**Zeiss Live Cell Imager – Quick Reference Guide for users**

For any help, please contact **Jiten Manji**

Login to computer:

Username: **PC8-94/Imaging User**

Password:**123LiVeLED**

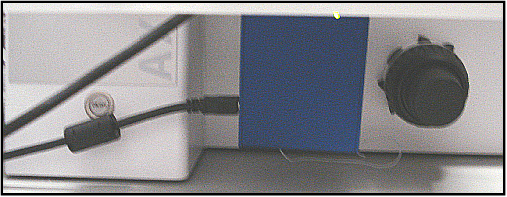
**Start-up Procedure**

1) Turn on both ‘Power Units’

2) Turn on the ‘Environmental Controller Units’ by the switch found on the base unit called “TempModule S”. The switch is located at the rear-right just above where the power lead is.

3) Switch on the Microscope by pressing the switch found behind the camera on the left side of the microscope

Camera



Focussing Wheel

FRONT

REAR

ON/OFF Switch

Caution

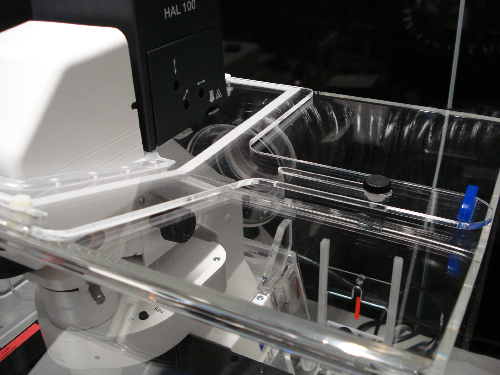
4) Open gas valves and gently adjust regulators to supply 1 Bar of both CO2 and N2 – please contact Jiten Manji to do this

5) Open the AxioVision Software on the desktop

6) Choose the appropriate stage insert

Available Microscope Inserts: **Image of inserts**

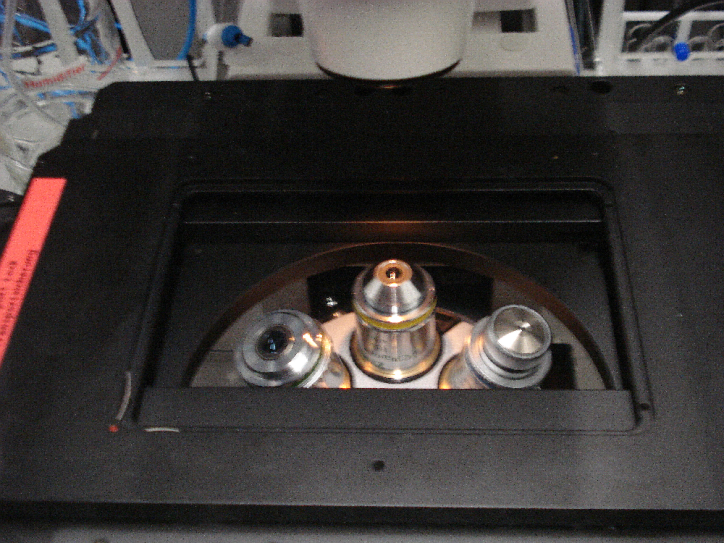
* Heated Petri Dish
* Heated 6 Well multiplate
* Heated 48 Well multiplate
* Non-heated multiplate insert

7) Unscrew ‘Lamp Support Chamber sealer clamp’ and slide away from the microscope Lamp Column

Lamp Support Chamber sealer clamp

10) Tilt the lamp support column backward to allow the stage insert to be correctly placed

11) Place the stage correctly into the Stage Insert Clips to ensure that the motorised stage is not damaged



