Zeiss LSM 510 META Guide

Getting Started

1. Ensure the **main power** is switched on (black knob on the far left of the laser module box).

2. Turn on the **System/PC** and **Components** switches (located on the grey switch box near the monitor).

3. Switch on the computer and log in with your UQ username and password.

4. Start the **Zen 2008** software. On the start-up window select "**Start System**".

Shutting Down

1. Lower the stage and remove your sample.

2. _Gently wipe_ any oil objectives you have used with _lens tissue_ (**do not use kim wipes to clean objectives**).

3. Turn off the lasers within Zen 2008 and **allow time to cool down** before shutting down the whole system – _you will hear the cooling fan turn off when ready to shut down – approx. 5 minutes._

4. Exit the software and copy your files to your _home or group network/ USB drive._

5. Shut down the computer.

6. Turn off the **System/PC** and **Components** switches.

Visualising a Sample Through the Oculars

1. Ensure microscope is in **VIS mode** (button on top left of microscope stand)

2. On the touch screen attached to the microscope press "**load position**" and position slide on stage. Pressing the button will return you to the working position.

3. After pressing "**microscope**" on the far left of the touch screen you will be able to change objectives, filtersets and switch between fluorescence and brightfield modes.

4. Open **RL-Shutter** to see fluorescence.

5. Adjust stage position and focus as necessary.

Zen 2008

Zen 2008 is designed with separate toolboxes to adjust each aspect of imaging. It is important to have the **pro** option enabled on each one of these boxes to be able to see all features.

Before starting expand all the **Setup Manager** boxes (**Lasers**, **Imaging Setup** and **Light Path**), then drag the **Online Acquisition** boxes (**Acquisition Mode**, **Channels** etc) into a new column – you can do this by clicking on the white "online acquisition" heading and dragging it to the right. Expand the **Acquisition Mode** and **Channels** boxes – you now have everything visible that is needed to take an image.
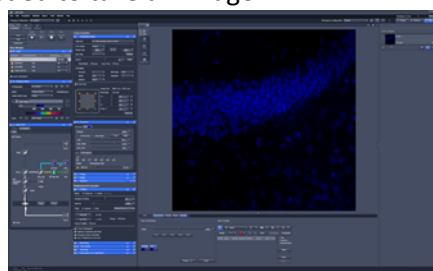
Zen 2008 - Setup Manager

1. **Lasers**
   - Here you need to switch on each laser necessary for imaging your sample.
     - **Argon/2** – typically for **GFP/YFP** (use 5 to 12%)
     - **HeNe543** – typically for **RFP/Cy3/Alexa546** (use 30 to 50%)
     - **HeNe633** – typically for **Alexa633/647/Cy5** (use 8 to 14%)
     - **Diode 405-30** – typically for **DAPI** (use 2 to 6%)
   - The Argon/2 and Blue Diode laser need to enter standby mode before they can be switched on.

2. **Imaging Setup** and **Light Path**
   - Here you need setup each light path you will be using to capture your images.
   - Each light path configuration is known as a **track**
   - You can capture multiple channels (using different detectors) within the same track – known as **simultaneous imaging** OR
   - You can capture multiple channels (using the same or different detectors) with different light paths, using separate tracks – known as **sequential imaging**
     - This is slower than simultaneous imaging but reduces crosstalk between channels

   - **Imaging Setup**
     - Here you can select and load/or save a light path configurations and view which laser/s each track is using and what wavelengths will be detected
     - To add a track use the + button at the bottom left of this window
     - Click on the name of an active track to bring up details about this track and its light path configuration
     - The tick box enables/disables that track for imaging.
     - It is best to disable other tracks adjusting the settings for one track in the live window

   - **Light Path**
     - Shows the light path configuration
     - Recommended instructions for light paths can be found on the laminated sheets near the microscope and are summarized below

Zen 2008 - Online Acquisition

1. **Acquisition Mode**
   - Here you select the resolution and quality of imaging – essentially you need to find a balance between image quality and speed while considering how much your sample will bleach

   - **Frame size**
     - 512x512 will give you a fast image but a resolution of at least 1024x1024 is recommended

   - **Speed**
     - A slower speed will give you a better image but will take more time
     - A scan speed of 7 or less is recommended

   - **Averaging**
     - Averaging will remove noise from the image
     - A slower scan speed will often require less averaging
     - Generally 2 – 4 x averaging will produce a good image

   - **Bit Depth**
     - 8 Bit uses 0-256 levels of grey - standard
     - 12 Bit uses 0-4096 levels of grey – producing a smoother image with more information for intensity analysis but will generate a larger file

