Olympus xCellence Software - basic user guide

This is a basic overview of setting up time lapse experiments using Olympus's xCellence software on BIU's IX81 inverted phase contrast system - the software allows you to program complex time lapse runs through a simple and intuitive user interface. A more comprehensive, BIU-specific instruction set is contained in the red folder and the original Olympus Manuals are also available.

Note: this software is new to BIU and available features may change with time.

Please log all runs and any problems in the IX81 log book.

CONTENTS / SECTIONS

- TURN SYSTEM ON
- INITIAL STARTUP AND SETUP
- DEFINE STAGE POSITIONS
- EXPERIMENT MANAGER
- DEFINE YOUR EXPERIMENT
- CHECK AND RUN THE EXPERIMENT
- AFTER THE RUN
- DESIGNING EXPERIMENTS FROM SCRATCH AND SAMPLE EXPERIMENT DESIGNS

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 1 of 27

TURN SYSTEM ON

If you need to image at 37°C, make sure that the chamber heater is switched on well ahead of time to ensure that the microscope is fully equilibrated to temperature (we routinely leave it on 24/7/365).

Insert battery into the back of the electronic (Red-y) CO_2 needle valve assembly and open the 5% CO_2 in air cylinder to 0.5 bar).

Switch on the microscope and stage control boxes - for fluorescence imaging, turn on the mercury lamp.

Check the humidifier bottle, refill with sterile dH_2O if necessary.

Check the moisture trap in the chamber, empty if necessary.

Adjust the electronic needle valve to set the CO_2 flow rate to a slow but steady bubble in the humidifier (eg. 0.05 l/min).

Check that there are no leaks in the gas delivery line by placing the end of the line into a vial of dH_2O

Check the correct sample holder is in place in the stage, place sample on the stage and attach and secure the gas line / needle / butterfly to it.

Start the PC - it is a stand alone (non domain) PC running Windows XP service pack 2. There is no login procedure, the PC starts straight into windows.

The PC currently has 4 internal hard drives (this may change):

> C: boot drive and program files only
> D: data storage
> F: data storage
> G: 110 GB solid state drive for fast data read/write - **use this for acquiring images**

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
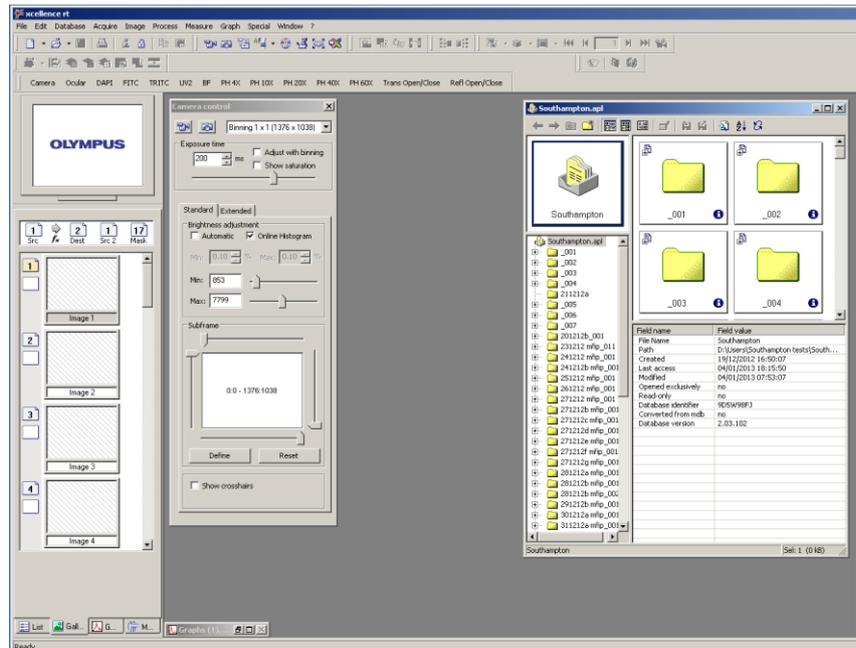page 2 of 27

INITIAL STARTUP AND SETUP

Start the XCellence software using the icon at top right of the LH monitor and resize the application window to fill both monitors.

All data files are stored in a database structure.

Database > Administration > new database... to create a database for your images. Name the database and browse to *G:\user temp\your folder* to create the database in.

The database window opens - resize it to fit at the RHS of the LH monitor.

Acquire > Microscope Settings... to open the Microscope Settings window

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 3 of 27

The *Microscope* window provides control of various microscope functions.

The top part shows the current focus level

The middle part allows you to specify / change the various automated components on the microscope:

- the desired objective.
- the fluorescence turret (set desired filter cube or no cube).
- the condenser (set the correct phase ring to match the objective - x4 = PhL, x10 and x20 = Ph1, x40 and x60 = Ph2, x100 (oil) = Ph3.
- the lamp voltage (set to 2.7v for general phase contrast imaging).

The bottom part has icons to control the light path and the light shutters and to set up different imaging modes (described below).
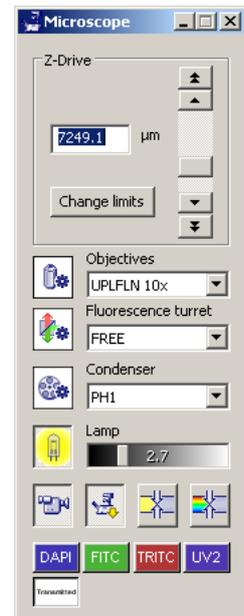
Image to eyepiece or camera.

Bottom port or side port active

Transmitted light shutter open / closed (open when icon looks depressed).
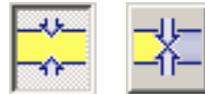
Reflected light shutter open / closed (open when icon looks depressed).
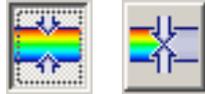
To get an image through the eyepieces, these 2 icons should be set and the relevant shutter opened.
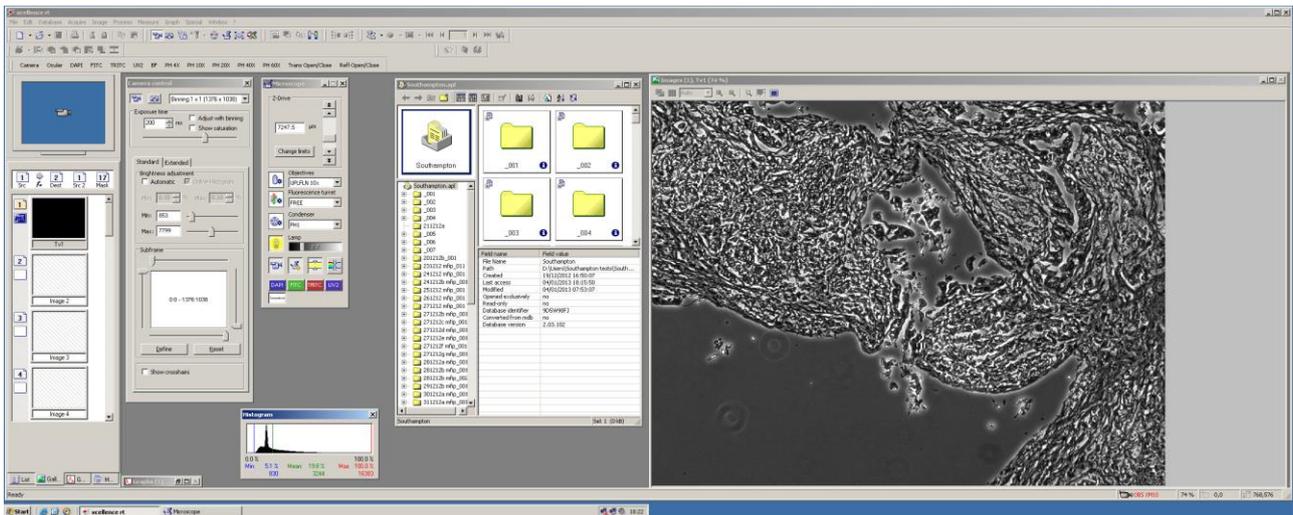
Fluorescence imaging with DAPI, FITC, TRITC or soft UV filter cube.

Transmitted light imaging (phase contrast or brightfield, depending on whether the required phase insert is in position in the condenser or not).

