Zeiss StereoDiscovery Guide

Getting Started

1. Switch on the appropriate light source.
   - ➢ Transmitted and incident light
       - ▪ Can be turned on by a switch at the **back left** of the LED control panel.
   - ➢ Fluorescent light
       - ▪ Can be turned on via the white (HBO) box above to the microscope.
       - ▪ This is a HBO mercury arc burner and should not be switched on and off quickly - <u>leave running for at least 10 minutes before switching it off</u>.
2. Switch on the computer and log in using your UQ username and password.
3. Switch on the colour camera at the powerpoint (behind microscope).
4. Double click the **AxioVision** icon.
   - ➢ If it is your first time using AxioVision on this microscope a window will appear – do <u>not</u> reconfigure the microscope – instead tick the box at bottom of this window (do not show this window again) and click "continue".

Shutting Down

1. Shut down the computer.
2. Switch off the LED lights and HBO fluorescent light if used.
3. Switch off the camera at the powerpoint (behind microscope).
4. Cover the microscope with the blue dust cover.

Using the LED illumination system
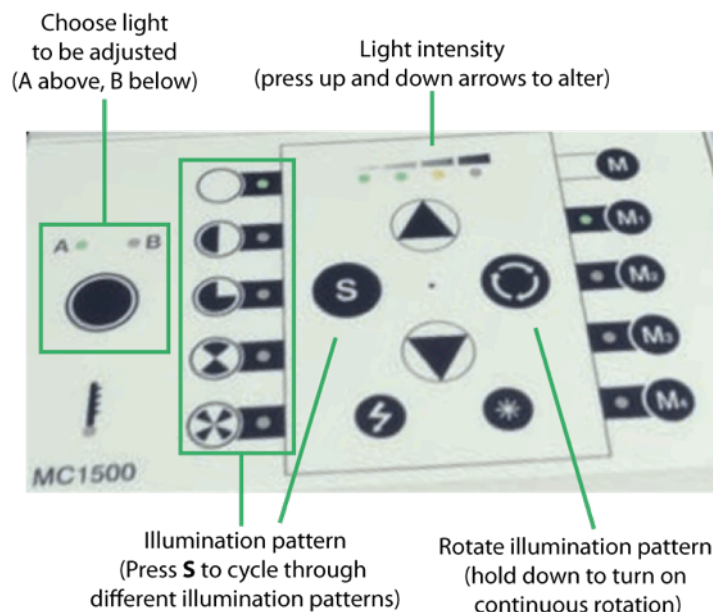
When using transmitted or incident light ensure you are using an empty filter turret position (<u>positions 4 and 5</u>)

Using fluorescent illumination

1. Ensure the fluorescent shutter is open
   - The shutter slider is located where the fluorescent light enters the microscope
   - Pulled all the way towards you is closed.
   - **The middle position (1 click back) is open for normal use**
   - The back position (2 clicks back) is a filter to remove red background light (if present)
2. Chose appropriate fluorescent filter set.
   - Position 1: GFP
   - Position 2: YFP
   - Position 3: RFP
3. Adjust the light path so light reaches the camera.
   - Toggle on top right of microscope.
4. Click on the **Live** icon in the menu. Click **Exposure** to automatically adjust the exposure

Visualising and Imaging a Sample

1. Ensure the appropriate light source is on and reaching the sample.
2. Adjust the focus and zoom as necessary.
3. Adjust the light path so light reaches the camera.
   - Toggle on top right of microscope.
4. Click on the **Live** icon in the menu. Click **Exposure** to automatically adjust the exposure.
5. Adjust exposure if required. To do this select "**Cameras**" in the workarea and choose the camera you are using. Exposure settings are under the **adjust tab**.
6. Click **Snap** to create an image. Note: the images are captured but not saved at this point.
   - *You can also use the foot peddle below the desk to take a Snapshot in Axiovision*
7. To add a scale bar click on the **scale bar** button (adjacent to Snap) and drag the cursor over the image where you would like the scale bar to appear. NOTE: ensure the **correct scaling option is selected** in the drop down list at the bottom of the **live image window**.
   - Tick "**burn-in annotations**" when saving if you want the scale bar to be saved on the image
8. To automatically fit captured images to the window size go to **Tools>Options>Display** and tick the **Fit image to window** box

Multidimensional Acquisition (multi-colour imaging)

Multidimensional acquisition is accessed through the Multidimensional Acquisition menu in the workarea (to the left of the screen). Here it is possible to create protocols for single and multiple channel.

You should use this feature if you wish to take: **1) Multi-channel images 2) Overlay a fluorescent channel with a brightfield image** or **3) Create a time-lapse image.**

NOTE: This microscope is *not motorised* and so is not capable of automatically taking multi-colour images.

