Zeiss Axiovert 135 Live Imaging Microscope Operating Procedures



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Access to the microscope

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

Turn it on



Turn it on – CO2



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.





- 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
- 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.
- 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 Heidenhain Length gauge

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



Stage	Window	Help		
Stage Add Go to Go to Revie Clear Clear Crea	Vindow Point o Next Poin o Previous I ew Points r Selected P r All Points te Points fr	Help t Point Points om Well	Overla	ዕ
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Zoor Zoor	n to Wells n to Stage /	Area		

Calibrate Stage...

Ocular/Camera light path switch





XYZ & shutters/filters control



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.

Length gauge to prevent Z drift



Shutters/Filters selector

2 = Brightfield shutter

1 = DAPI 2 = FITC 3 = TxRd





Focus Motor on/off

Reset the Z coordinate

Focus on your specimen, Then set focus to zero



Selecting the XYZ points for imaging

Stage	Window He	lp
Add Go to Go to Revie Clear Clear Creat Save Resto	Point Next Point Previous Point W Points Selected Point All Points te Points from Points pre Points	企業A] t [well Overlay
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Make Enab Clear Make Apply Save Resto	e Well Overlay le Selected We Well Overlay Focus Map y Focus Map Overlay overlay	Is
Set R Set R	OI Top–Left OI Bottom–Rig	ht
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Calib	rate Stage	

- 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



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Channel Set-up



Acquisition Set-up: Channels/Z

Video Stage Window Help	Acquisition Setup		
Smooth Zoom	Channels/Z Time Points Stitch Autofocus Reference Rules Notes		
Show Scale Show Saturation Show Focus	Title:		
Crop Video CCD Active Area	Channel I: BF \Rightarrow \bigcirc + \bigcirc Capture with this Z spacing: 1 µm 2 Cl 1 2: DAPI \Rightarrow \bigcirc - \bigcirc Capture this many slices: 1 Cl 3: FITC \Rightarrow \bigcirc - Scan direction Up (Recommended) \Rightarrow		
Live Video 企業V • XY Stage 企業X Calibrate	Channel 4: TRITC Use Auto-Exposure for each channel Use Auto-Exposure for each channel Channel 4: TRITC Channel 4: TRIT		
Acquisition Setup Capture Frame Start Recording Pause Recording Create New Event	Summary : Capture for 4 hours. Acquire at a fixed rate of 150 seconds per timepoint. Capture 4 channels by changing light paths. Visit multiple XY positions using "Ludl XY Stage". Autofocus at a custom rate at each position. Shutters will be managed for balanced protection and speed. Save Restore Default Cancel		
Preview Options Scale Options	1. Add/remove channels		
Measure Device Latencies	 Select Focus option – set this to NONE unless doing z stacks 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

	Acquisition Setup
Cł	annels/Z Time Points Stitch Autofocus Reference Rules Notes
Rate	
 Set manually Use 150 	Seconds per Timepoint 💠 1
○ Variable	Set the initial timelapse rate to 0 Maximum Speed +
Duration	
Capture: OUnt	4 hours 2
mmary	
apture for 4 hours. Ac udl XY Stage". Autofoc	uire at a fixed rate of 150 seconds per timepoint. Capture 4 channels by changing light paths. Visit multiple XY positions usin is at a custom rate at each position. Shutters will be managed for balanced protection and speed.
Save Resto	re Default Cancel OF

- 1. Choose time interval
- 2. Choose length of experiment

Acquisition Set-up: XY Points

Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rules Notes Change XY using Ludl XY Stage	
Summary	1
: Capture for 4 hours. Acquire at a fixed rate of 150 seconds per timepoint. Capture 4 channels by changing light paths. Visit multiple XY positions using "Ludl XY Stage". Autofocus at a custom rate at each position. Shutters will be managed for balanced protection and speed.	
Save Restore Default Cancel OK	
1. Select Ludi XY Stage	

Acquisition Set-up

Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rules Notes	
Change XY using None \$	
Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rules Notes	
Autofocus using Don't Autofocus 💠	
Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rules Notes	
Capture a reference image from channel None 💠	
Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rules Notes	
Add F	tule

- 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

Acquisition Setup	
Channels/Z Time Points Stitch Autofocus Reference Rule User Name:	s Notes
Description:	
Summary	
: Capture for 4 hours. Acquire at a fixed rate of 150 seconds per timepoint. Capture 4 channels by changing light paths. "Ludl XY Stage". Shutters will be managed for balanced protection and speed.	Visit multiple XY positions using
Save 3 Restore Default	Cancel OK

- 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 ...

LICIP
15:09:59
197 - 220 0.9 dB
2 150 Seconds per Ti +
Start 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 ...

Channels (c):	1		Channels (c):	1
Slices (z):	1		Slices (z):	1
Frames (t):	1		Frames (t):	1
Note: c*z*t mus	t equal 1		Note: c*z*t mus	t equal 1
Unit of length:	pixel		Unit of length:	um
Pixel width:	1.0000		Pixel width:	0.467
Pixel height:	1.0000	\longrightarrow	Pixel height:	0.467
Voxel depth:	1.0000		Voxel depth:	0
Frame interval:	0 sec		Frame interval:	300 sec
Origin (pixels):	0,0		Origin (pixels):	0,0
🗖 Global			🗌 Global	
OK Cancel			0	K Cancel