Widefield 1

Switching on

1. Ignite DG5 lamp - **must be switched on first** (if previous user has switched off, wait 30 min before igniting)
2. Wait 5s and then turn on the main DG5 controller switch.
3. DG5 shutter
4. Piezo controller (optional – for capturing z-stacks)
5. Ludl shutter/filterwheel controller
6. Photometrics camera controller – for front port camera. Note there is another on/off switch on the top of the photometrics camera which is normally left on
7. Side port Qimaging camera – on/off switch on side of camera (optional – large field of view)
8. Switch on microscope
9. Log into computer using UoB login
10. Start Metamorph software. This is found within the Meta Imaging Series 7.0 folder on the desktop

(If you want to do ratiometric imaging you should open the Metafluor software – please see separate guide)

Note that on starting software you will get 1 or 2 error messages which can be ignored:

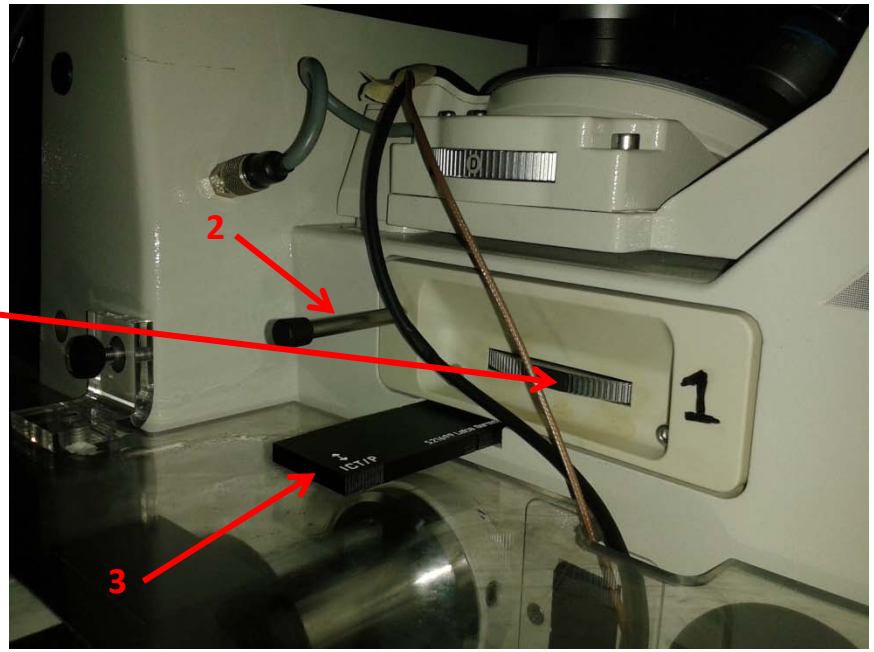
1. "no cameras could be opened" – when asked if you want to try again click **no**.
2. "Leica microscope could not be detected" – when asked if you would like to try to connect click **cancel.** *(the software does not need to directly control the microscope).*

NB. Switches 1 (DG5 lamp) and 2 (main DG5 controller) are next to each other on back of DG5 box (1 RHS, 2 LHS)

The heater is left on at 37°C. People wanting to use the system at room temperature should specify this on their booking and include sufficient for the system to cool.

Selecting the correct filter cube for fluorescence

There are 2 filter carousels with 7 different filter cubes. Filter carousel 1 should be left in by default – if you use carousel 2 please put carousel 1 back in when you finish. The carousels should be put in their boxes on the shelf when not in use.

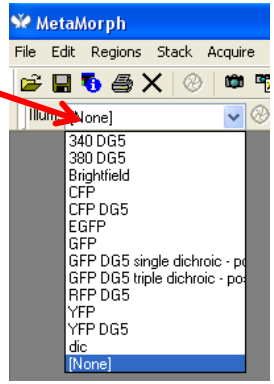


1. Select the cube position you want by turning the dial

2. Ensure the manual fluorescent shutter is pulled out and open.

3. Ensure the DIC analyser is pulled out of the light path (as shown) as it will decrease the brightness of your signal

You should now also select the correct Illumination setting in the software for the cube you have selected.



Notes

- **If using GFP and RFP** select **filter cube 1** in **filter carousel 1** and use methods **RFP DG5** and **GFP DG5 triple dichroic.**
- **If using GFP only** it is better to use **filter cube 2** in **filter carousel 1** and the method **GFP DG5 single dichroic** (the cube is more efficient than filter cube 1.)
- For **CFP/YFP FRET**, you need to use **filter cube 3** in **filter carousel 3**. **Do not look down the eyepieces when using this cube** – only view the image on screen as the emission filter are in front of the camera and not in front of the eyepieces.