To get a live image onscreen on the RH monitor, click on the acquire icon on the top toolbar.
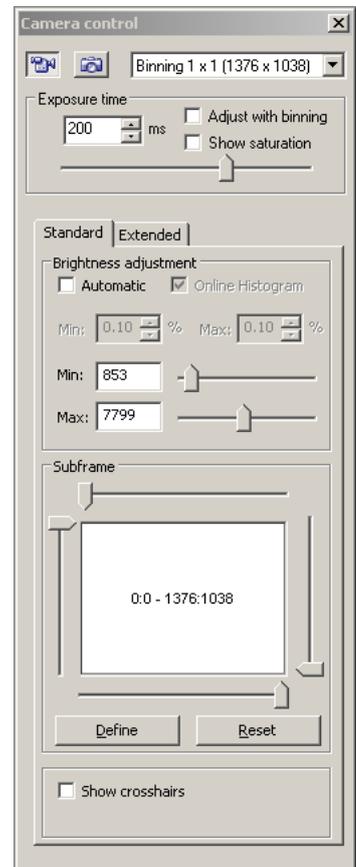
Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 4 of 27

Acquire > Camera Control to open the *Camera Control* window.

Use the *Camera control* window to adjust the exposure time to the correct level.

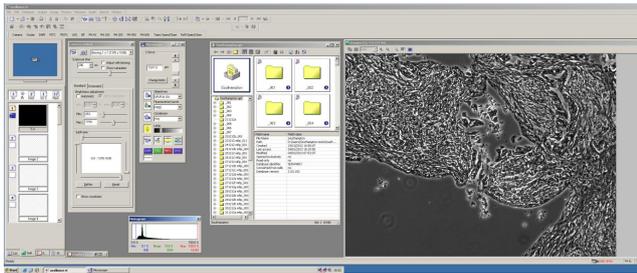Set *min* to 0 and *max* to 16383

Adjust the exposure time to the correct level for the live image (the shortest time at which the red line on the *Histogram* display reaches close to 100%).
.
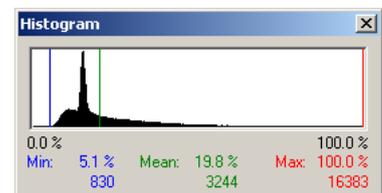
Using a **fixed** field of view, and each of the imaging modes that you want to use for your run, determine and write down the correct exposure time and focus level for the mode and sample.
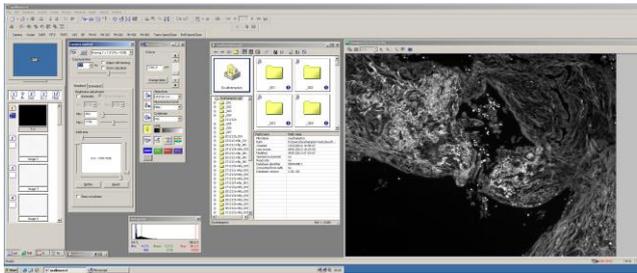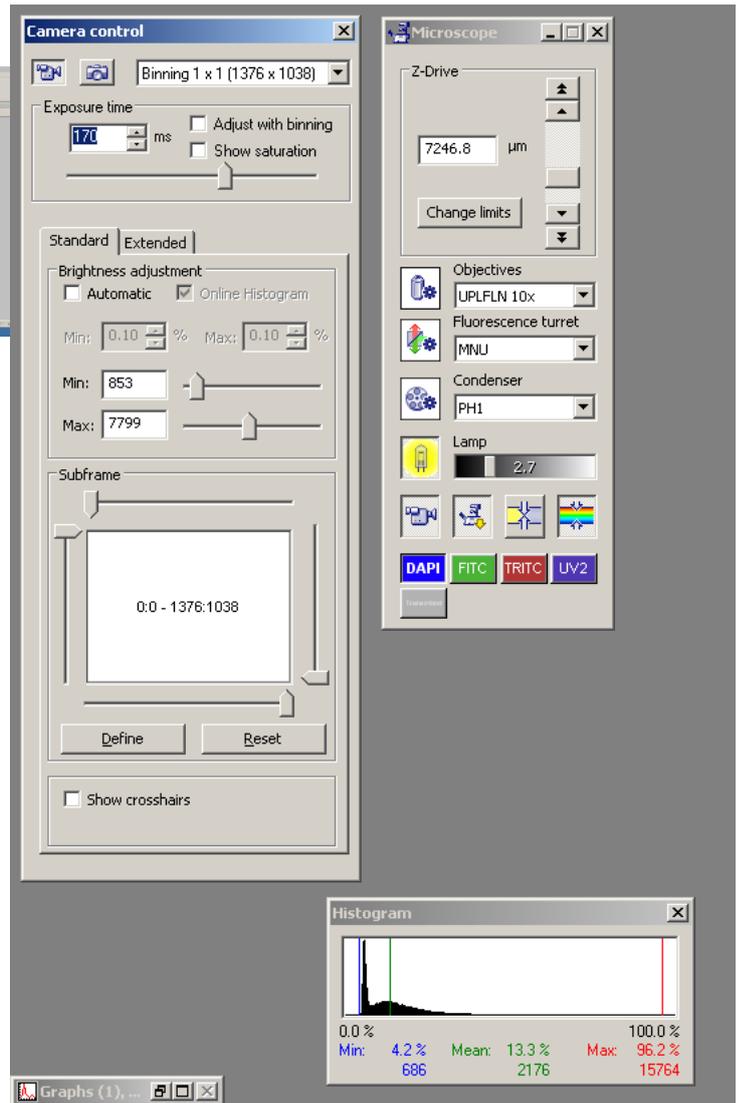
For example:

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 5 of 27

Phase Contrast

exposure = 200ms
focus = 7247.5

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 6 of 27

DAPI fluorescence

exposure = 170ms
focus = 7246.8

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 7 of 27

FITC fluorescence
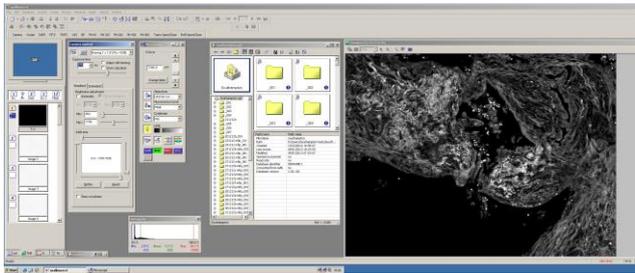
exposure = 150ms
focus = 7248.8

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 8 of 27

TRITC fluorescence
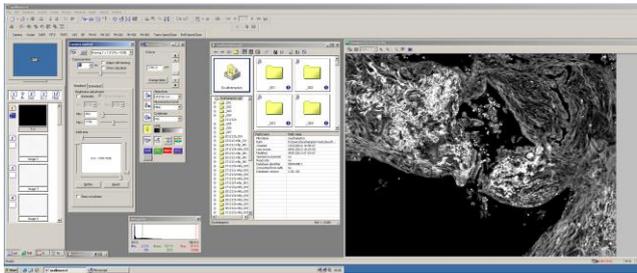
exposure = 52ms
focus = 7246.0
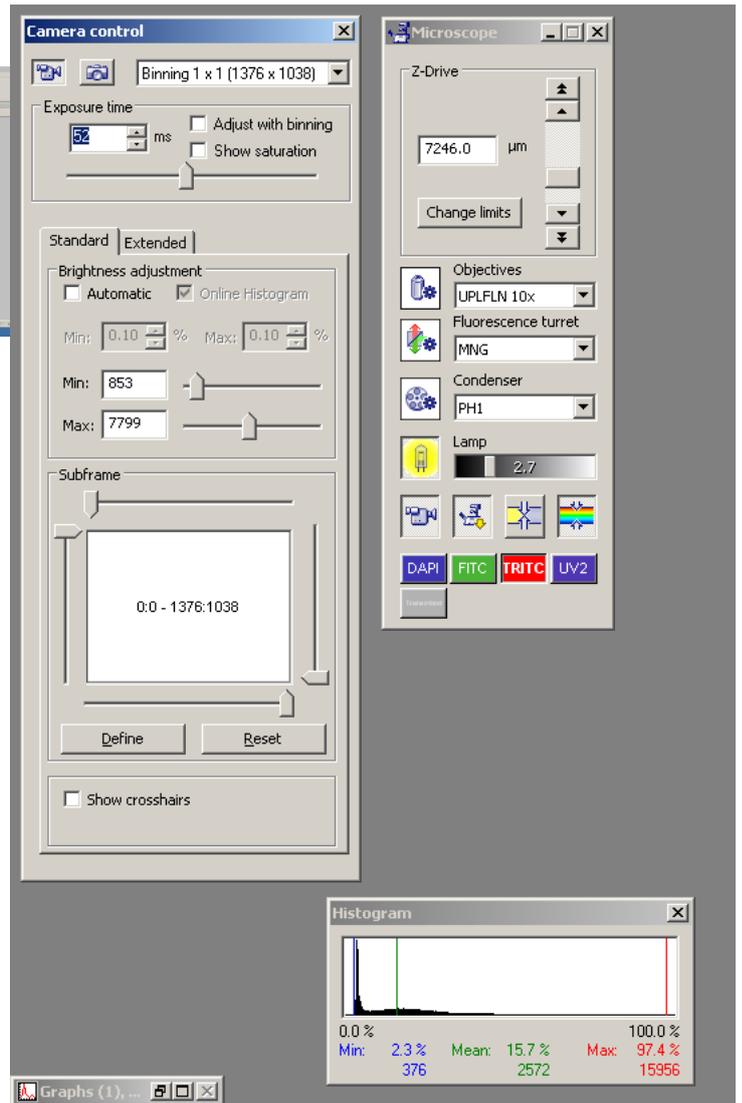
Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 9 of 27

DEFINE STAGE POSITIONS

Open the *Stage* list window using the icon on the top toolbar.

