

Brief instructions for setting up phase contrast time lapse experiments on the Olympus IX81, with notes on Fastcam operation

Fastcam notes are in blue

Much more comprehensive instructions are available in the red file kept by the microscope.

Very important: if for any reason the system should crash during a run, under no circumstances restart the Cell[^]P software as it will delete your data from Cell[^]P's temporary folder. Use "my computer" to browse to D\Cell[^]P_temp and move all files out of this folder to another folder before starting Cell[^]P.

Turn On

1. Switch on the chamber heater well ahead of time (preferably the previous evening) to ensure that the microscope is fully equilibrated to temperature.
2. Switch on the CO₂ control box 15 minutes ahead of time to let it warm up, do not open the CO₂ cylinder yet (the controller can be a bit moody, find DAJ if you are having problems).
3. Switch on the microscope and stage control boxes.
4. 15 minutes after switching on the CO₂ controller, adjust the black calibration knob on the back of the controller so that the display reads 0%. Then open the CO₂ cylinder main valve (the second stage of the regulator is pre-set to 1.5 bar) and check / set the CO₂ level (note: the controller is only specified to work up to 8.5%, we cannot guarantee accuracy if you set it to 10%).
5. Check the humidifier bottle, refill with sterile dH₂O if necessary.
6. Check the moisture trap, empty if necessary.
7. Check that there are no leaks in the gas delivery line by placing the end of the line into a vial of water.
8. Check that 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.
9. Check that the desk fan under the bench is switched on at speed 2 and blowing into the computer carcass (important to prevent overheating).
10. Check that the network cable is disconnected from back of the PC
11. Start the PC and log on to the computer (NOT to SOTON domain) as *Administrator*

- with password *BIU*. Clear any startup warnings you see about graphics card drivers
12. In windows explorer create a new folder within your data folder under D:\Users\
(we recommend that you use your name and the date eg. *David010610*) and use
this same prefix for all the Cell[^]P configuration files that you will be setting up.
 13. start Cell[^]P, and ignore or clear the 3 warnings that appear about Hamamatsu and
Camedia cameras.

Setup Cell[^]P and microscope

1. Log into the microscope (Acquire > IX Microscope > Log On..., password is *a*).
2. Bring up the "IX Control Panel" window (Acquire > IX Microscope > Microscope
Control...).
3. Bring up the "Camera control" window (Acquire > Camera Control...).
4. Click on image 1 in the image buffer column at LHS of the LHS monitor and drag
the numbered box at its top LHS onto the RHS monitor (legend at top of RHS
monitor window changes to say Image 1).
5. Open the transmitted light shutter using the "IX Control Panel".
6. Set the lamp voltage to 2.7V in the "General" tab of the "IX Control Panel". [For
phase contrast with x60 for Fastcam, set it to 12 volts \(full on\) and check that
there are no filters in the top arm of the light path.](#)
7. Select the required objective and phase contrast in the "Observation" tab of the "IX
Control Panel" (or use the softkeys at top of the LHS monitor).
8. Set the image destination to the bottom port in the "General" tab of the "IX Control
Panel". [For phase contrast with x60 for Fastcam, set it to the camera port \(split
between eyepieces and LHS camera port\).](#)
9. Acquire a live image (Acquire > Acquire), focus on screen using the microscope
focus knob and adjust the camera exposure to a suitable level using the "Camera
control" window. [For phase contrast with x60 for Fastcam, use the Fastcam PC for
the live preview - focus level on polyp samples is somewhere around the 7000-
8500 \$\mu\$ m setting.](#)
10. Bring up the "Define Image Sequence" window (Acquire > Define ISP...).
11. If the "Virtual Stage" window appears, click on the X axis line and then on the "Init"
button to initialise the X and Y stage axis drivers - swing the condenser up out of
the way before hitting "OK" in the "PRIOR ProScan" window that appears and
reposition it after initialisation is complete. **Note:** To force a stage initialisation if

the software doesn't ask for it, click F8 to open the preferences window, click the stage tab, highlight the Virtual Stage1 option, click connect, click limits and proceed as above, close preferences window when done.

Setup the ISP "Info" tab:

1. Provide a name for the run in the "Process" box (use your name and date as above).
2. If desired, provide a description of the experiment in the "Description" box (eg. which sequences (positions monitored) correspond to which experimental conditions).

Setup the ISP "Flow" tab:

1. Check that "Start acquisition" is set to "immediately".
2. Set the required number of cycles and frame interval.
3. Check that "Acquisition Mode" is set to "Frame".
4. Check that "Save image" is set to "Folder", and then click on the "..." button and navigate to the folder that you set up earlier to select it as the destination.

Setup the ISP "Settings" tab

1. You can accept the "default" setting or:
2. Click the "New..." button, to bring up the "New Stage Path" window
3. Provide a name for the run in the "Name" box (use your name and date as above)

Setup the ISP "Macro" tab

1. Check that none of the boxes are ticked. For phase contrast with x60 for Fastcam, check that the "process" and "sequence" pre and post boxes are ticked, then click on the define buttons for these and check that the following text is entered in the appropriate boxes:

In sequence preprocess box

//-----

// seq pre

```
//-----  
IXC::ixSetShutterOpen(TRUE,FALSE);  
sysWait(1);  
VM110::Digital_1_Pulse();  
sysWait(35);  
//adjust syswait line according to record time and  
//file save time on fastcam PC  
//for 500 frames at 250 per second need 35 sec
```

In sequence post process box

```
//-----  
//seq post  
//-----
```

In process pre process box

```
//-----  
//process pre  
//-----  
VM110::Trigger_Arm_Silent ();
```

In process post process box

```
//-----  
//process post  
//-----  
VM110::Trigger_Arm_Silent ();
```

The text for these commands can be found in a file called "*fastcam macros.txt*" on the desktop. **Note:** you may need to change the sysWait value in the sequence pre process macro - this value (in seconds) must be greater than the combined time needed for image acquisition and file saving on the Fastcam PC.