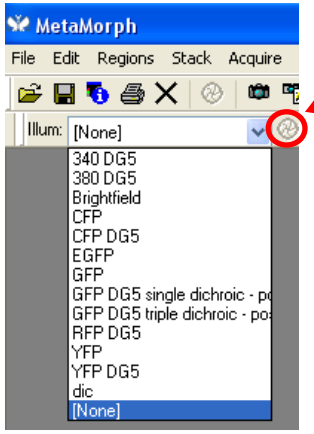
Filter carousel 1

Position	Example fluorophore	Excitation filter in cube	Excitation filter in DG5 (select in software)	Dichroic mirror	Emission filter in cube
1	DAPI FITC TRITC	none none none	380 485 550	RKP 400	450/10 520/13 595/22
2	GFP	470/40	none	RKP 495	525/50
3	FURA-2	none none none	340 or 380	RKP 430	510/40
4	CFP	440/21	none	RKP 455	480/30

Filter carousel 2

Position	Example fluorophore	Excitation filter in cube	Excitation filter in DG5 (select in software)	Dichroic mirror	Emission filter in cube
1	CFP	440/21	none	455	480/30
2	YFP	500/25	none	525	545/35
3	CFP/YFP FRET	none	none	455	none
4					

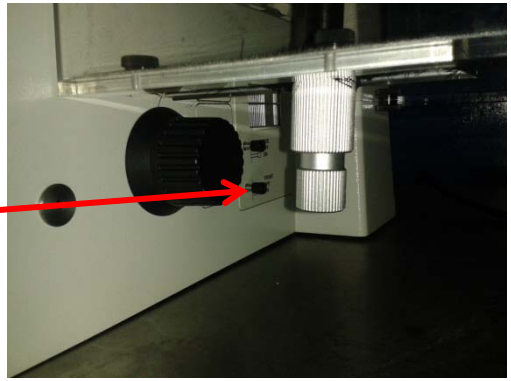
More microscope info…



When you have selected your illumination setting, open and close the shutter on the microscope by clicking here (green when open).

When using the Photometric camera (front port), push the slider to the left of the eyepieces in to divert light to the eyepieces and pull out fully to divert light to the camera

- If using the **Photometric camera** (front port), check that the bottom slider on the right side is **pushed in.**
- If using the **Qimaging camera** (side port) this bottom slide must be **pulled out** to divert light to the camera.



Running Metamorph

Commonly used controls are have been put in a taskbar on the desktop. If this task bar has been removed you can find it under **Journal**

taskbar

load

main taskbar

1. Select which camera you want to use – Photometrics = front port = sensitive
   Qimaging = side port = large chip

2. Select Acquire (for capturing single channel snapshots) or

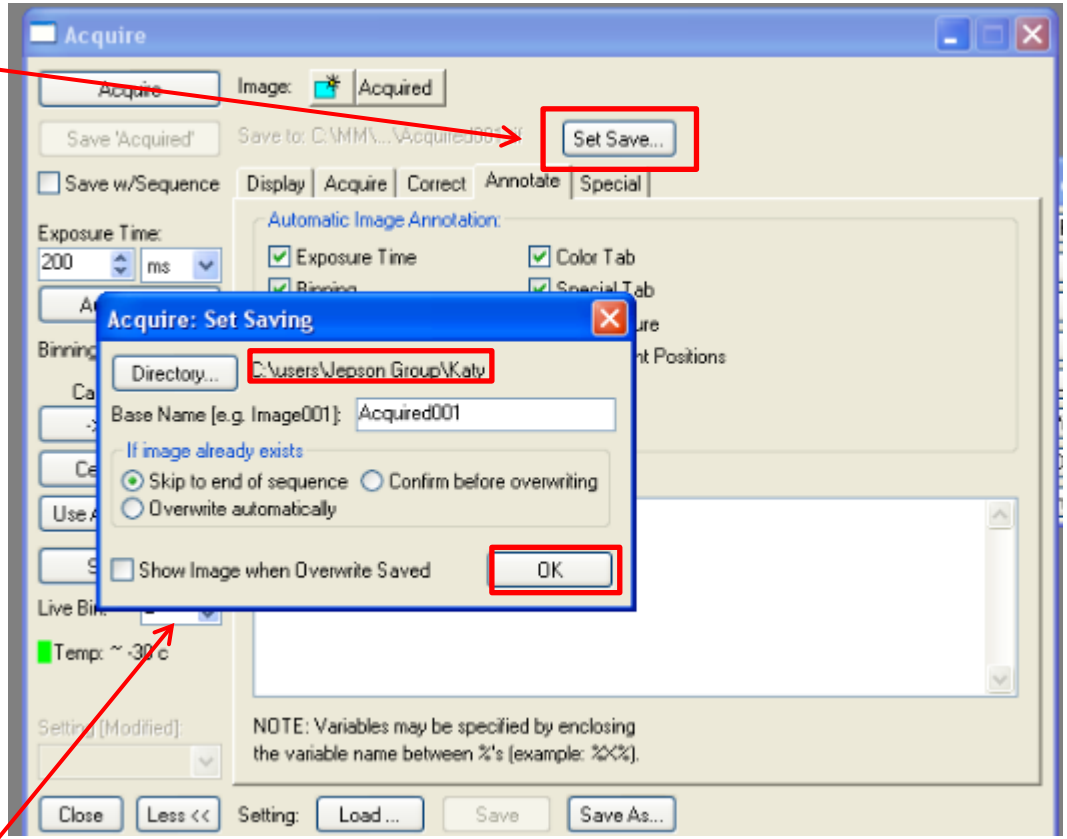
3. Multi Dimensional Acquisition (for multiple channels, z-stacks, timecourses)