2. **Channels**
   - This is the control window for adjusting the detector sensitivity and pinhole size and needs to be set _for each channel._

Zen 2008 - Online Acquisition

2. **Channels - cont.**
   - ➢ This is the control window for adjusting the detector sensitivity and pinhole size and needs to be set _for each channel._
   - ➢ **Pinhole**
     - ▪ The pinhole setting always defaults to wide open. So for each channel you need to press the **1 AU button** (1 airy unit) to ensure you are taking a confocal slice

   - ➢ **Gain**
     - ▪ Controls the sensitivity of the detector
     - ▪ The higher the setting the brighter the white
     - ▪ Drag the controller to about half way – any higher than this and you begin to introduce noise. If it is too bright when you take an image you can drag it back towards zero – if the image is still too dim you may need to increase your laser power.

   - ➢ **Amp Offset**
     - ▪ Controls the black level of your image – and defaults to 0.1
     - ▪ The higher the setting the less black the background will be
     - ▪ You will need to make fine adjustments to this while the image is scanning (usually to around 0)

   - ➢ Using **Find** will automatically adjust the Gain and Amp Offset to roughly the right places for the channels/tracks currently active

Capturing an Image

Once Zen has been setup to capture each channel you can take a **single** image or use **continuous** imaging to finely adjust the capture settings.

Using **Fast** imaging is useful for moving around or adjusting focus onscreen.