It opens with the most recently used set of stage coordinates shown.

Click on the down arrowhead at bottom right of the *Stage* window to access the stage calibration buttons.
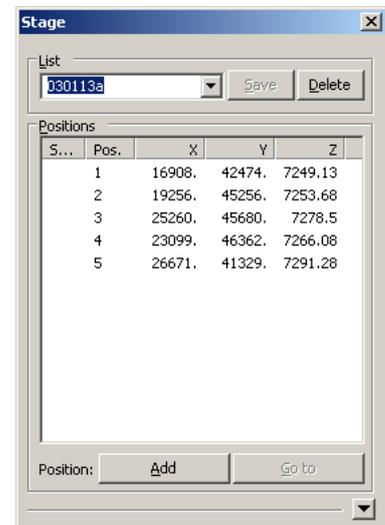
Select *<New List>* in the list box.

Recalibrate the stage before each run.

Click on the refresh icon to the right of the green *Connected* field.

Click on *Auto Calibrate*.
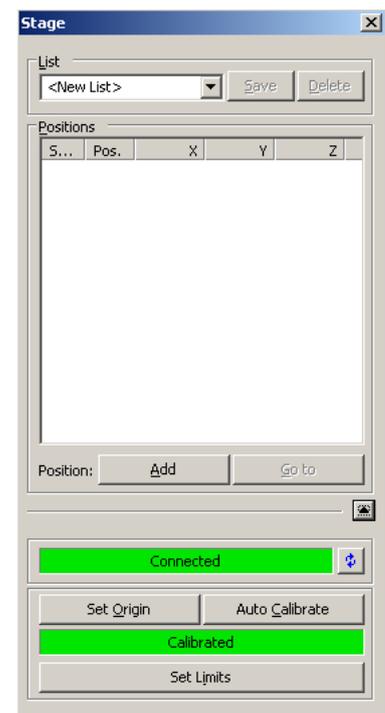
A stage movement warning window appears - check that there is nothing on the stage that will hit the condenser as the stage moves (hinge the condenser up out of the way if necessary) and click on *OK*.

The stage will move to its end point and back.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 10 of 27

Under phase contrast imaging, use the joystick to move your sample to a desired field of view, focus and click on *Add* in the *Stage* window to log the XYZ position of that field.

Repeat for each desired field to be recorded during the experiment (here 10 fields).

Type a new list name into the List box and click on *Save* to save the locations as a stage file. We suggest that you use your name and the date - e.g. *daj030113*.

S...	Pos.	X	Y	Z
	1	27061.	39377.	7299.95
	2	22348.	44915.	7262.8
	3	18981.	45297.	7251.56
	4	21298.	44089.	7262.73
	5	25465.	44089.	7277.5
	6	29114.	44204.	7289.61
	7	31481.	43302.	7302.5
	8	34868.	41608.	7322.09
	9	34868.	40232.	7322.6
	10	37753.	38391.	7340.45

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 11 of 27
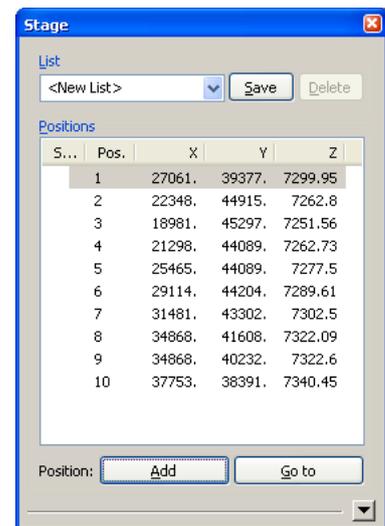
EXPERIMENT MANAGER

Open the Experiment Manager using the icon on the top toolbar.

The Experiment Manager window opens.

This provides a graphical interface for designing experiments by adding components and linking them together within boxes and by timelines.

Depending on how the software was last used, it may open blank or it may show the last experiment used.

Icons for the different experiment components are found on the toolbar.

The principle functions are described below.
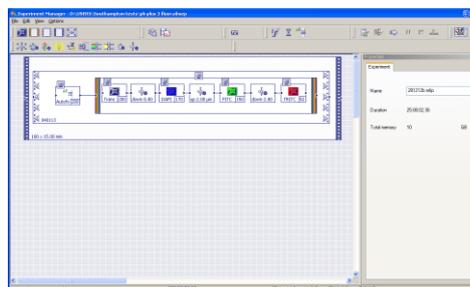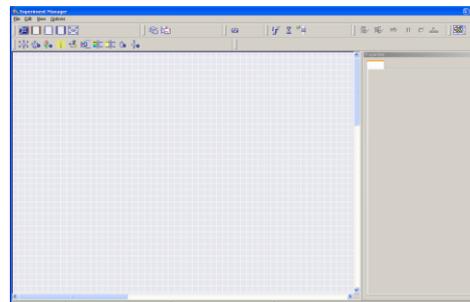
L to R:
- Do image acquisition
- Do multichannel image (box around the images to combine)
- Do Z stack (box around the images to do Z stack for)
- Do time lapse cycle (box around images to cycle)
- Do stored stage coordinates (box around image modes to repeat at different, defined fields of view)
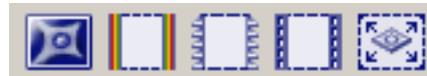
L to R:
- Move stage (absolute or relative in XYZ)
- Change condenser insert
- Change filter cube
- Transmitted lamp on / off
- Select side or bottom port
- Select eyepiece or camera port
- Reflected light shutter open / closed
- Transmitted light shutter open / close
- Change objective
- Change focus (absolute or relative)

Do autofocus

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 12 of 27

In addition, for more complex experiments, it is possible to set trigger events that activate external devices at specific times during a run. For example:

- an automatic pump that adds test chemicals at specific times.
- a second camera on the side port - e.g. a high speed video system to capture a short burst of high speed video at specific times during a long run.

To create an experiment, the icons are selected and added to the gridded area of the *Experiment Manager* window as discrete events or as boxes surrounding events. Events are linked with lines to specify the order in which they are performed (L to R), boxes can be nested.

For Example, consider the following experiment:
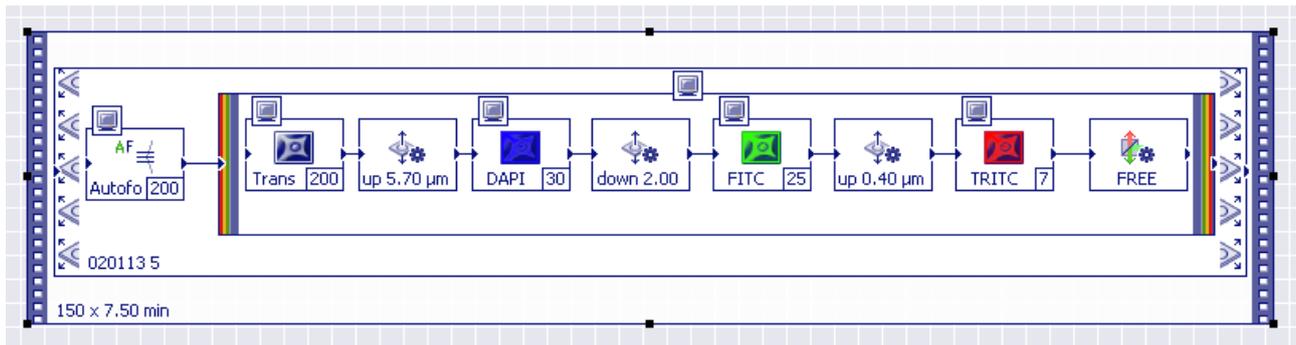
This does the following:

> In sequence:
> > **Inner box**
> > - Takes a transmitted light image at 200 ms exposure, and shows it on screen.
> > - Refocuses up 5.7 um.
> > - Takes a 30 ms exposure fluorescence image with DAPI cube, and shows it on screen.
> > - Refocuses down 2.0 um.
> > - Takes a 25 ms exposure fluorescence image with FITC cube and shows it on screen.
> > - Refocuses up 0.4 um.
> > - Takes a 7 ms exposure fluorescence image with TRITC cube and shows it on screen.
> > - Moves the filter cube to an empty position ready for the next phase image.
> > - Combines the 4 images into a false colour composite image and shows it on screen
> >
> > **Middle box**
> > - It repeats the 4 image acquisition for each stage position stored in stage file *020113 5*.
> > - At each stage position at each cycle it performs a software autofocus using 200 ms exposure times before capturing the set of 4 images and shows the autofocus procedure live on screen.
> >
> > **Outer box**
> > - It repeats this entire cycle 150 times at 7.5 minute intervals.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 13 of 27

DEFINE YOUR EXPERIMENT

Whilst you are learning to use the software it is probably easier for you to edit an existing program, rather than create a new one from scratch. See the end of this guide for more information on designing experiments from scratch.