4. Select PiFOC if you need to control the Piezo focus device.

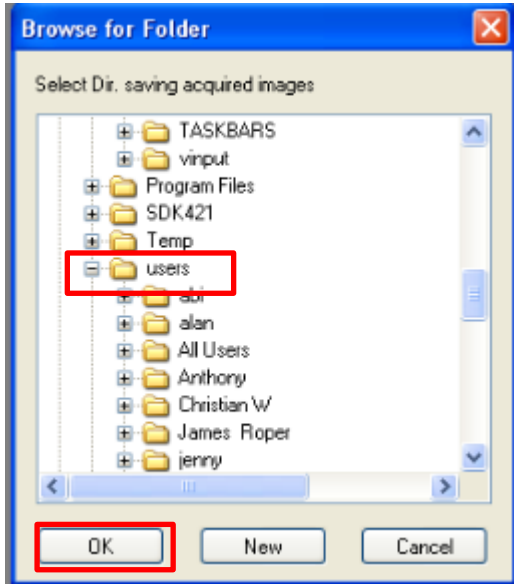
Using Acquire

First select where you want your images to be saved to:

1. Select "Set Save"

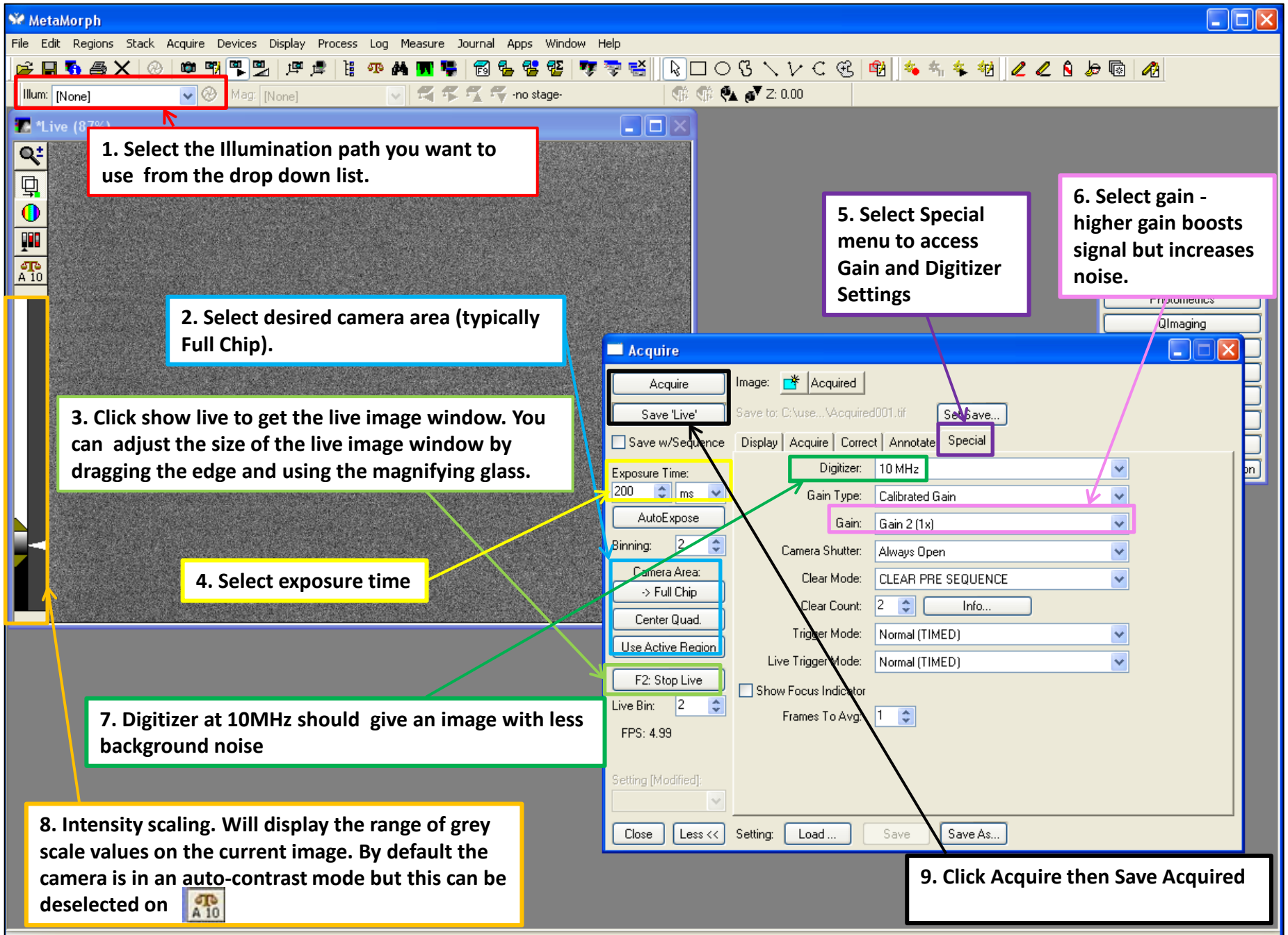


2. "Browse for Folder" window will open. Select the folder you want to save the images in from "users" and click "OK"