Setup your sequences in the "Define Image Sequence" window

1. Click on the "Delete all Sequences" button (1st button) to delete the previously used sequence set.
2. Click on the "Add Sequence" button ((2nd button) to add a new sequence.

Biomedical Imaging Unit

3. A new sequence (sequence 1) appears in the sequence frame.
4. Using the joystick, select the location to be monitored for this sequence and focus on it on screen. [For phase contrast with x60 for Fastcam, use the Fastcam PC to preview and set up the live image.](#)
5. Click on the "Read Device Settings" button (5th button) to bring up the "Read device settings" window. **Note:** If you want to use the autofocus facility, write down the Z position value for the sequence.
6. Check that the "Stage", "Camera" and "Microscope" boxes are all checked in the "Read device settings" window.
7. Click on "OK" to read the microscope settings to the ISP and close the "Read device settings" window.
8. repeat steps 2 to 7 for each position to be monitored.

Setup / Switch off Autofocus

1. On the "Flow" Tab of the "IX Control Panel" click on the "Define" button in the "Acquisition" section to bring up the "Define Frames" window.
2. If you do not want to use autofocus, uncheck the "Autofocus" option and click the "OK" button. [For phase contrast with x60 for Fastcam, autofocus should be switched off.](#)
3. If you do want to use autofocus, check that the "Autofocus" option is ticked and click on the "Define" button to bring up the "Define Autofocus" window.
4. look at the list of values for the Z positions of each sequence that you have set up and written down and identify the highest and lowest values in the series.
5. Calculate the midpoint between the highest and lowest values and the range between them.
6. In the "Define Autofocus" window, enter the range value **plus 20 μm** into the "Focus Range" box.
7. In the "IX control Panel" enter the midpoint value in the "Z position" box to the RHS of the microscope graphic and press the "Return" key on the keyboard to move the microscope to this focus level.
8. Click the "Set Focus Center" button on the "Define Autofocus" window to register this position as the midpoint of the focal range.
9. Check that the "Velocity" box is set to 20 $\mu\text{m/s}$.
10. Check that the "Delay" is set to 400 ms.

11. Click the "OK" button to close the "Define Autofocus" window.
12. Click the "OK" button to close the "Define Frames" window.

e.g. if the lowest and highest Z positions are 600 and 700 μm respectively, set the focus range to 120 μm $((700 - 600) + 20)$ and the Focus Center to 650 μm .

For phase contrast with x60 for Fastcam, setup the Fastcam PC with the desired settings; 500 frames / 250 frames per second / external trigger and check that the USB trigger box is connected to both PCs.

Save settings and Start run

1. Save the run settings by clicking the "File" button at bottom left of the "Define Image Sequence" window.
2. Provide a name for the ISP file in box at the top of the "Save/Load ISP" window (use your name and date as above) and click on the "Save" button.
3. For phase contrast with x60 for Fastcam, activate the external USB trigger using the "Connect USB trigger" button ("1") on Cell \wedge P's FastCamControl toolbar, then close the connection confirmation window. If the toolbar is not visible, right click on Cell \wedge P's toolbar region to display a list of possible toolbars, scroll down to FastCamControl and left click.
4. For phase contrast with x60 for Fastcam, click on the record button to start recording (waiting for external trigger). Click on the "Start" button in the "Define Image Sequence" window to start the run.
5. The "IS Process Control" window may appear immediately, or at the end of the first cycle.
6. fill in the log book with your name, the date and your run conditions (number of sequences, frames, frame interval). This is very important as it can allow image recovery in the rare event of a system crash.

Image Retrieval

1. At the end of the run, a message box will appear to report successful completion of the run. Click the "Close" button and close Cell \wedge P.
2. The folder that you specified in the "Flow" tab of the "Define Image Sequence" window will contain a tiff file for each sequence that contains all the cycles for

that sequence.

3. Log off from the PC, and login again, this time to the Soton domain using your Soton user name and password. Copy the image files to your folder on the BIU file server to archive them. If you do not have an ISS login, you cannot access the server and you must take full responsibility for archiving up your own data on your own systems. **It is a user's responsibility to ensure that their data is backed up.**
4. Copy the files to a portable hard drive to transfer them to your computer (they will probably be too large for the scratch drive and copying many GB of data back from the server (at Highfield) can be slow).
5. Once you have verified that you have an intact copy of the data, delete the files from the IX81's PC. It only has a small hard drive and if we need to create space on it we will delete any data we find without consultation.

Notes:

1. BIU has 2 portable USB hard drives that you can borrow to move large files. These must be signed for and returned **immediately** after use.
2. The images from Cell[^]P are 16 bit, multilayered Tiff format image files where each layer is the image of a separate time point in the run. Windows picture viewer cannot cope with 16 bit TIFF files (multilayered or single layered). You will need to open the images with Cell[^]P or ImageJ (<http://rsbweb.nih.gov/ij/>) or similar software in order to analyse your data and / or convert it to 8 bit format.

If in doubt, please don't hesitate to ask, we are here to help