**RIGHT**

**LEFT**

Objectives

Stage insert clips

Illustration of procedure to correctly mount the Stage Insert

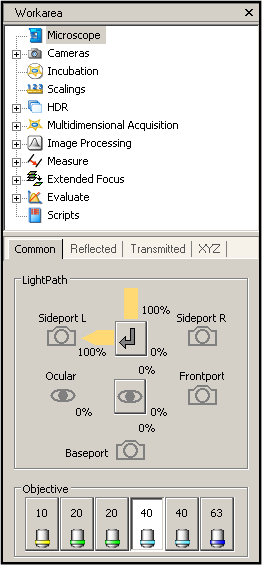
**RIGHT**

**LEFT**

**Inserting a Sample and Environmental Controls**

toolbar

* Live – Opens a window with a live camera feed
* Snap – Captures an image of the live camera feed
* Scale bar – Annotates a calibrated scale bar onto an opened image
* Text – Annotates with text onto an opened image
* Navigator – Defines a viewing region in images and live camera feeds
* Workflow – Opens a defined path of work for achieving specific aims
* Workarea – Opens window for all options for working
* Splitter – Opens a viewing mode where multiple images/series can be viewed simultaneously
* Gallery – Allows selection of which opened/captured image/series to view
* Dapi/GFP/txred – All three will set the microscope to image in the selected channel
* Closed – turns all imaging modes off
* Incubation – Opens settings window for heated elements and atmospheric controls



Control Section

Microscope Controls

*(these can also be accessed via touchscreen control panel)*

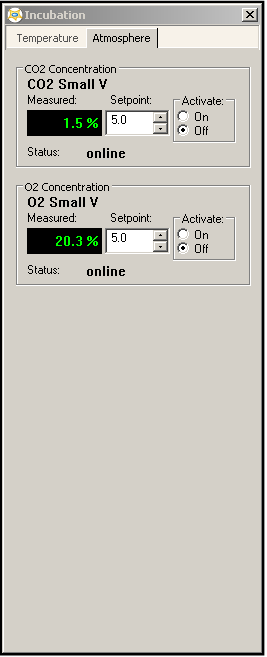
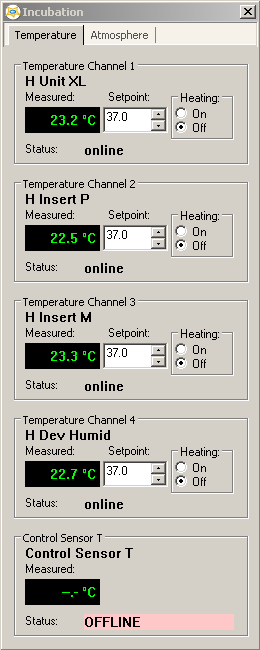
**Incubation Controls**

**H Unit XL** – Heats the air inside the Incubation Chamber

**H Insert P** – Heats the ‘Heated Petri-Dish Insert’

**H Insert M** – Heats the ‘Heated 6 Well Multiplate Insert’

**H Dev Humid** – Heats an accessory bottle warmer – **Not needed**



1) Open the Incubation Settings from the Toolbar

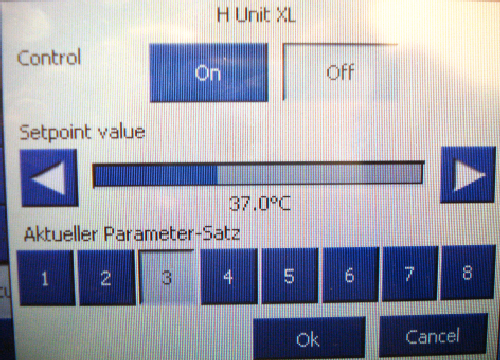
2) Set Temperatures desired (alternatively this can be done with the Microscope controls unit)

3) Leave system on to **warm for 1 hour** so all components are warmed equally

4) Insert sample dish into holder



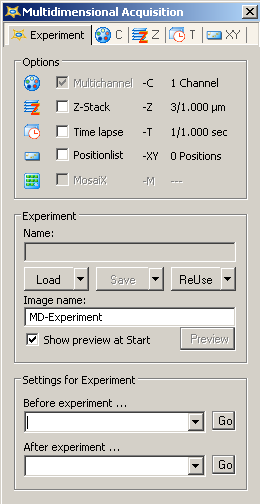
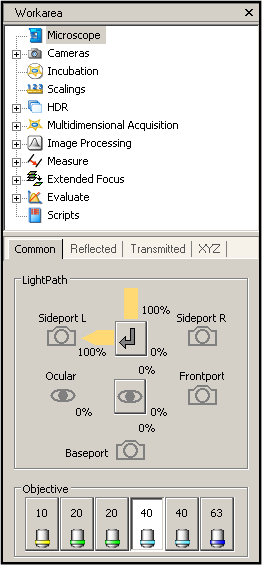
Component Selection