1. Expand the **Multidimensional Acquisition** menu in the work area and click on **Multidimensional Acquisition.**
2. Click on the **C/colour wheel tab** of the multidimensional acquisition menu.
3. Select the appropriate marker/dye you are using from the first drop down menu. AxioVision will assign this dye a colour (you may change this colour if you wish in the second drop down menu).
4. Ensure you have the corresponding filter in place if fluorescence (chose an empty filter position if using LEDs)
5. Leave **Hardware Settings** menus empty.
6. Under **Exposure Settings**:

Multidimensional Acquisition (multi-colour imaging)

7. Under **Exposure Settings**:
   - Click on **Measure –** the exposure will be adjusted automatically and your sample should become visible. Adjust the exposure further if necessary in the top left hand corner of the window. When you close this window your exposure time will be saved.
   - Ensure the image will be captured with the **linear histogram** option (graph icon with a diagonal red line running through it at the bottom of the window) if fluorescence.

8. You can save these settings by clicking **Save to Channel Pool** in the lower left corner.

9. Duplicate this channel and adjust the new channel to suit your other fluorescent marker/brightfield image.

10. Once you have created all your channels you can generate a multi-dimensional image by clicking **snap** under each channel tab - ensuring you have manually chosen the correct filter and light source for this channel - this will generate a multi-colour image.

QBI Fluorescent Marker Guide
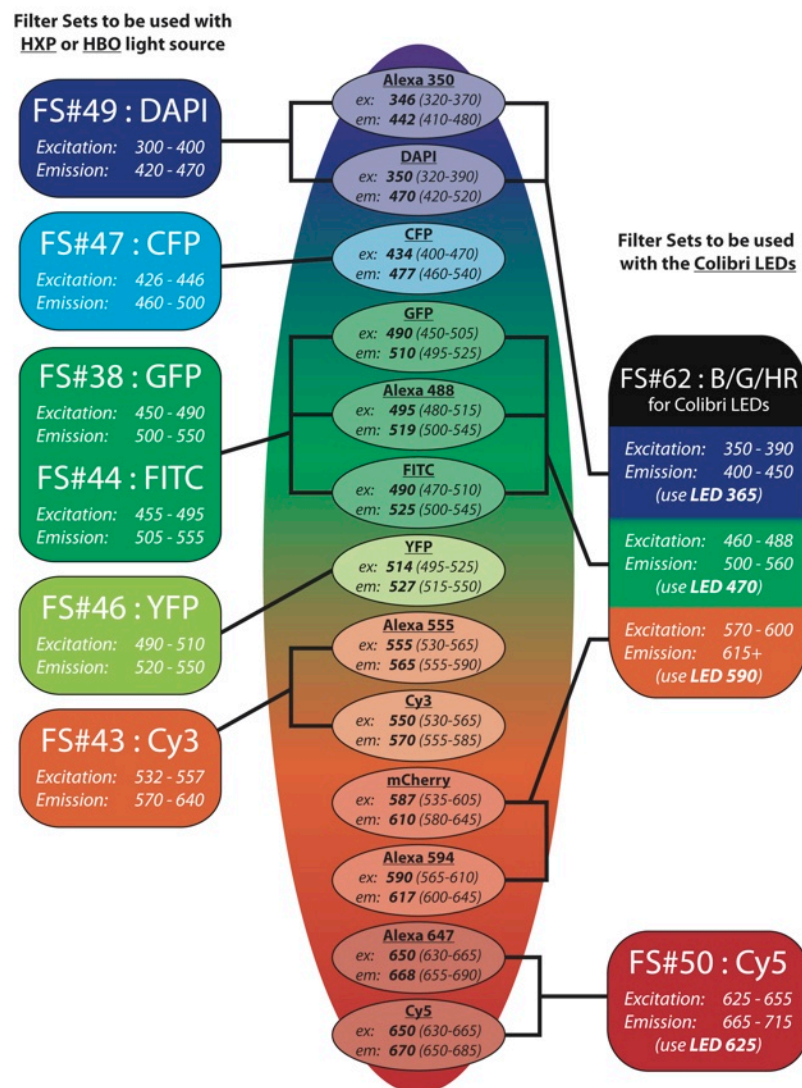
Saving Images

- To save an Image you can click the **save** button in the top menu.
  - ➢ This will save the image as a *.ZVI file, which can be only opened by Axiovision and FIJI (free downloadable software)
  - ➢ It is best to keep the ZVI file as your original data but if you need the image for a powerpoint presentation or want to open it in photoshop you can export your ZVI files as TIF files - see below.
- You can save your images to the desktop - but at the end of each session, either:
  - ➢ Move your files to your group share or personal network share.
  - ➢ Move your files to drive D: for storage there. Or,
  - ➢ Move your files to a USB stick / portable harddrive
- You can set up automatic prefixes and suffixes for your naming your images under **Tools>Options>Naming**
  - ➢ Chose the category you wish to change the settings for as there are separate specifications for each category (for example single acquisition versus multidimensional acquisition).
- You can also set the software to automatically save every image you capture in **Tools>Options>Storage**

Exporting *.ZVI files as TIFs

If you save files directly as TIFs you may loose data and have no way of getting it back, also any changes you save to TIFs are permanent. It is best to save a ZVI file then convert to TIF format.