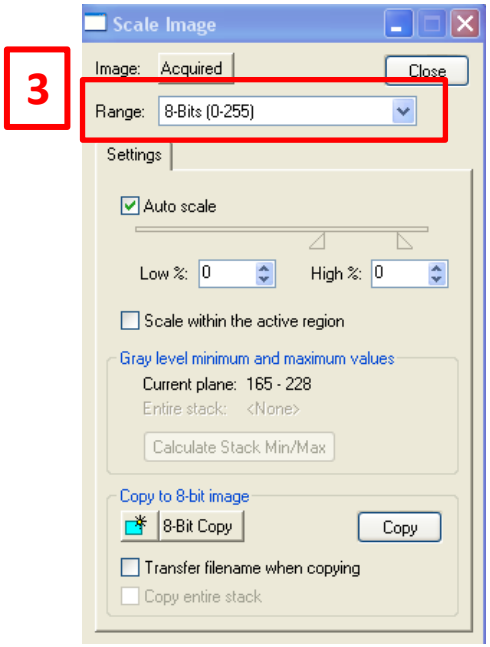
3. The directory you have chosen will appear. Change base name as required. Click "OK" to save images here.

Using Acquire continued…

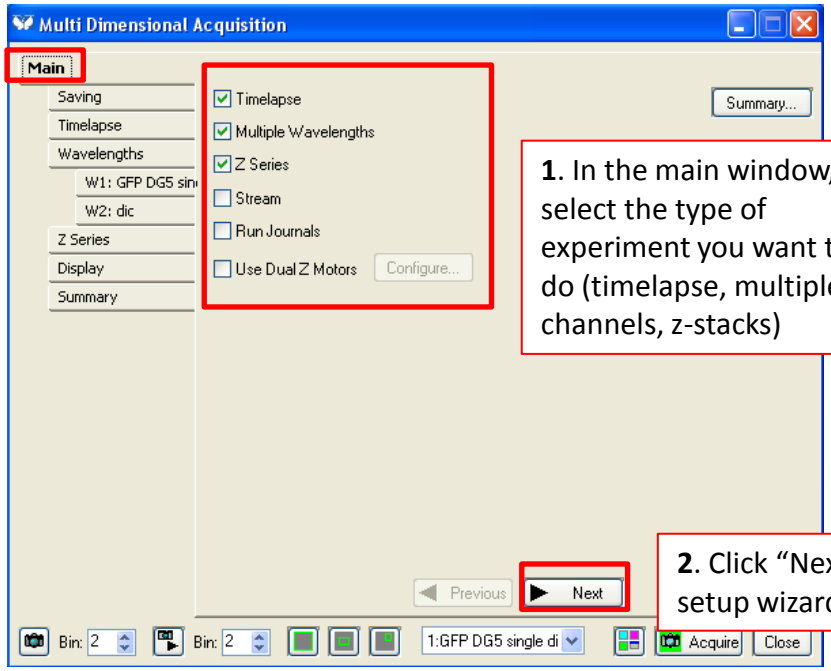


Using Acquire continued…

Note that images are saved in a 14 bit format by default so cannot be viewed easily outside of imaging software. You can however save an 8 bit copy by clicking on  then making an 8 bit copy in the scale image window.



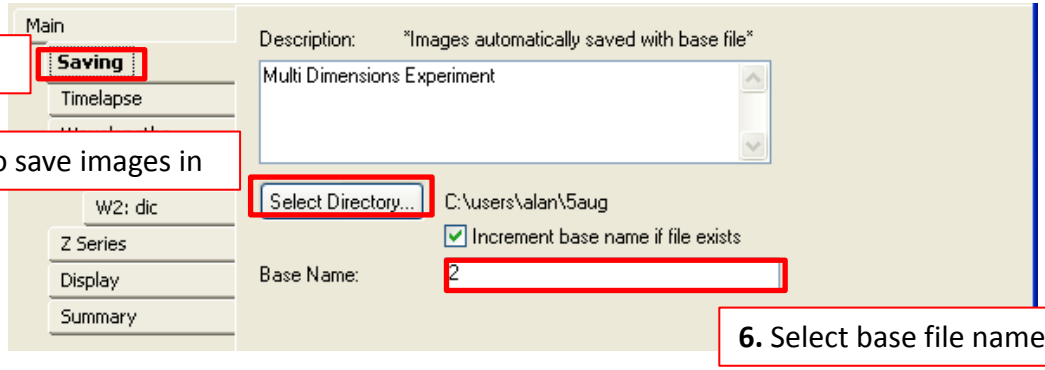
Using Multi Dimensional Acquisition



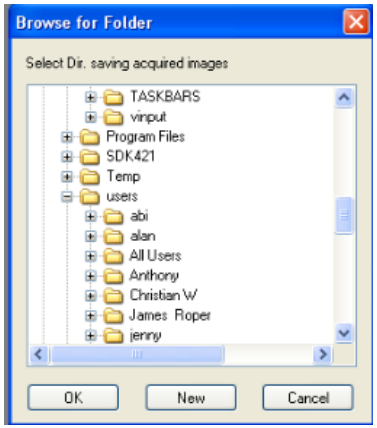
1. In the main window, select the type of experiment you want to do (timelapse, multiple channels, z-stacks)