Optimising the Dynamic Range

1. Use **continuous** or **live** imaging so the image is constantly updated**.**
2. In the bottom left, below the image, click the channel colour box (**red circle below**). This will change the palette to "range indicator".
   - ➢ The range indicator shows over-saturated pixels as **red** and true black pixels as **blue**
3. Adjust the _master gain_ until no/little red is seen and the _digital offset_ until small amount of blue is visible (usually 0.0)
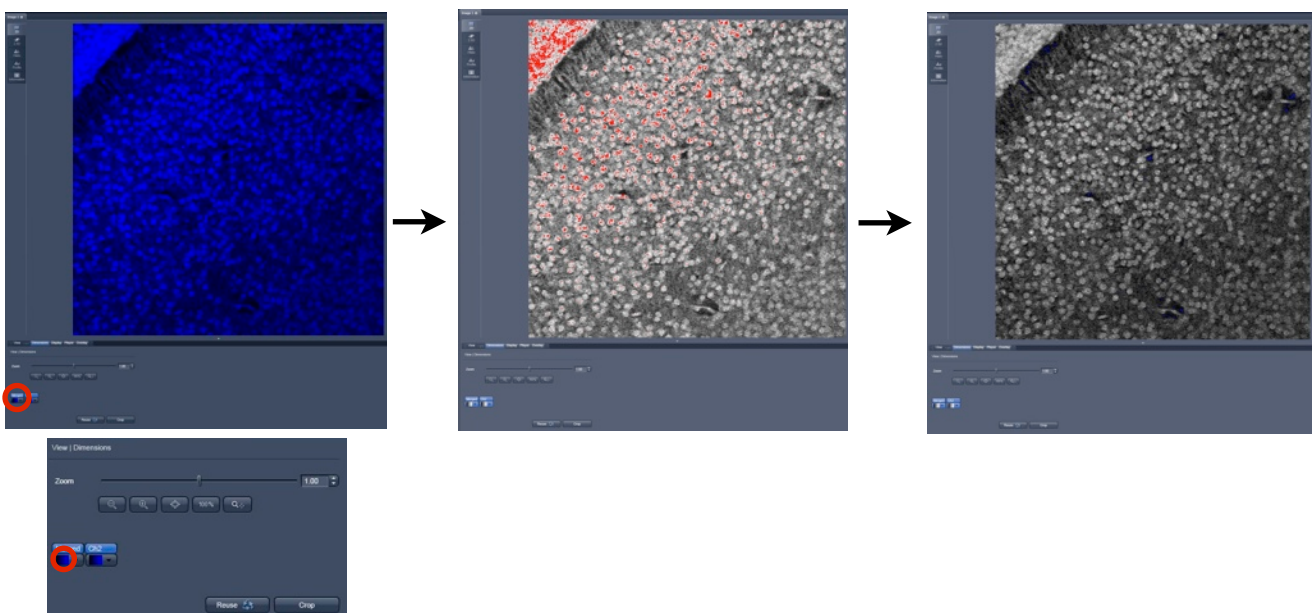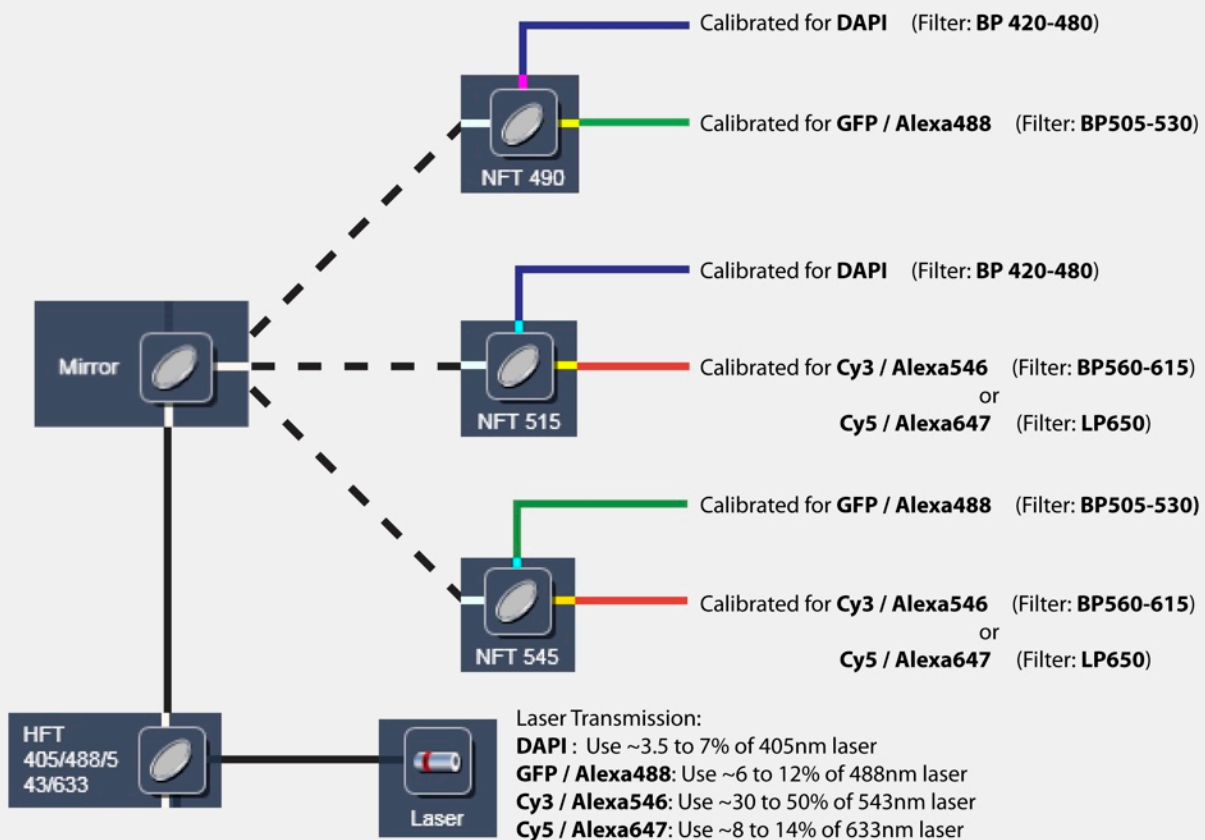
Getting an Image