On/Off

Temperature

**Acquisition of Images**



Control Tabs

Option Selections

Re-use a previously saved experiment

The most common way to acquire a multi-parameter image is to use the Multi-dimensional acquisition tab found within the workarea. These options can be used separately or in conjunction with one another. I.e. 3 Colours, multiple points and time lapse etc…

**Multichannel** – multiple coloured fluorophores

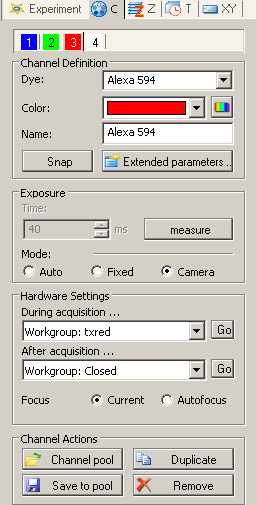
**Z-Stack** – Imaging through the Z plane

**Time Lapse** – Multiple time points

**Position List** – Multiple positions

**C-Tab for multichannel imaging**

The default setting is for all 3 fluorescent channels and a phase contrast ‘channel’. These can be de-selected by right clicking on the separate channel boxes at the top.

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**Channel Selection**

**Dye Definition**

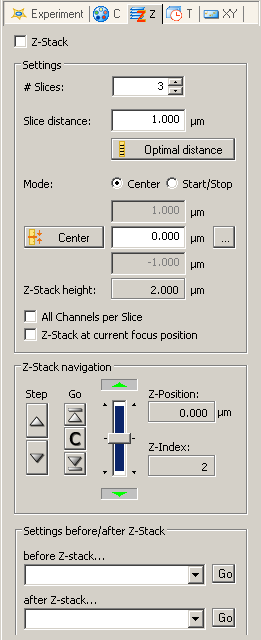
**Exposure Measurement**

1) Select desired channels depending on the fluorochromes used in you protocol

2) Click on measure and adjust the camera exposure accordingly for each channel separately

**Z-tab for imaging through the z plane**

You can stipulate the number of stacks desired, or you can allow the system to calculate depending on the thickness of your specimen.

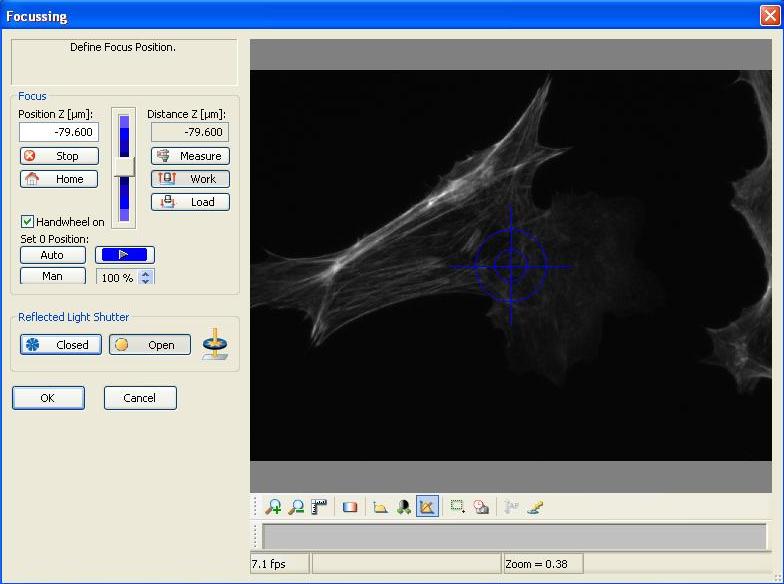


**Focussing window**

**Stack dimension selection options**

1. Choose Start/stop and open the focussing window next to the ‘Start’ box

* *This window provides a live image feed of the sample which can be focussed manually or using the slider to the left of the window.*

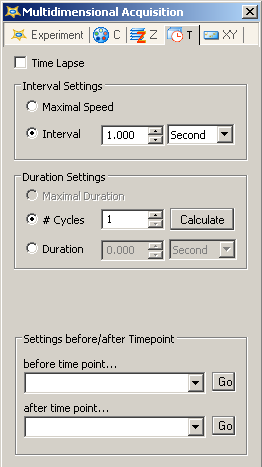


1. Focus to a position above cells
2. Click ok in focussing window
3. Repeat for ‘Stop’ by focussing beneath sample/cells

**T-tab for Time Lapse microscopy**

Open the T tab if you desire to collect a time lapse/live video tick the ‘Time Lapse’ Box

Select the appropriate interval and duration/cycle number for time lapse experiment – Remember that if the interval is too short to complete all commands the interval will lengthen regardless.