2. Click "Next" to work through the setup wizard

3. The "Saving" window will appear



4. Select the directory to save images in



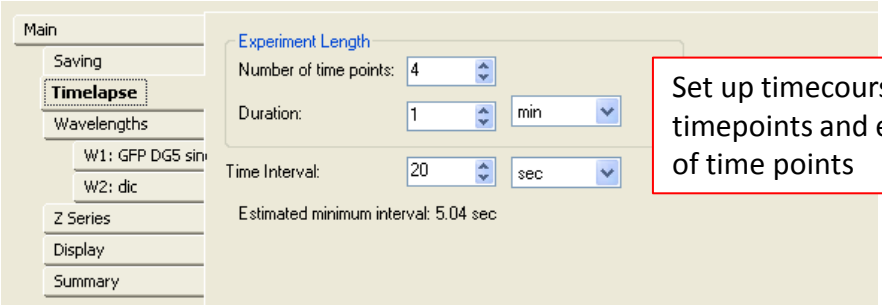
5. "Browse for Folder" window will open. Select the folder you want to save the images in from "users" and click "OK"

6. Select base file name

Note: by default images are written to the hard disk as they are captured.

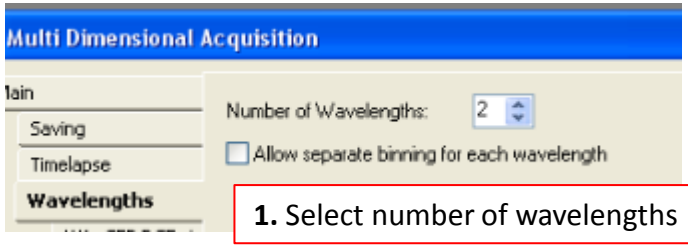
Using Multi Dimensional Acquisition continued…

Timelapse

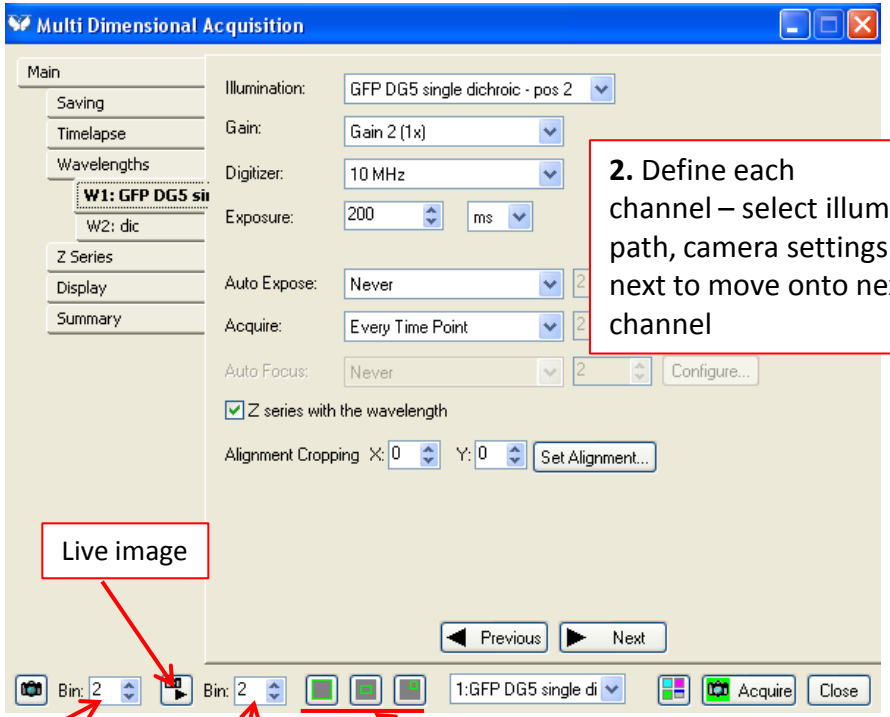


Set up timecourse by defining the interval between timepoints and either the experiment duration or number of time points