1. Switch on the lasers you need to use under the **laser** toolbox.
   - ➢ The argon and 405 laser need to put in stand-by mode first.
2. Open the **Imaging Setup** and **Light Path** toolboxes.
   - ➢ Ensure the **"pro"** setting **(1)** is enabled for each toolbox.
3. Under **Light Path** set up a light path for your first maker (e.g. Alexa 488) using the recommended light path guide (see over).
4. Save the light path/track **(2)**.
5. If you have a second marker, add a track by clicking the + button **(3)** and set up a new light path.
   - ➢ It is possible to image multiple markers in one track (**simultaneous imaging**) - this is faster, however increases the chance of cross-talk between the channels. **Sequential imaging**, using a separate track for each channel, is slower but minimises cross-talk.
6. You can also save these two tracks together as one **"configuration"** this allows you to load in the same complete experimental set up next time you use the microscope.
7. Open the **Acquisition Mode** and **Channels** toolboxes in a new column.
   - ➢ You can create a new column by clicking on the white heading "online acquisition" and dragging it to the right.
8. For each channel under **Channels**:
   - ➢ Ensure that the "1AU" (1 airy unit) button is pressed **(4)**
     - ▪ This closes down the pinhole to allow optical sectioning
   - ➢ The master gain is set to 650 **(5)**
     - ▪ At 0 you will not be able to see anything
     - ▪ Any higher than this and you begin to introduce noise to your image
   - ➢ The digital offset is 0.0 **(6)**
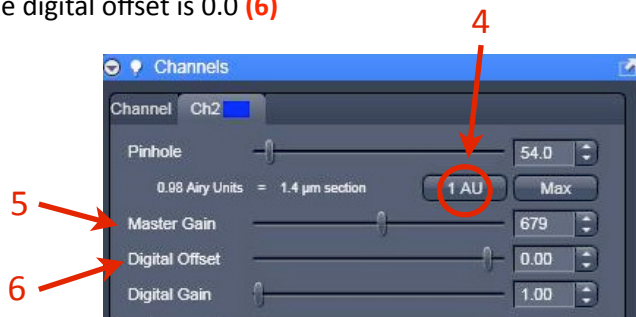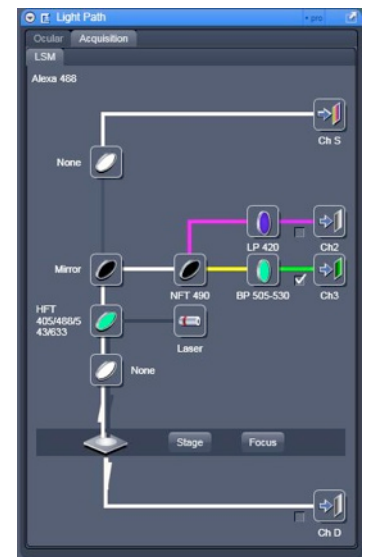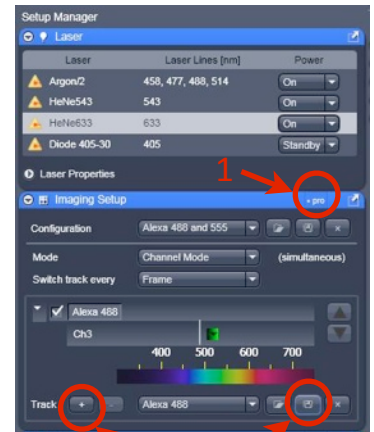
9. Optimise the gain and offset settings for each channel separately as mentioned in "optimising the dynamic range" on the previous page.
   - ➢ To disable/enable tracks click the check box next to the track in the **Imaging Setup** toolbox.
   - ➢ If you save a track/configuration after you have adjusted the Gain, pinhole and offset settings then these will be saved as well.
10. You are now ready to take an image. Under the **Acquisition Mode** toolbox choose the resolution **(7)**, scan speed **(8)** and amount of averaging. **(9)**
   - ➢ 1024x1024 at a speed of 7, averaging 4 times is a good start.

Optimised light paths for LSM 510 META (Violet)

Calibrated for **DAPI**   (Filter: **BP 420-480**)

Calibrated for **GFP / Alexa488**   (Filter: **BP505-530**)

NFT 490

Calibrated for **DAPI**   (Filter: **BP 420-480**)

Calibrated for **Cy3 / Alexa546**   (Filter: **BP560-615**)
or
Cy5 / Alexa647   (Filter: **LP650**)

NFT 515

Calibrated for **GFP / Alexa488**   (Filter: **BP505-530**)

Calibrated for **Cy3 / Alexa546**   (Filter: **BP560-615**)
or
Cy5 / Alexa647   (Filter: **LP650**)

NFT 545

Mirror

HFT
405/488/5
43/633

Laser

Laser Transmission:
DAPI : Use ~3.5 to 7% of 405nm laser
GFP / Alexa488: Use ~6 to 12% of 488nm laser
Cy3 / Alexa546: Use ~30 to 50% of 543nm laser
Cy5 / Alexa647: Use ~8 to 14% of 633nm laser

Regions of Interest / ROIs

Can be used to image a small section of the image frame or for bleaching experiments.