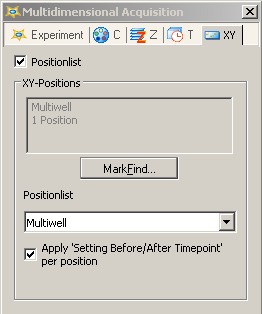


**Select the Interval**

**No of cycles**

**XY-Tab for multi-point imaging**

This option is used to capture multiple points at once, exceptionally useful if conducting multi-well differing condition experiments

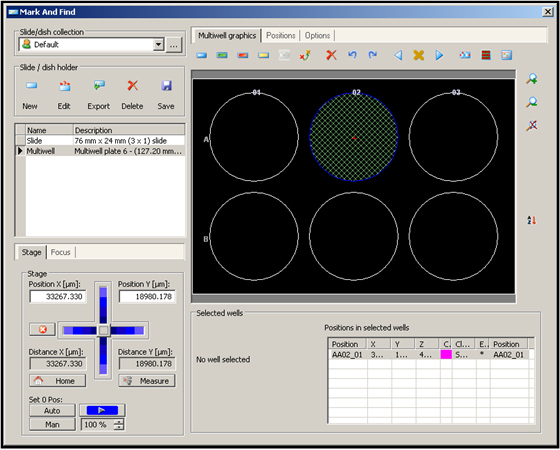
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MarkFind Window

List Selector

1. Open the Markfind Window

Create positions



Create Positions

2) Select the correct insert/slide type by highlighting existing selection and clicking on edit in the box above

3) Select the wells desired (hold Ctrl and click on the wells)

4) Click on the ‘Create Positions’ button and select the number of positions desired within each well

5) Open a ‘Live’ image feed window

6) Navigate your specimen to an area of interest wished to be imaged

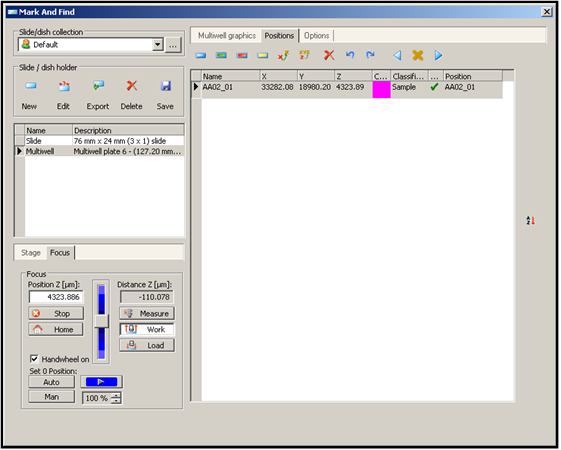
7) Select the Positions tab in the MarkFind Window (below)

Create Positions

Assign Position Location

Previous Position

Next Position



Select the positions by clicking on the ‘next position’ and ‘previous position’ buttons.

8) With each position move the stage with the XY Controller and focussing wheel and find a position to image.

9) Click on the ‘Assign Position Location’ button

10) Select a location and assign for each ‘position’

Once you have set how to acquire your image click on the Start button at the bottom of the MDA Window. Double checking all settings first is recommended.

**SHUT DOWN**

Once you have saved and exported your files safely the system needs to be shutdown safely.

1) Disconnect the blue gas tube from the bubbler flask located on the rear left inside the incubation chamber. This is achieved by pressing the tube inwards from the tube and pulling the tube out. It is designed to lock in place so two hands are required. All users will have been shown how to do this.