Wavelengths



1. Select number of wavelengths



2. Define each channel – select illumination path, camera settings. Select next to move onto next channel

Live image

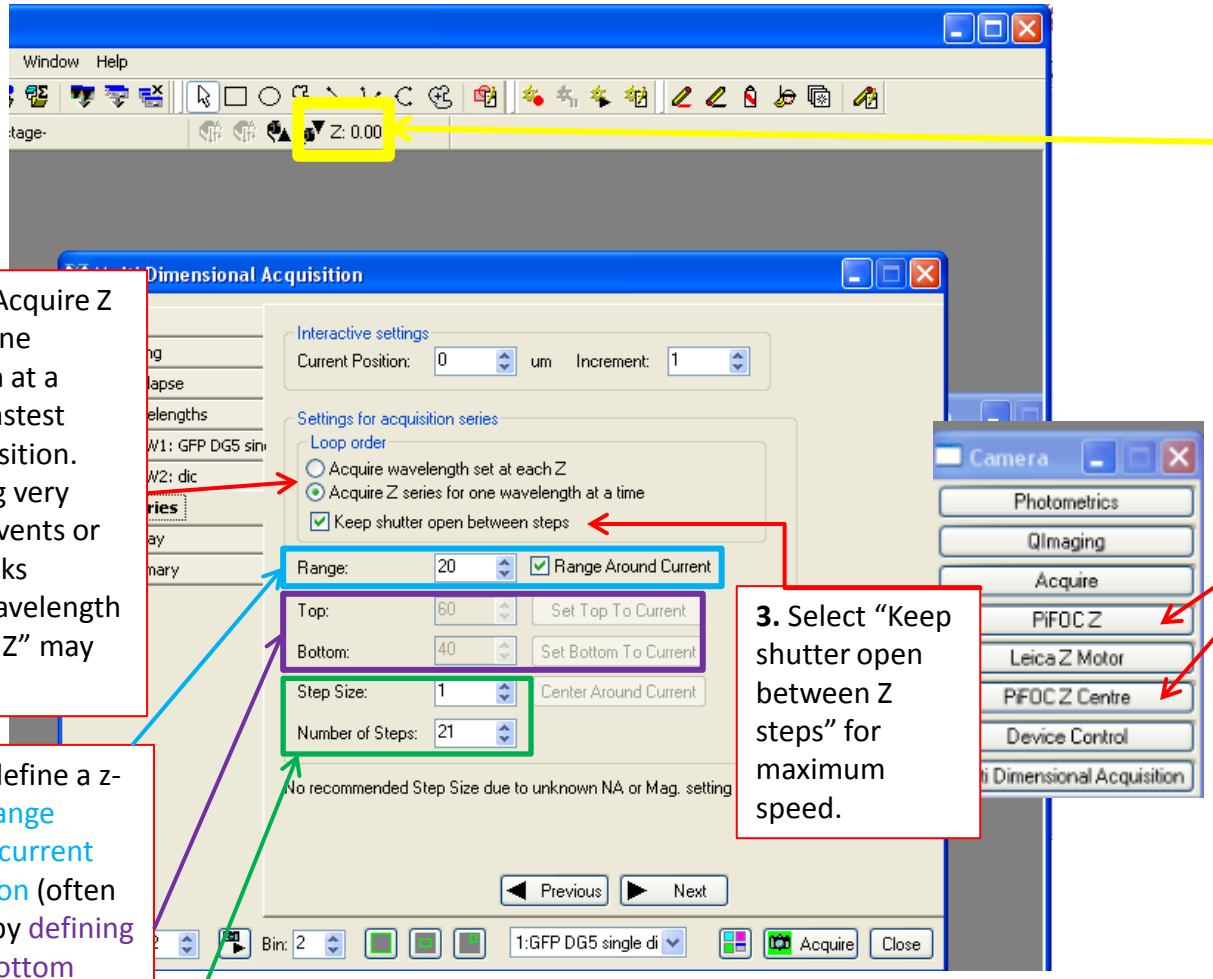
Set binning for captured images if required.

Set binning for live images if required.

Active camera area

Note: please see page 11 for instructions on how to change the intensity of the excitation light if required

Z series



1. Select the PiFOC Z motor and centre it.
The PiFOC device has a range of 100 microns and it's best to start off in the middle of this range (at 50 microns).
Once you have done this, the Z position at Z: 0.00 should read 50.

2. Select "Acquire Z series for one wavelength at a time" for fastest stack acquisition. For imaging very rapid cell events or thick Z-stacks "Acquire wavelength set at each Z" may be best.