On the *Experiment Manager* toolbar do *File > Open* and navigate to: G*:* and select file *AF phase and 3.obsep* - this program includes everything that you are likely to need for most runs.

First save the experiment in G*:\user temp\your folder* under a new name (we suggest that you use your name and the date), then edit / delete elements from it as required.

Clicking on any box or action in the experiment selects it and brings up its settings at the RHS of the *Experiment Manager* window, allowing you to edit the settings. Selected components can be deleted and new components added.

Looking at each step of the above experiment in more detail:

Click on the gridded area outside the outermost box to bring up the *Experiment* tab.

Here you name the experiment (the name it will be given in the database - we suggest you use your name and the date).
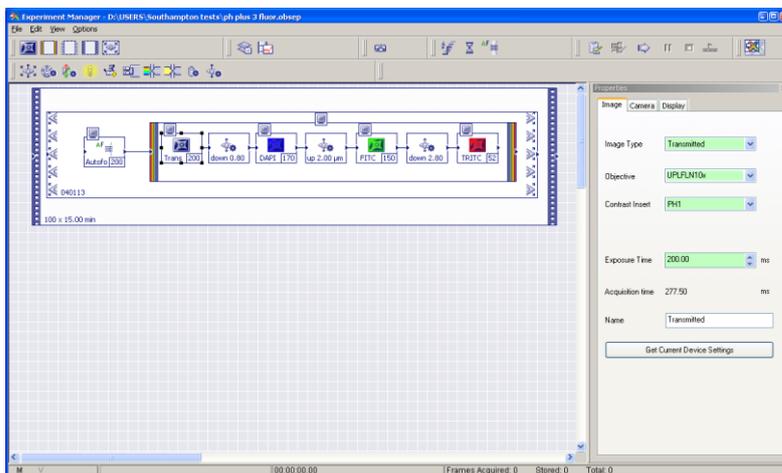
This tab also shows the total experiment run time and data set size (which will be determined by the number of fields, cycles, images etc.).
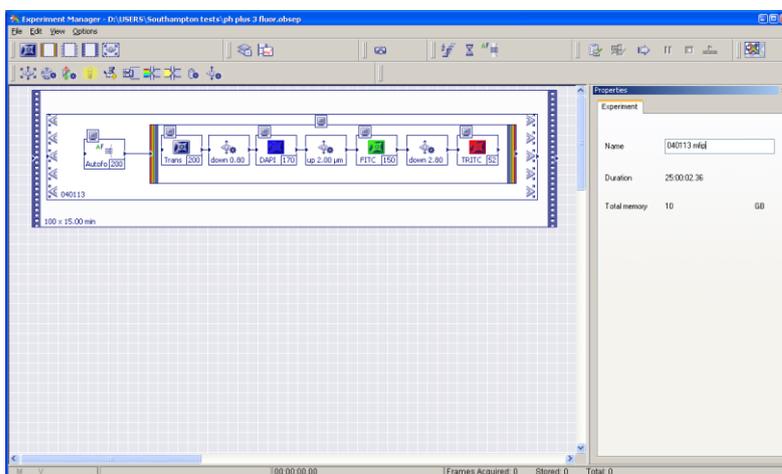
Click on the *Trans* image icon to bring up its settings.

Here you set the imaging mode (transmitted or fluorescence), the objective magnification, exposure time and condenser setting (brightfield or phase ring inserted).

Here we are specifying a 200 ms exposure, phase contrast image using the x10 objective.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 14 of 27
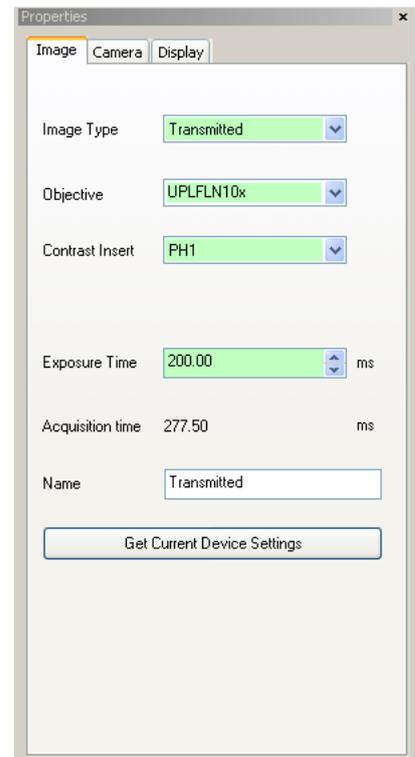
Click on the *refocus* icon to bring up its settings.

Here we are specifying to refocus down (minus) 0.8 um between the phase image and the DAPI image.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 15 of 27
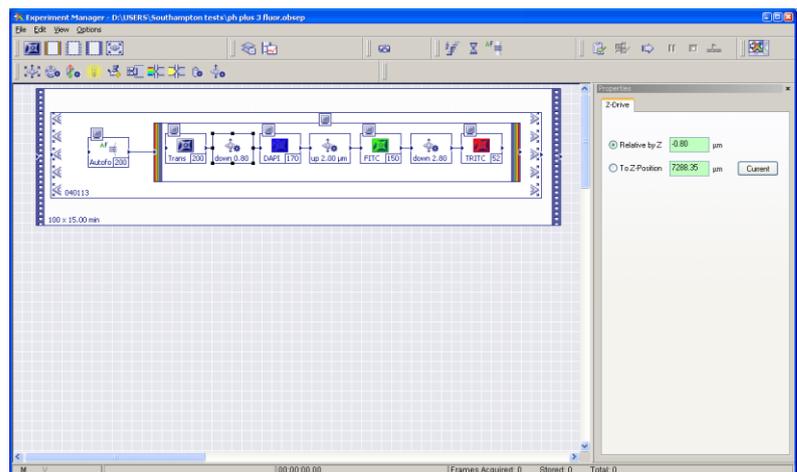
Click on the *DAPI* image icon to bring up its settings.

Here we are specifying a 170 ms DAPI fluorescence image using the x10 objective.

(The correct filter cubes and lamp shutter settings for DAPI, FITC, TRITC and soft UV fluorescence images have been predefined).

The contrast insert setting doesn't matter as fluorescence images are captured using epi-illumination.

Click on the next *refocus* icon to bring up its settings.

