

Bosch Microscope Users Guide

Standard Operating Procedures

- 1. Startup and Shutdown**
- 2. Create your own Archive**
- 3. Acquisition Settings**
- 4. Live Image**
- 5. Saving, Scaling & Superimposing**

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| Bosch Microscope Facility Bosch Building, Room 205 | Laser Capture Microdissection Facility Blackburn Building, Room 663a Tel: 9351 6181 |

1. Startup and Shutdown

Startup

- Computer on. Login 'Olympus', password 'elkus_1807'
- Microscope on (RHS rear switch)
- Mercury or xenon lamp on if required. Make sure it has cooled for 30mins - 1 hour prior to turning on. Leave on for at least 1 hour
- Note starting time on log sheet
- IM1000 program open

Shutdown

- IM1000 program close
- Microscope off
- Fluorescence lamp off if no users within 1 hour (refer to web calendar)
- Note finishing time on log sheet
- Clean oil lenses with lens tissue and 70% EtOH. Do not clean dry lenses
- Backup files using the CD burner. Do not rely on this computer for storage of all your images
- Log off or shutdown computer if no users
- Report any concerns immediately to the lab supervisor

2. Create your own Archive

Open IM1000

- Opens last archive used

Create new Archive

- *File, New Archive*
- *Archive Wizard* window appears
- Select *Create a new archive*. Do not use template because they are difficult to modify
- Create hierarchy of entries. Press *Add* to add folder 1 (eg. User info) then repeat to add subfolder (eg. Sample), then another subfolder (eg. Image)
- Press *Next*
- Define fields for each level eg. For *Image window*, enter 3 fields: Image name (text), Image (image) and Comments (text)
- Select *Identifier* field for each level by highlighting and checking box (eg. last field of each level)
- Press *Finish*
- Save as archive file (.iaa) in C:Documents and Settings\All Users\Documents\Leica\IM\Archives

3. Acquisition Settings

Configure AutoSave

- *Options, AutoSave, Image window*
- Check *Activate box*
- Define image labelling, format and save destination

Configure Image Acquisition

- *Image Acquisition, Configure Image Acquisition*
- Choose the mode (*Direct* or *Superimpose*)
- Choose the source (*Camera, Clipboard* or *File*)
- For bright field and fluorescence single-labelling choose *Direct* and *Camera*
- For fluorescence multiple-labelling choose *Superimpose* and *File* (refer to section 5)

Create a new Datasheet

- Click on window of interest (eg. User, Sample, Image)
- To create new datasheet click *blank sheet icon* or right click and select *New Datasheet*
- Fill in new parameters
- Fill in Image window parameters after image acquisition

Configure Source

- Click on the *spanner icon*, this accesses the camera
- *Live Image window* appears

4. Live Image

- Pull out *silver knob* for 100% light to camera
- For bright field, make sure the filter wheel (front of scope) is set to 6 (the blank filter space)
- Set parameters for *Live Image* and *Captured Image*
- Right click on each parameter to view options

Live Image

- *Binning*, greater than 1x1 increases signal but reduces live image resolution
- *Mode*, Standard
- *Under/overexposure*, displays as overlay colours
- *Find Focus*, displays focus level using a sliding bar
- *Check colour*, average colour within blue box is shown on the *Colour Correction chart*. Do not change.

Captured Image

- *White balance*, select small background region within image with mouse, select *white balance*
- *Shading*, none
- *Colour depth*, 8 or 16 bit (8 usually adequate)
- *Image type*, colour or greyscale
- *Image size*, 1300 x 1030, 1 shot
- *Sharpen*, do not check
- *Crop to ROI*, only if required

To acquire image

- *Exposure*, adjust so histogram spread is greatest. Side arrows should be either side of histogram and middle arrow adjusts contrast
- *Gain*, keep at zero
- Press *Acquire*

5. Saving, Scaling & Superimposing

Saving

- *Select Calibration window* appears
- Choose *objective lens/ image size*
- Press **OK**
- Fill in *Image* field parameters

Scaling

- Apply scaling, measurements, labels, arrows, shapes
- Right click image, select *Measurement*
- Use icon of interest
- Eg. For scaling, go to *Simple Measurements* window, select *scale bar icon*, apply to image, right click to format, then select *burn icon* to merge annotation onto image

Superimposing images

- *Image Acquisition, Configure Image Acquisition*
- Select *Superimpose* mode and *File* source
- *Image Acquisition, Acquire Image into new datasheet*
- To overlay images, select a *colour box*, press *Add* button, choose image file to overlay
- Click **OK** to save superimposed image to archive