To make things easier you can save ZVI files as you go and then convert all of these files to TIFs in a batch at the end of your session.

To do this:

1. In the top menu click **File** and then select **Export**
2. In the export window that opens choose where you would like your TIF files to save **(1)** (e.g. a TIF export folder on your desktop).
3. Uncheck the **Create project folder (2)** (unless you are exporting Z-Stacks/mosaiX images) .
4. Click the **batch** button **(3)**, then choose the ZVI files you want to convert by clicking **Add files... (4)** (you can select multiple files using the shift key, or whole folders).
5. Tick **on** all 4 top options **(5)**
   - ➢ If you are using more than 3 colours (i.e. not RGB) uncheck merged images only.
6. Choose TIF in the file type selection (JPEG format compresses you images and reduces image quality)
7. Tick on: **(6)**
   - ➢ **Apply display mappings** - keeps any adjustments you have made to the histogram/brightness/contrast
   - ➢ **Burn in annotations** - keeps scale bars visible in image
8. Click **Run Batch (7)** (*not Start*)
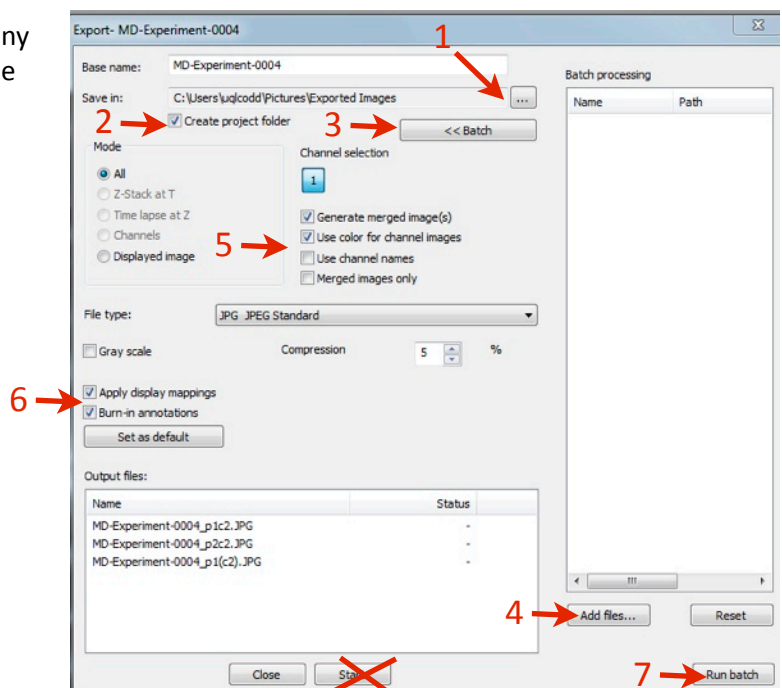
WORKING WITH GENERAL MICROSCOPES

Ergonomics: Use of mouse and keyboard / viewing computer screen – Prolonged use of the microscope and microscope computer without breaks can increase the risk of muscular strain.

Eye strain and fatigue – Viewing samples through microscope eye piece or computer monitor over lengthy periods of time can result in eyestrain and headaches.

Exposure to sharps – Expsoure to razor blades, scalpels, forceps, cover slips, glass slides could result in cuts or puncture wounds to hands or other areas of the body. Any microscope slide shards or glass debris must be disposed of in the appropriate shapes disposable bin in accordance with PC2 regulations.

Exposure to intense fluorescent light –Xenon or mercury light sources are attached to this microscope and are the source of intense and potentially dangerous light. Under no circumstances should any optical elements be removed from the microscope light path or fail-safe switches be circumvented. Do not attempt to adjust the light path, or illumination modules in any way. Avoid direct exposure to the light.

Scope

This procedure details the method for using the general microscopes.

Safety Considerations

Personal Protective Equipment (PPE):

Laboratory coat, **latex gloves** and **closed in shoes** should be worn to prevent injury.

Ergonomics and Risk Exposure:

Appropriate ergonomics, including adjustment of the seat, computer screen and microscope oculars should be undertaken to reduce risk of strain injuries.

Emergency Procedures:

First aid may be required for:

Exposure to sharps – Contact the nearest first aid officer from the list that is beside all first aid kits and on safety notice board.

Exposure to intense fluorescent and laser light – Seek immediate medical assistance if you have been exposed to intense direct light or laser light.

All incidents must be reported to the OH&S Manager and on UQs online incident reporting system.

Contacts: Security x53333 or OH&S Manager Ross Dixon 0401 673 654