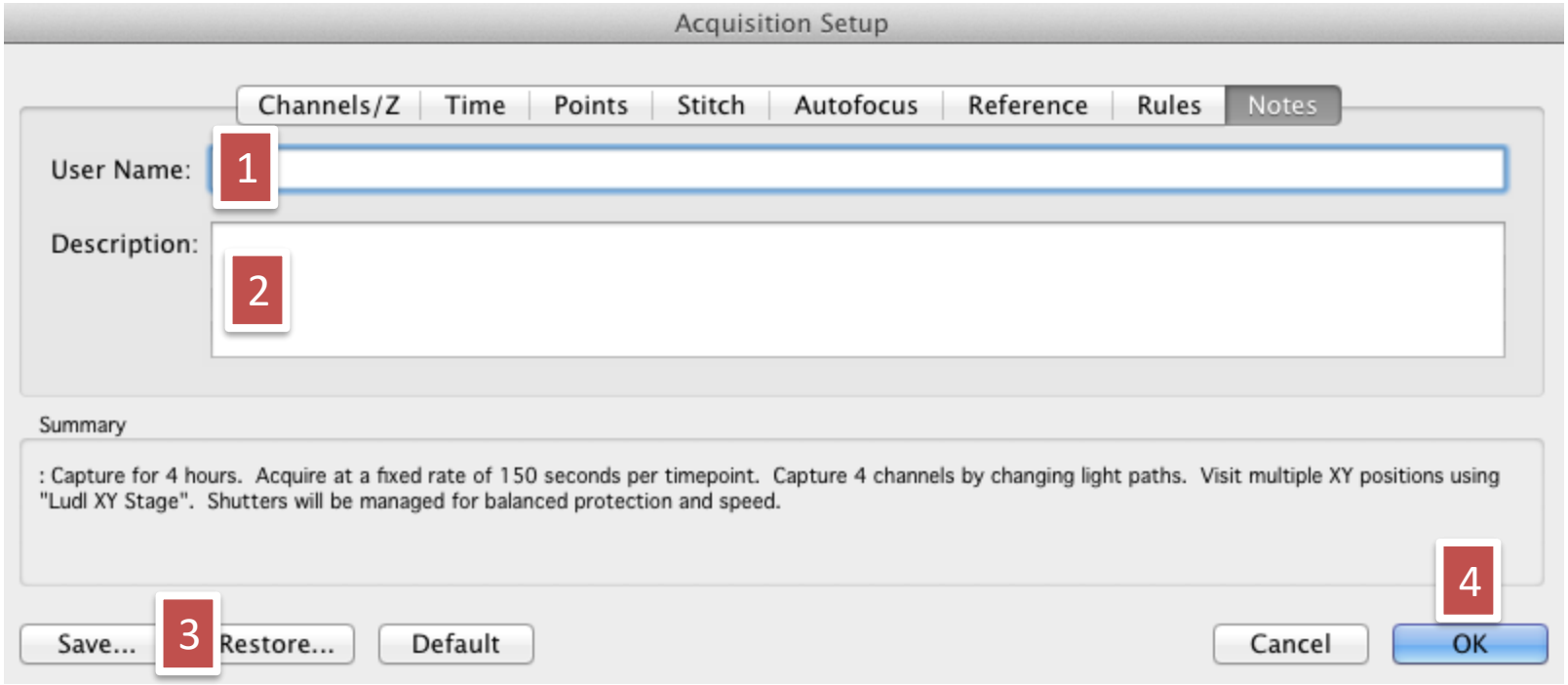
Acquisition Set-up



1. No "Stitch"
2. No "Autofocus"
3. No "Reference"
4. No "Rules"

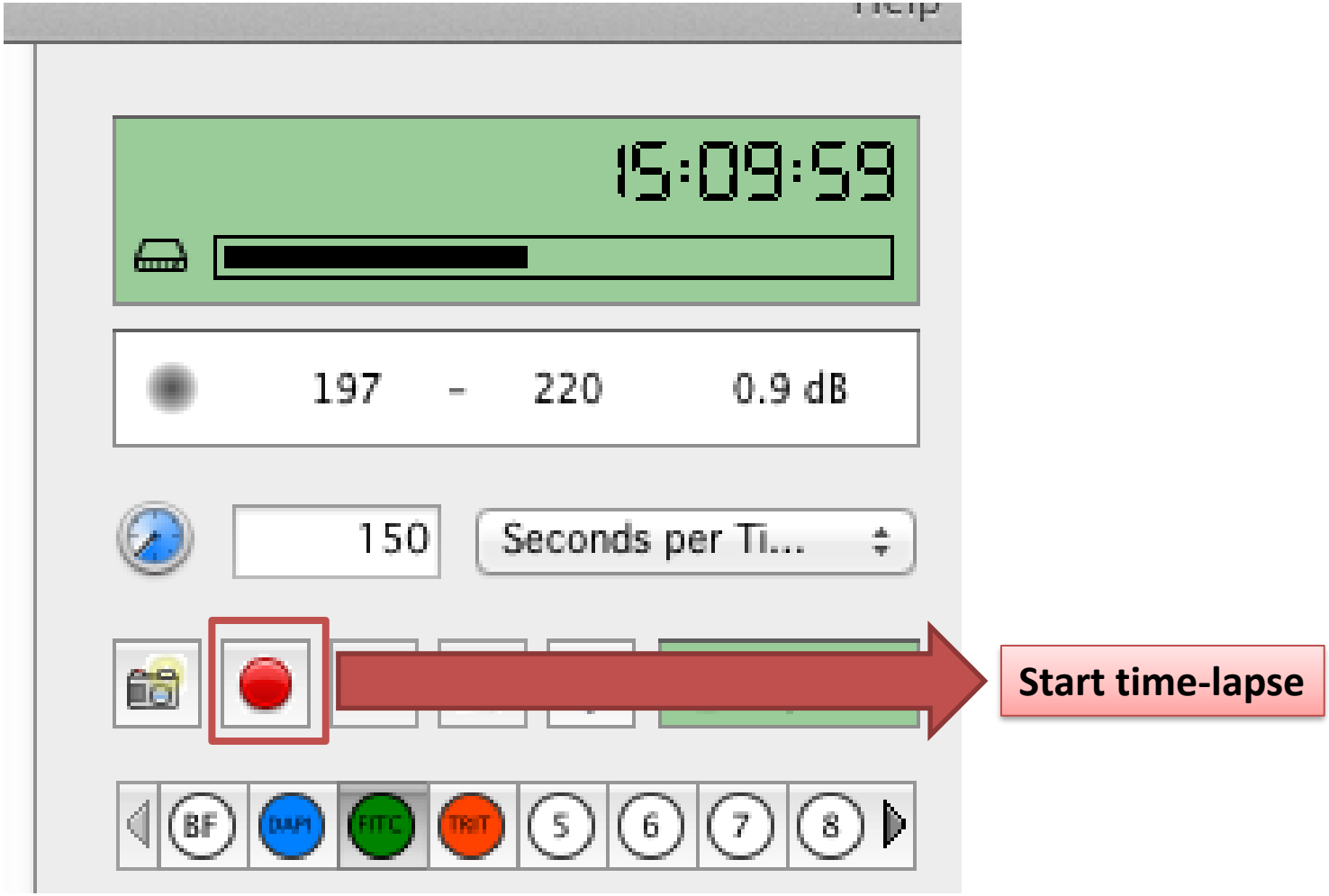
Note: if doing z stacks, set up XYZ as usual. Set a z-stack at position 1.
All subsequent positions will do the same size stack, but at the appropriate focus position.

Acquisition Set-up: Notes



1. User Name
2. Description of experiment (optional)
3. Save acquisition setup or load previously saved acquisition setup
4. Exit Acquisition Setup

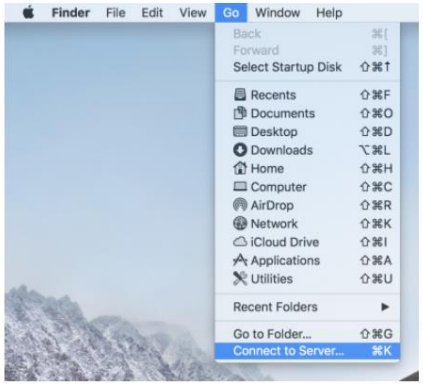
And Start the time-lapse …



Data Management

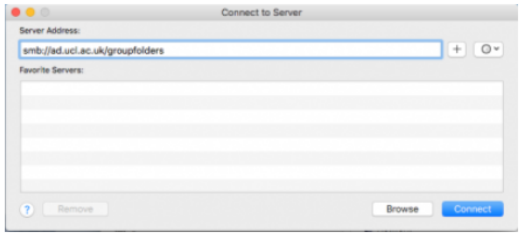
- Save your data in the DATA folder
- Data more than a month old will be deleted
- Transfer your data using UCL S drive…

1. In the **Finder**, click on the **Go** menu and select **Connect to Server..**



2. Enter the path as follows:

`smb://ad.ucl.ac.uk/groupfolders`



3. Click the **+** button to save the path for future reference

4. Click **Connect**

5. Supply your credentials:

- **Name:** enter your UCL Username with "ad"\ prefix. e.g. *ad\ccaabbc*
- **Password:** enter your UCL password

6. A new icon should appear on the desktop. That is your mapped network drive.

Scale

	Hamamatsu
Axiovert 135	Full Frame bin 1x1
objective	µm/pixel
x10 NA	1.031
x20 NA	0.467
x32 NA	0.318
x40 NA	0.228

Change image unit in Fiji/ImageJ:
Image>Properties …