2) **Turn off all components**

a) Microscope

b) Environmental Control Units (switch at rear of basal unit – Fig 1)

c) Both Power Units (Fig 1)

d) Shutdown computer and turn off monitor

3) If you are the last user that day and have used the gas please have the gas turned off – users will all be shown how to do this

**POST-ACQUISITION OPTIMISATION**

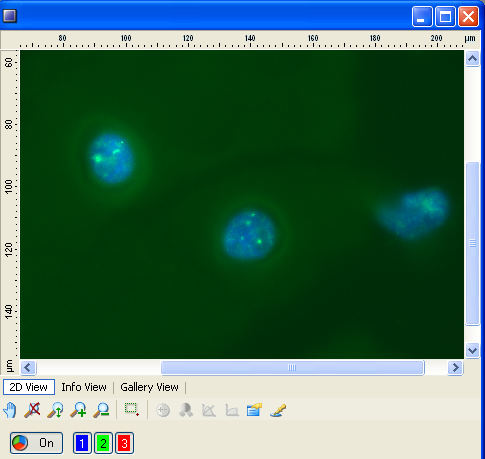
Once data has been collected the image can be enhanced so more detail is visible and also blurry z stacks can be reduced to a single plane image which uses in focus from all of the stack.

**1 – Brightness/Contrast Enhancement**

One of the first enhancements that is nearly always an improvement to an image is to adjust the brightness and contrast of each channel collected so it is the most clearly visible.

* The acquired image/series should be present as a tab just beneath the main toolbar – select the desired image/data
* Save as a ‘.zvi’ file immediately to prevent any data loss – it is best to export the file as desired for publication after saving this way so that if changes are necessary these can be made without data loss.

**Fig 25 – Image Window – 2D View**



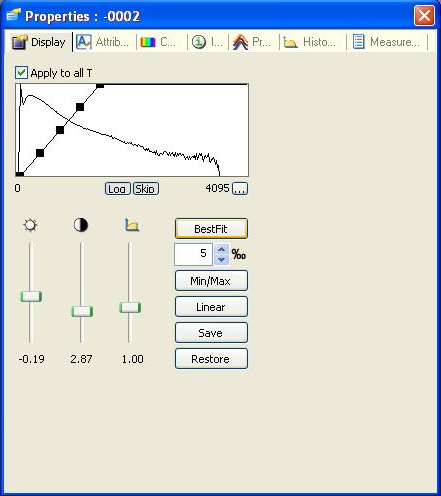
Psuedo-coloring

Tools

Channel Selection

1. Turn off Psuedo-colouring switch
2. Press **Alt+Enter** or right click on image and select ‘Properties’ to open an image properties window

**Figure 26 - Image Properties/Display Window**



Intensity Histogram

Restore Display properties

Automatic Brightness/

Contrasting controls

Save Display properties

Brightness

Contrast

Gamma Sliders

1. Alter brightness and contrasting by either moving display indicator directly, using the sliders or using an automatic selection. Linear is the default and is used to reset any changes. The gamma slider (furthest in from edge) causes a curved display indicator which is useful to reduce background or low level staining without removal.
2. Choose and repeat for each channel.

**2 – Selecting a Region of Interest (ROI)**

It is possible to select a smaller area of your image / sequence / series to crop and enlarge. This is defined with the ROI tool.

This is found in the tools section of an image window (Fig 25)

The ROI boundary is defaulted to encompass the entire image

* Select a smaller area by moving the boundaries to the edges of the area desired
* Select Copy ROI in Edit (main tabs) or press **ctrl+shift+c**.
* Now simply pressing **ctrl+v** or **Edit → Paste** and a new image is produced of just the ROI selected.

This process may be especially useful when a long capture has occurred and the file is very large but only a small area has any valuable information.

**3 – Annotations**

1. Adding a scale bar
   * Open image
   * From menu tabs select **Annotations → Scale Bar**
   * Draw the length of scale bar desired
   * Open Image Properties (Fig 26)
   * Select second tab
   * Adjust colour/size/font as desired
2. Adding a time mark
   * Select **Annotations → Frequently used annotations → Time Mark**

Time marks annotate the time at which images were collected. An alternative is relative time mark which shows sequence duration.

* Remember to select ‘Burn in annotations’ when exporting

**4 – Exporting As Another File Type**

Once a file has been captured is always best to save it as a **.zvi** file but it is not always best to transport such large files and mostly these cannot be used to publish.

To export a file it must be opened and being viewed.

* Open **File → Export**
* Select a file type to export as
* Select the desired percentage of compression – if none is desired select 0%
* Choose a name and location in relevant areas of the Export window.
* Click on **Start**

The default location for files to be exported is on the desktop.

Please transport files via the X-Drive or use a memory device.