It specifies to refocus up (positive) 2 um between the DAPI and FITC images.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 16 of 27
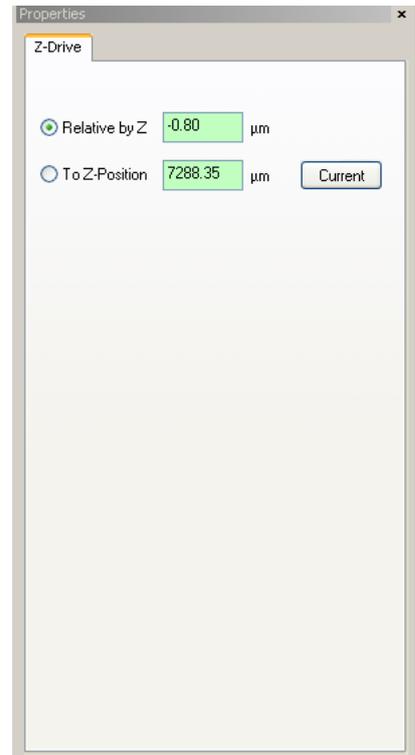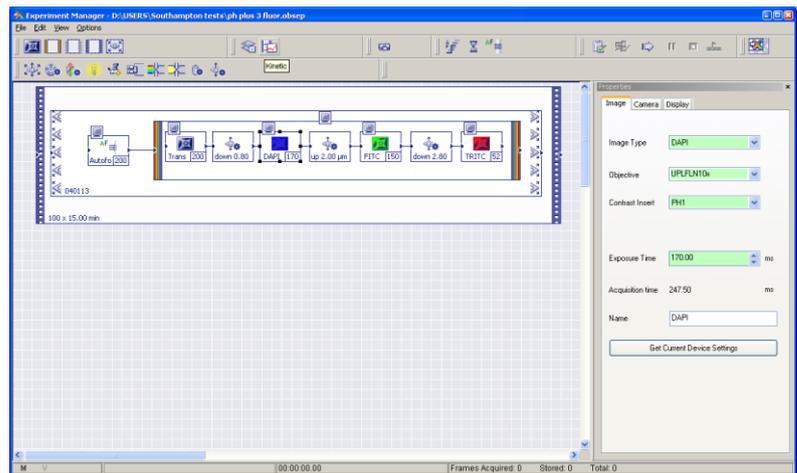
Click on the *FITC* image icon to bring up its settings:

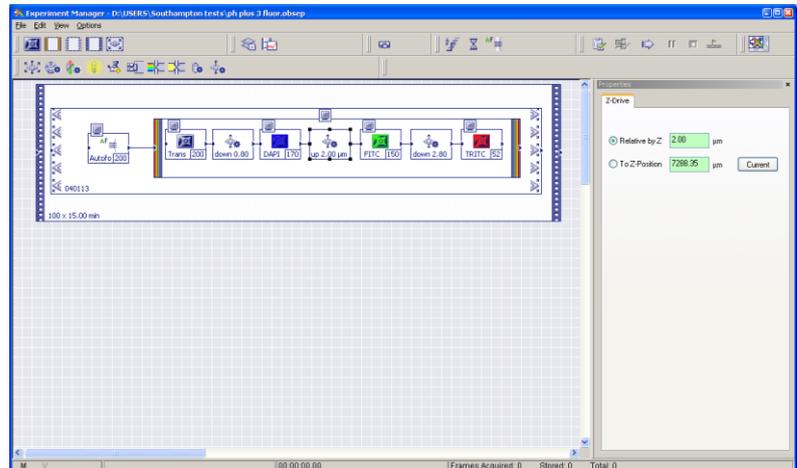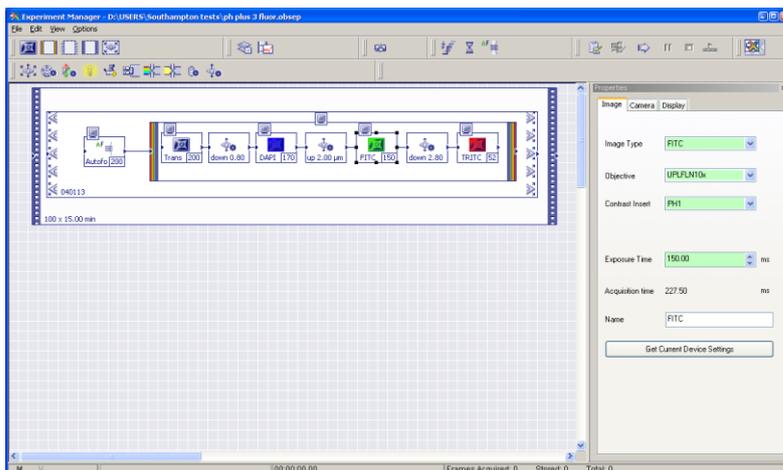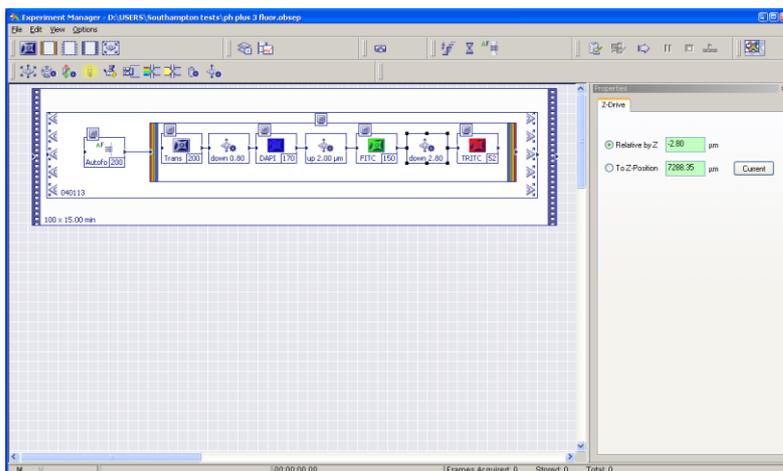Here we are specifying a 150 ms FITC fluorescence image using the x10 objective.

Click on the next *refocus* icon to bring up its settings.

It specifies to refocus down (negative) 2.8 um between the FITC and TRITC images.

Note: when setting up a new experiment, use the exposure times that you determined for the live image under the different imaging modes (above) and calculate the focus offset / correction for the different focus levels that you recorded.

For the imaging modes and sample that we looked at earlier:

mode	exposure	focus	focus change required before image
Phase	**200 ms**	7247.5 um	none - focus specified in stage list (and autofocus, if active)
DAPI	**170 ms**	7246.8 um	**-0.8 um** (7246.8 - 7247.5)
FITC	**150 ms**	7248.8 um	**+2.0 um** (7248.8 - 7246.8)
TRITC	**52 ms**	7246.0 um	**- 2,8 um** (7246.0 - 7248.8)

These are the values which we would set for this experiment.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 17 of 27

For each of the image acquisition icons, its *Display* tab allows you to specify whether you want the captured image to be displayed on screen during the experiment or not (*Online Display* is checked / unchecked respectively). If *Online Display* is checked, a small monitor icon is shown at top left on the relevant image acquisition icon.
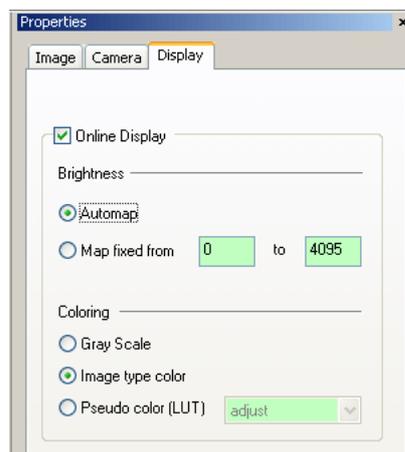
Note: after acquiring the image set, you should specify an additional action to set the filter cube turret to an empty position so that the next phase image isn't captured with the transmitted light still passing through the final filter cube of the fluorescent image series (as this would affect exposure time).
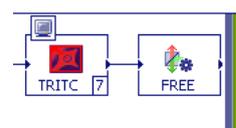
Click in the *Multichannel image* box.

Here you give a name to the composite (multichannel) acquisition image (this name forms part of the file name that is recorded in the database).
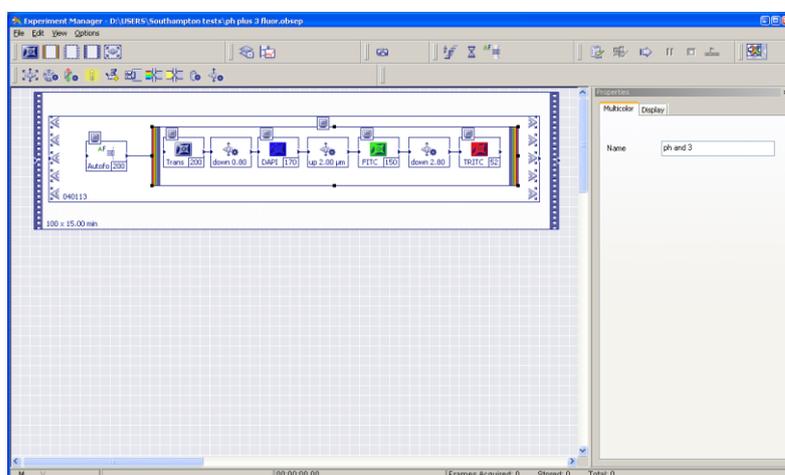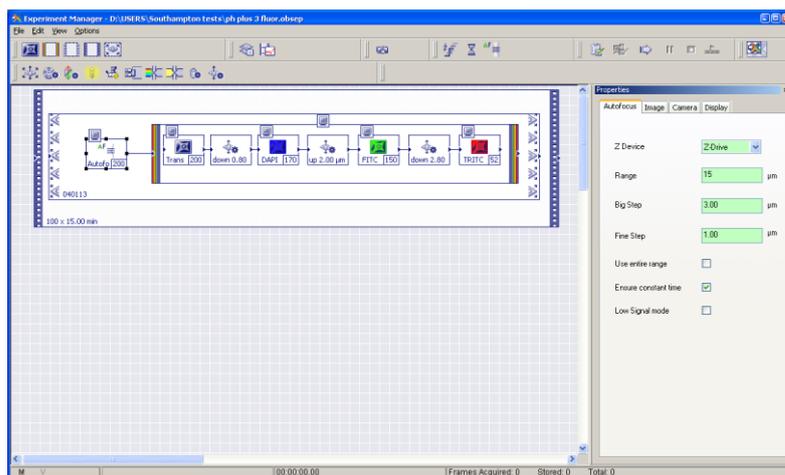
Again, the *Display* tab allows you to specify whether the merged image is shown live on screen or not.

