OLYMPUS IX81 REAL TIME MICROSCOPE

CellR SYSTEM: START UP – SET UP

Switch on incubator temperature control box on left hand side 30 minutes prior to imaging session.

Images are only stored in the buffer, not saved, so save as you go.

Single snapped images and monochrome movie clips should be saved in Tagged Image Format (*.tif) format. Monochrome movie clips should be "Converted" to 8-bit prior to saving and transferring. RGB composite movies should be saved in the TIFF Split File Sequence Format (*.TIFF) format.

Normal log-in:
Type in your User Name, Password and IMBPC.
Save images in a folder in your group partition.
Fall back log-in:
Name – cell user; password – c1; this computer Lab Dyna.
Save images in a folder you create in D:\ User Data.

Computer on your right hand side should always be left on. This preserves the "dongle" key access.

Switch on IX2-UCB box on right hand side if it has been switched off.

Click on CellR icon to start software.

Click on Camera Control icon and drag to RHS screen.
Camera Control: Exposure, automatic brightness adjustment, subframes.
Click on "Automatic" and "Histogram" as this will satisfy the vast majority of image capturing. Generate an image within the confines of maximum exposure time, intensity of illumination, clarity of material of interest and dynamic range spread from the histogram.

Click on Illumination Settings icon and drag to RHS screen.
Illumination Settings: Burner (Xenon) On/Off, excitation filters, shutter Open/Closed, neutral density filtering.

Click on Microscope Settings icon and drag to RHS screen.
Microscope Settings: Objective selection, fluorescent turret with DIC and paired emission filters (GFP-RFP or CFP-YFP), condenser BF/DIC, bright-field lamp, ocular view/camera view, Z-drive focus (use central roller).
Brightness adjust – Automatic versus Online Histogram?

To capture an image: **Remember images are only stored in the buffer.**

Brightfield:
Microscope Panel:
(a) Click on lamp icon; Turret - UMDICT ; Condenser - BF

Fluorescence:
Illumination Systems Panel:
(a) Switch burner on.
(b) Select required excitation and emission filter.
(c) Open shutter.
(d) Click acquire icon in Camera Control panel.
(e) Focus.
(f) Capture image via Snapshot.

Care when changing objectives: use software.
If in manual mode make sure you hit the ESCAPE button, lowering the objective, before you change objectives to ensure you do not crash the objective into the stage. This happens automatically when focus is driven by software.

Experiment manager:
Set up a database folder as a repository for images captured *.apl.
New database created from Experimental Manager – File – New Database.
Open the Experimental Manager via icon **X**.
Drop or Drag the various command symbols to drag and command frames to drop to assemble your experimental protocol. Some examples are Image Acquisition, Multicolour frame, Time loop frame, Switch transmission lamp on/off.
Image acquisition items allow you to collect fluorescence and brightfield images individually or sequentially.
Multicolour frame applied merges series as an RGB composite.
Time lapse icon allows movie making.
Z-series icon allows stacks to be gathered.
Pauses can be inserted.
The brightfield lamp can be turned on and off to allow fluorescence imaging in conjunction with DIC or brightfield images. Cycle time is long when fluorescence and brightfield are combined.
Verify, Prepare and Initiate your protocol.
Experimental protocols can be saved as *.obsep files in your folder.

To view images on other computers “Save As” Split File Sequence Format *.TIFF and choose “Channel” option.

SHUT DOWN: Turn Xenon burner off prior to exiting CellR software.
Log off is essential.
Switch off MT20 box when prompted.
You may be prompted to switch off Xenon burner if it has been left on.
Leave the computer and the IX2-UCB box switched on.