3. Select "Keep shutter open between Z steps" for maximum speed.

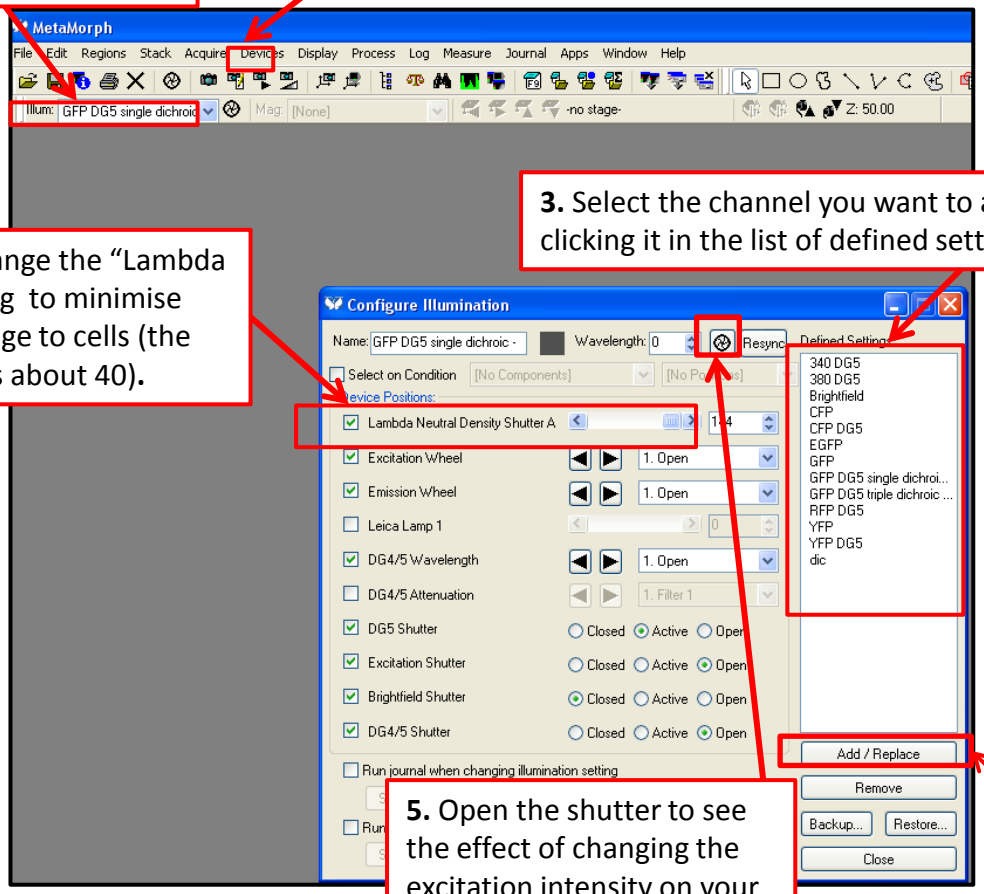
4. You can define a z-stack by a range around the current focus position (often easiest) or by defining a top and bottom position. Then select Step Size or Number of Steps required.

Continue to the end of the Multi Dimensional Acquisition pages then click "Acquire"

Configure Illumination settings

1.The standard illumination setting are available from the drop down menu

2. You may want to define you own illumination settings or adjust the standard ones – for example by the default the fluorescent excitation light intensity on each channel is set to its maximum. To change this, go to the devices menu and select **"configure illumination."**



3. Select the channel you want to adjust by double clicking it in the list of defined settings.

4. You may want to change the "Lambda Neutral Density" setting to minimise the risk of photo-damage to cells (the lowest usable setting is about 40).

5. Open the shutter to see the effect of changing the excitation intensity on your live image

6. Click "Add/Replace" to overwrite the previous setting

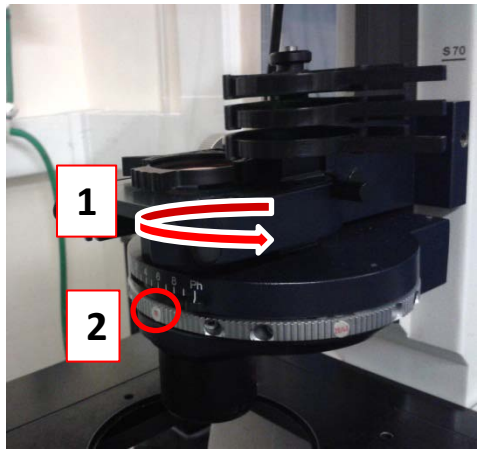
DIC

1. Ensure the polariser on the condensor is in the lightpath.

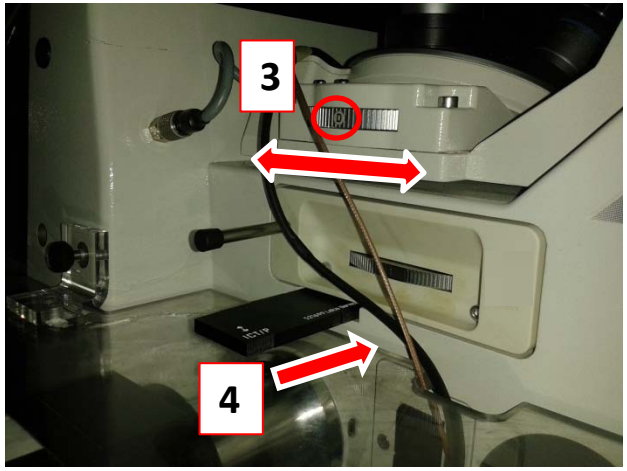
2. Select the appropriate DIC condensor prism for the lens used (see table below).