Click on the *autofocus* icon to bring up its settings.

Software autofocus is a 2 pass process.

You specify the range and step size for the first pass (*Big Step*) and the second pass (*Fine Step*).

Make sure that *Ensure constant time* is checked to make cycle timings exact.

For each field of view, autofocus starts from the Z position specified for that field in the stage list file.

Again, the *Display* tab allows you to specify whether the autofocus process is shown live on screen or not.

> **Note**: autofocus is quite a slow process, taking about 1 minute per field of view, so if you need a fast cycle time you may not

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 18 of 27

be able to use it.
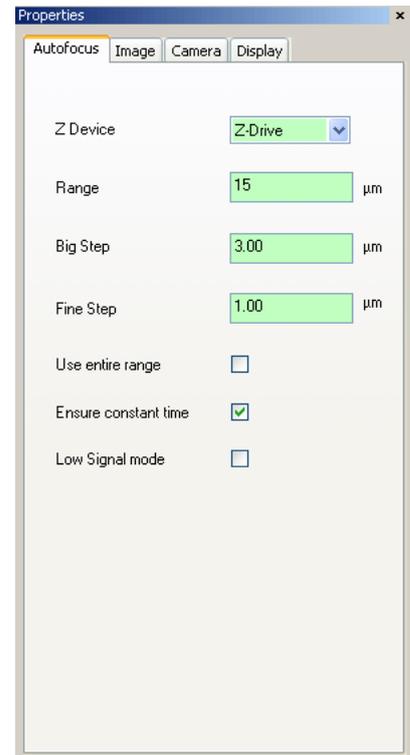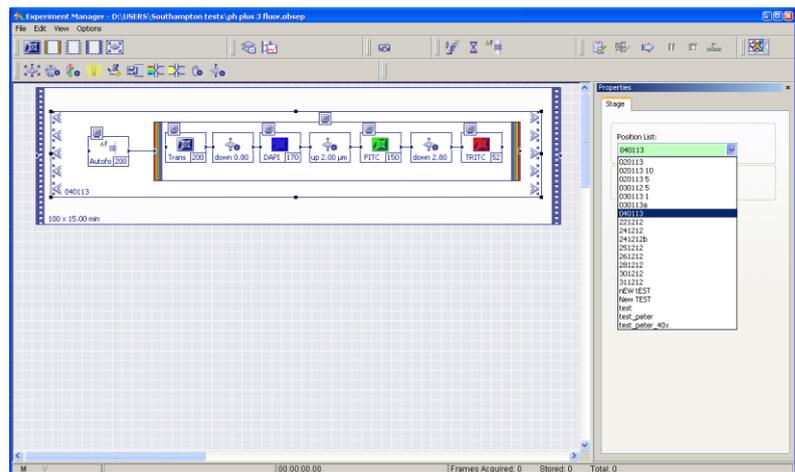

Click in the *Stage* box to access a dropdown menu where you select the stage list of field of view coordinates (XYZ) that you specified earlier.

Click in the *Cycle* box to bring up its settings.

Here you specify the number of cycles that you want to run, and the interval between them.

The software estimates the time that a single cycle will take (to guide your settings of cycle time) and the total run time.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 19 of 27
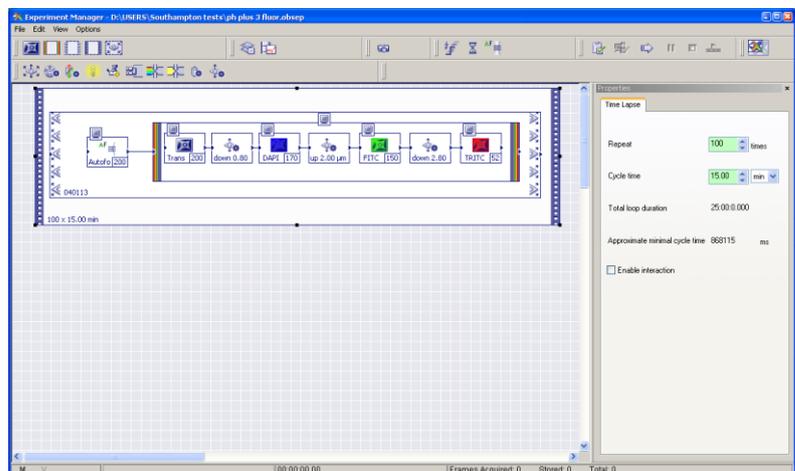
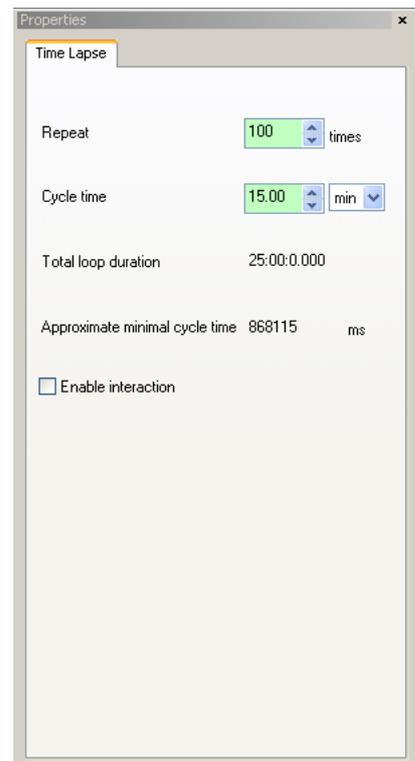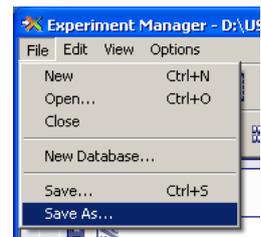Once you have edited all of the settings for your experiment, save the experiment file under a new file name (.obsep file extension). Again we suggest that you use your name and the date.

This allows you to reuse an experimental design by reopening it and editing its settings, rather than having to redesign and create each experiment from scratch.

Now you are ready to check and run your experiment.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 20 of 27

CHECK AND RUN THE EXPERIMENT

Click on the *Verify* icon - the software checks on your experiment design for errors (e.g. too short a cycle time).

If the experiment design passes its check, the *Prepare* icon becomes active. Click the *Prepare* icon to set up the system ready to run.

The RHS monitor divides into sections for each of the different images that you have specified to display during the run - here one for each of autofocus, the 4 individual images (phase, DAPI, FITC, TRITC) and the composite image.
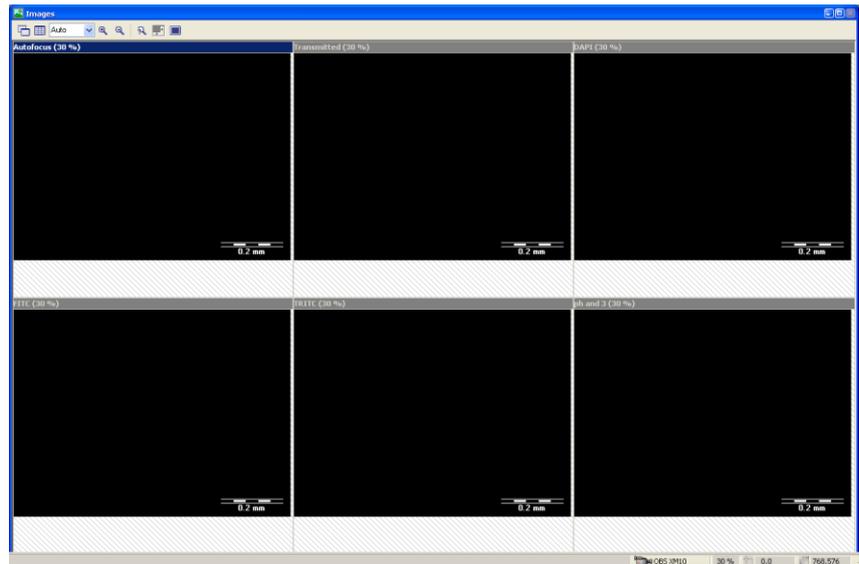
Once the system is prepared, the *Start Run* icon becomes active.

You can now minimise the *Experiment Manager* window to a smaller window (the *Execution Center* window) by clicking on the *X* icon (and restore the *Experiment Manager* window by doing the same in the *Execution Center* window).
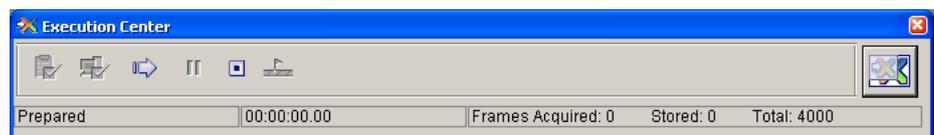
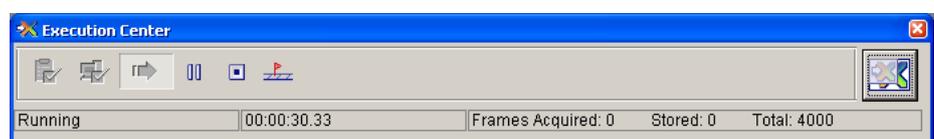This shows that the system is prepared to run and will capture a total of 4000 frames.

Click on the *Start* icon to start the run.

The *Execution Centre* window shows that the experiment is running and displays the elapsed time and the number of frames acquired.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
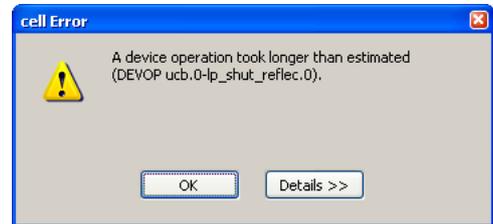page 21 of 27

As the experiment runs, the RH monitor shows the captured images as they are acquired (depending on which ones were specified to be displayed live in the experiment design). Here it is showing the autofocus, phase and 3 fluorescent images for a field.

> **Note:** the composite image tends to lag behind somewhat and also can show multiple fields superimposed on each other - do not worry, the actual captured and stored images are fine.
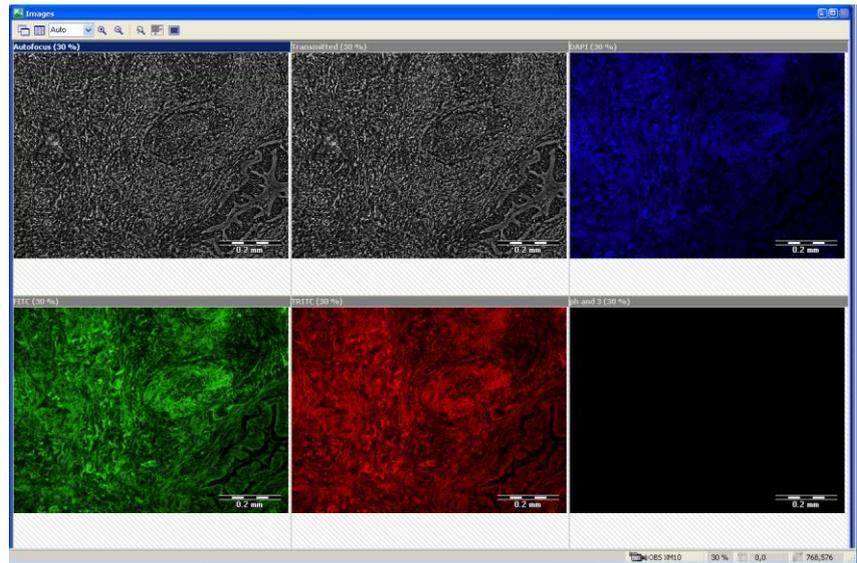
At the end of the run, the software always reports a device operation error - it can be ignored (click *OK* to close the window).

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 22 of 27

AFTER THE RUN

The captured images are listed in the database in a folder structure that is named after the run name specified in the *Experiment* tab of the *Experiment Manager*.

The individual fields are auto-named after their position in the stage list and, if multichannel images were created, also by the name for the composite image specified in the *Multicolor* tab.

Records physically exist as discrete files in a folder structure specified by the database. They can be loaded into the software to be viewed and played, exported as image stacks or movies etc..

Multichannel images can be separated into their component parts and individual images can be merged into multichannel ones.
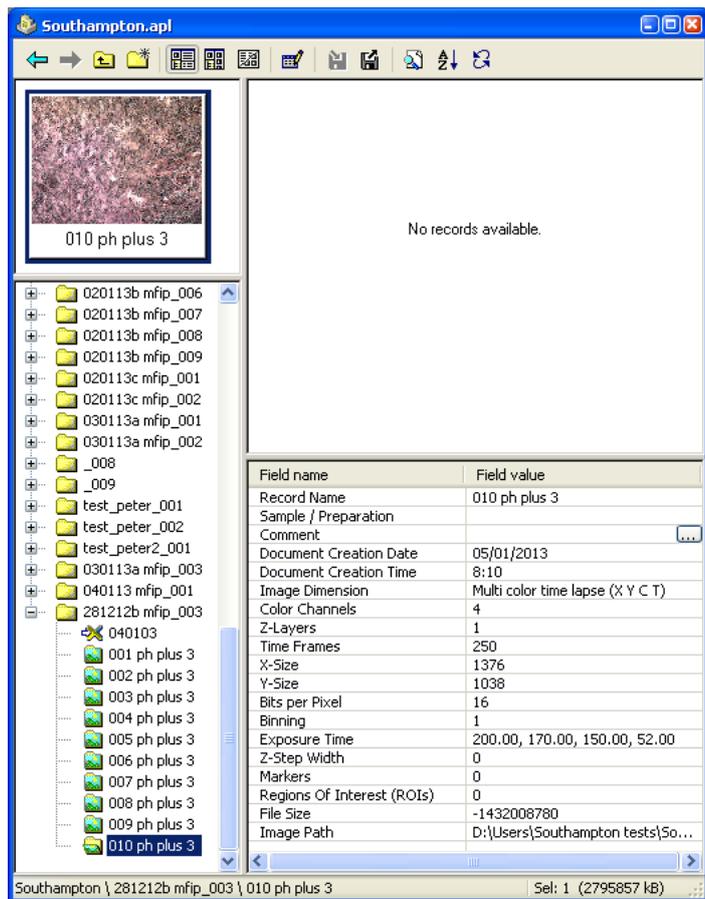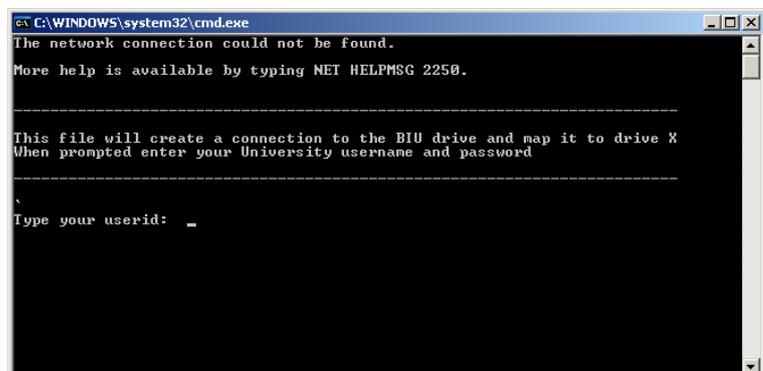