3. Select the appropriate DIC objective prism for the lens used (see table below).

4. Analyser must be pushed in for viewing DIC down the eyepieces but can be removed from the lightpath when capturing images with the Photometrics camera for which there is an analyser in the filter wheel



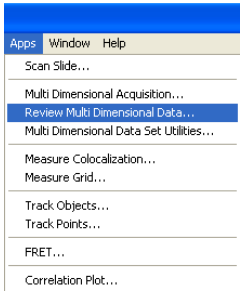
Objective lens	Objective Prism	Condenser Prism
10	D1	10
20	D, D1	10, 20/40
40	D	20/40
63	D	20/40



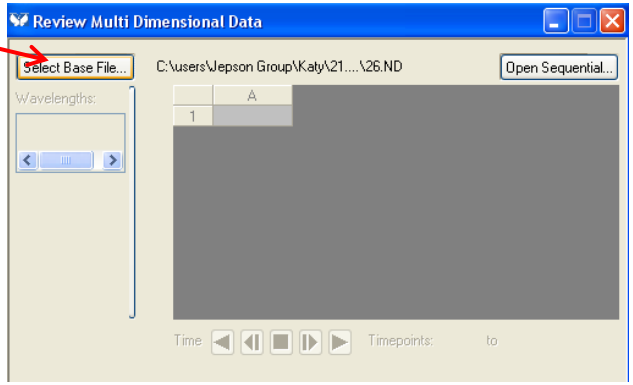
You will need to adjust both the polariser position (**1**) and condensor prism (**3**) to get the best contrast.

Viewing images captured with Multi Dimensional Acquisition

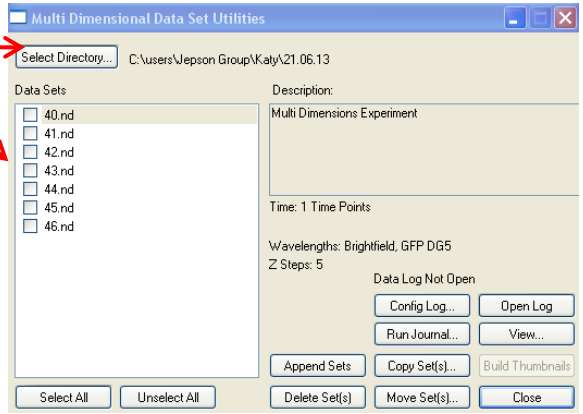
1. Go to the Apps menu and select “Review Multi Dimensional Data”



2. Select base file

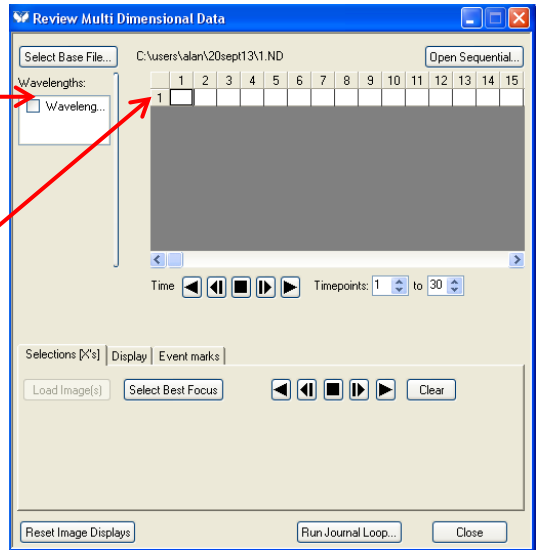


3. Select directory and file



4. Select channel(s)

5. Select timepoint(s) (right click here to select all timepoints)



Note: you can review captured images in an ongoing timecourse using this method. You’ll need to repeat steps 2 – 5 to get the software to update what images are viewable as the timecourse proceeds.

Saving images

Files are saved by default as multi dimensional tiffs which have an accompanying .nd file which holds the metadata for your images. Files (or just .nd fiile) can be dragged into Volocity software for analysis.

Note - currently images are not calibrated in terms of size.
Pixel sizes for most lenses are however given in the adjacent table.

Lens	Pixel width at 1x binning (um). Photometrics camera	Pixel width at 1x binning (um). Qimaging camera
10X	0.65	0.74
20X	0.32	0.37
40X	0.16	0.19
63X	0.10	0.12

Finishing a session

Check on Google calendar to see if the system is booked on after you**. If someone is booked on within 3 hours leave the DG5 light source on**.

If not it can be switched off **BUT** must to be switched off in the correct order:

1. Close Metamorph software
2. Switch off peripheral devices – microscope, camera controller, LUDL shutter controller, Piezo/PiFO, CDG5 shutter.
3. Switch off the main controller button on the DG5 box **BEFORE** switching off the lamp. (see diagram on page 1)

NB: Once this lamp is switched off it must be left off for at least 30 min before it can be turned on again.

Fill in the Excel log sheet, clean used surfaces with ethanol, clean oil off lens, log out of computer (but do not shut down)
Take any sample waste away with you in a sealed container for disposal in your own lab.

The system is left at 37 ^{o}C – if you want it at room temperature please note it on your booking. If you want the system at room temperature it is your responsibility to make sure the heater has been turned off in time (book time for cooling if necessary) and to turn the heater back on to 37 ^{o}C after you finished (unless someone is using it immediately after you at room temperature).