It is your responsibility to move and backup your own data.
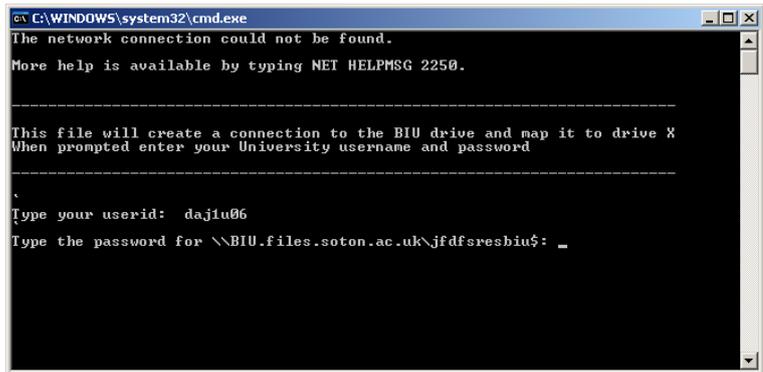
Use the *Connect to BIU filestore* desktop icon to access a command prompt login to the filestore to move your files.
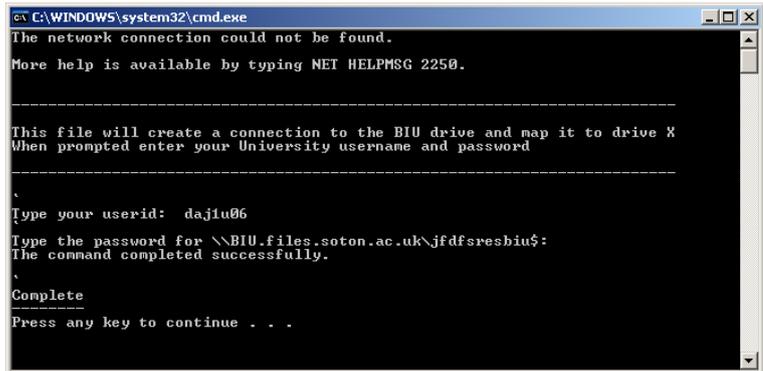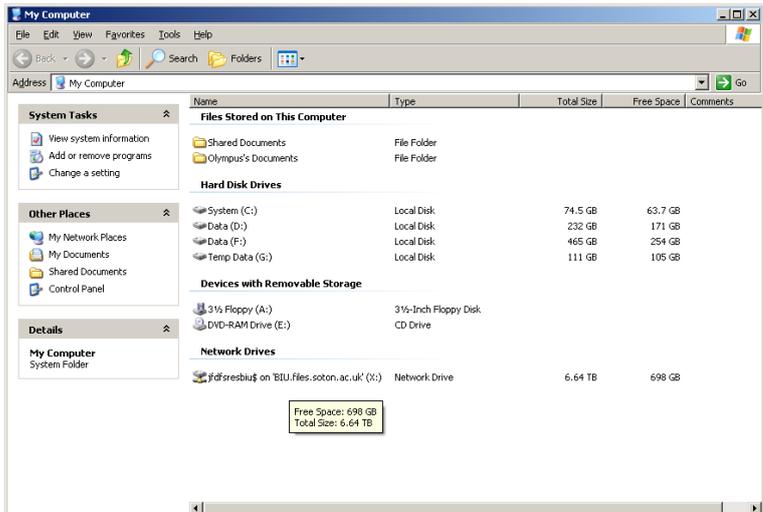

Type your UoS username.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 23 of 27

Type your UoS password.

Press any key to close the batch file.

The mapped drive will now appear under *My Computer* as drive *X*:.

Alternately, use a virus checked, portable hard drive (available from BIU) to copy your files onto.

Copy the files to the F drive as an additional backup.

Then delete your files from the G: drive - this solid state drive only has a small capacity and it will be regularly cleaned up.

Remove your sample and dispose of it according to GLP. Remove any needle or butterfly used to deliver the 5%CO_2 in air mix and place it in a sharps bin.

Turn off the 5%CO_2 in air cylinder and disconnect the battery from the needle valve.

Close down the PC and turn off the microscope control box, stage control box and mercury bulb starter (if used).

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 24 of 27

DESIGNING EXPERIMENTS FROM SCRATCH AND SAMPLE EXPERIMENT DESIGNS

To design an experiment from scratch, do *File > New* on the *Experiment Manager* toolbar to open a blank experiment.

Select the acquisition event icon, move the mouse onto the gridded area and left click to insert an acquisition event. Edit its parameters as required.

Repeat for any other imaging modes required.

You can select any element, left click and hold to move it around within the window.

The different acquisition events need to be joined to specify the order (L to R) in which they are captured. Move them into line in order.
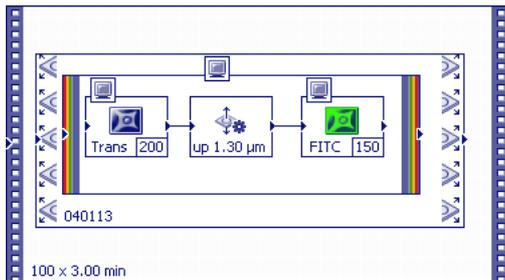
Move the cursor over the directional arrow head at the L or R side of an event and it will change to a cross hair. Left click and hold and draw a line to the arrowhead at the edge of the acquisition event that you want to join it to. A line now links the events.

To add a stage list, multichannel merge or time lapse frame, select its icon on the *Experiment Manager* toolbar and draw a box around the events to include.

> **Note:** you can't add a box and then move it over existing events, or move existing events into a box, you have to draw the box around existing events or create new events within an existing box.

Some sample experiment designs are shown below.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 25 of 27

Sample Experiment Design 1

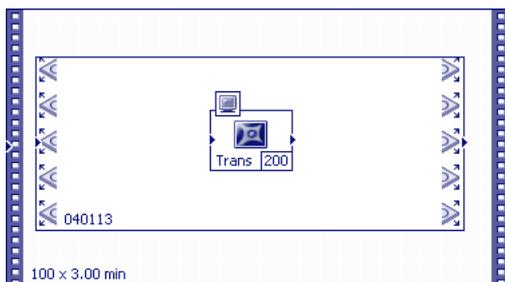Transmitted image (200 ms) - refocus up 1.3 um - FITC image (150 ms)

Create composite.

Capture these images for each location specified in stage list 040113.

Repeat 100 times at 3 minute intervals.

> **Note:** no autofocus, so a fast cycle time is possible.
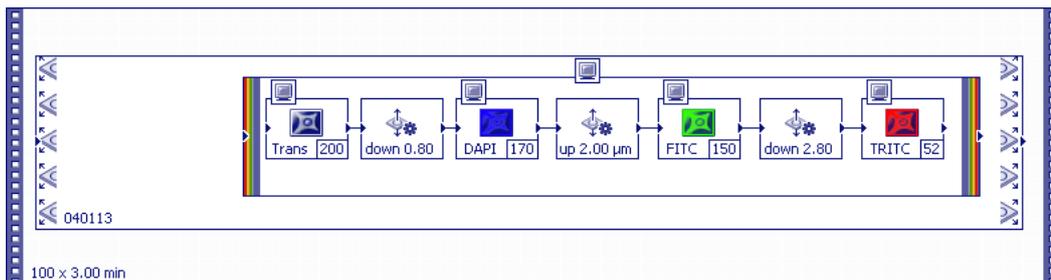
Sample Experiment Design 2

Transmitted image (200 ms) only.

Capture this image for each location specified in stage list 040113.

Repeat 100 times at 3 minute intervals.

> **Note:** no autofocus, so a fast cycle time is possible.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 26 of 27

Sample Experiment Design 3

Transmitted image (200 ms) - refocus down 0.8 um - DAPI image (170 ms) - refocus up 2.0 um - FITC image (150 ms) - refocus down 2.8 um - TRITC image (52 ms).

Create composite.

Capture these images for each location specified in stage list 040113.

Repeat 100 times at 3 minute intervals.

> **Note:** no autofocus, so a fast cycle time is possible despite a complex design.

Sample Experiment Design 4

Transmitted image (200 ms) - refocus down 0.8 um - DAPI image (170 ms) - refocus up 2.0 um - FITC image (150 ms) - refocus down 2.8 um - TRITC image (52 ms).
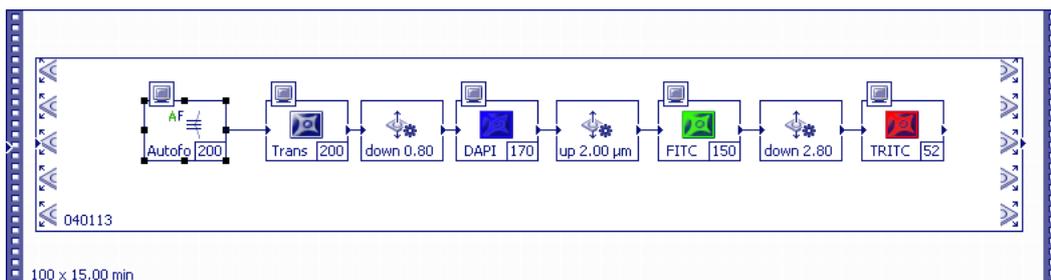
No composite image created - separate data files are created for each imaging mode and position.

Capture these images for each location specified in stage list 040113.

Repeat 100 times at 15 minute intervals, performing autofocus at each stage position at each cycle before taking the 4 images for that position.

> **Note:** autofocus, so a longer cycle time is required.

Olympus xCellence - basic starter guide
Author David Johnston - Biomedical Imaging Unit, University of Southampton – v2
page 27 of 27