1. Open the **regions** toolbox.

2. Chose the region shape. **(1)**

3. Draw the region over the image.

4. Tick the "acquisition" box. **(2)**

5. Tick the "fit frame size to bounding rectangle of regions" box. **(3)**

6. Click single/continuous to image the region of interest.
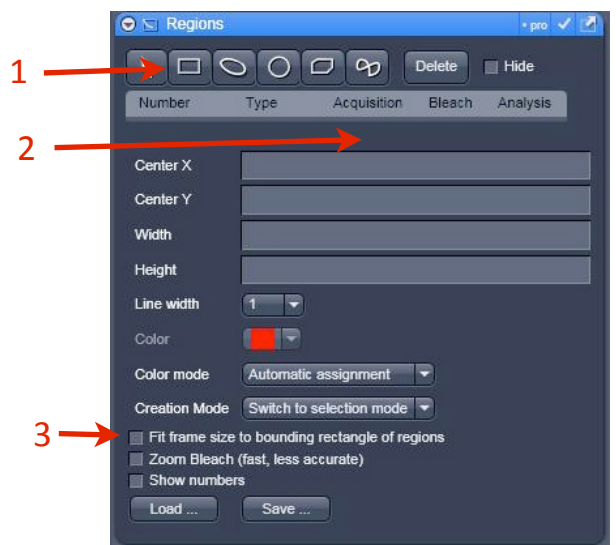
Z-Stacks

1. Open the **Z-Stack** toolbox (ensure the pro box is enabled).
2. Select **First/Last** imaging Mode.
3. Whilst **Fast Scanning** move the focus to the top of your sample and click **Set Last. (1)**
4. Now move the focus to the bottom of your sample and click **Set First. (2)**
5. Click **Optimal Interval. (3)**
    - The optimal interval is half the slice thickness (determined by the pinhole settings and objective).
    - You can increase the interval size to be the same as the slice thickness - this will speed up taking a Z-stack but the Z-resolution will be very poor. For better Z-resolution make the interval smaller than the optimal interval.
    - For best results you should check each channel in the **Channels** toolbox and ensure they all have the same *optical section thickness.* This will mean adjusting the pinhole settings slightly for each channel (remember to recheck the gain settings after doing this).
6. Check the tick mark (enables Z-stacking). **(4)**
7. Click **Start** instead of single to take a Z-stack .
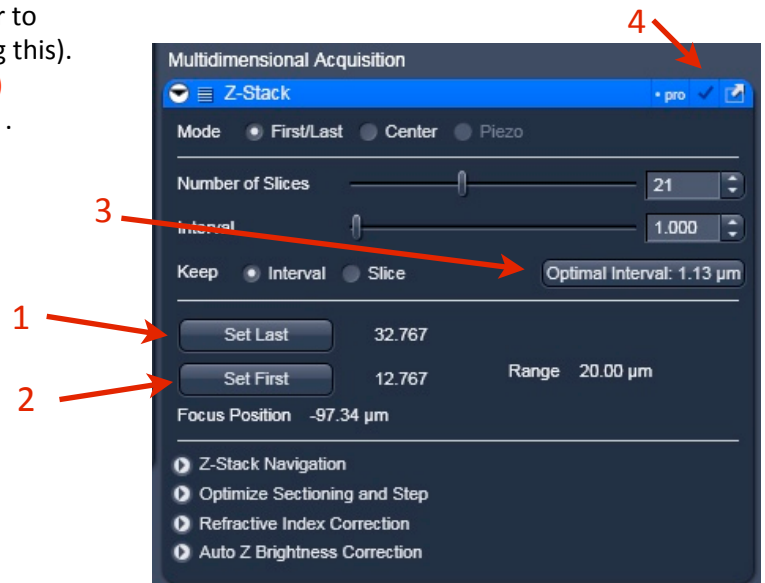
Saving Images

- Images are saved as .lsm files as default - you can open these in ImageJ on PC and Mac, or if you are using a PC you can open them in Zen LE (free download)
- If you want to save an image as it appears on the screen choose **Export** under the **File** menu. Select *.tif as the file format and choose **"Contents of Image Window - Single Plane"**

Heating and CO2 units

The heating & CO2 units can be controlled either by the TFT touchpad or through the software.

It is recommended to pre-heat the chamber for at least 30 minutes prior to a booking which is automatically added when incubation is selected in the PPMS booking system.

*Ensure the power switches for the Heating and CO2 units are ON before turning on the microscope power otherwise the microscope will not recognize these components on start-up.

1. On the TFT Touchpad
   a. Select "Incubation" **(1)**
   b. Select unit to control
      i. H Insert controls temperature of the heated stage insert (if present)
      ii. H Unit XL controls temperature of the large incubation chamber (if present)
      iii. CO2 Small V controls the concentration of CO2 pumped through the CO2 cover plate (if present)
2. Set required environmental conditions
3. Set to "On"
4. Select "Ok"

5. Set all Incubation modules to "Off" when finished your imaging session and shut down normally.
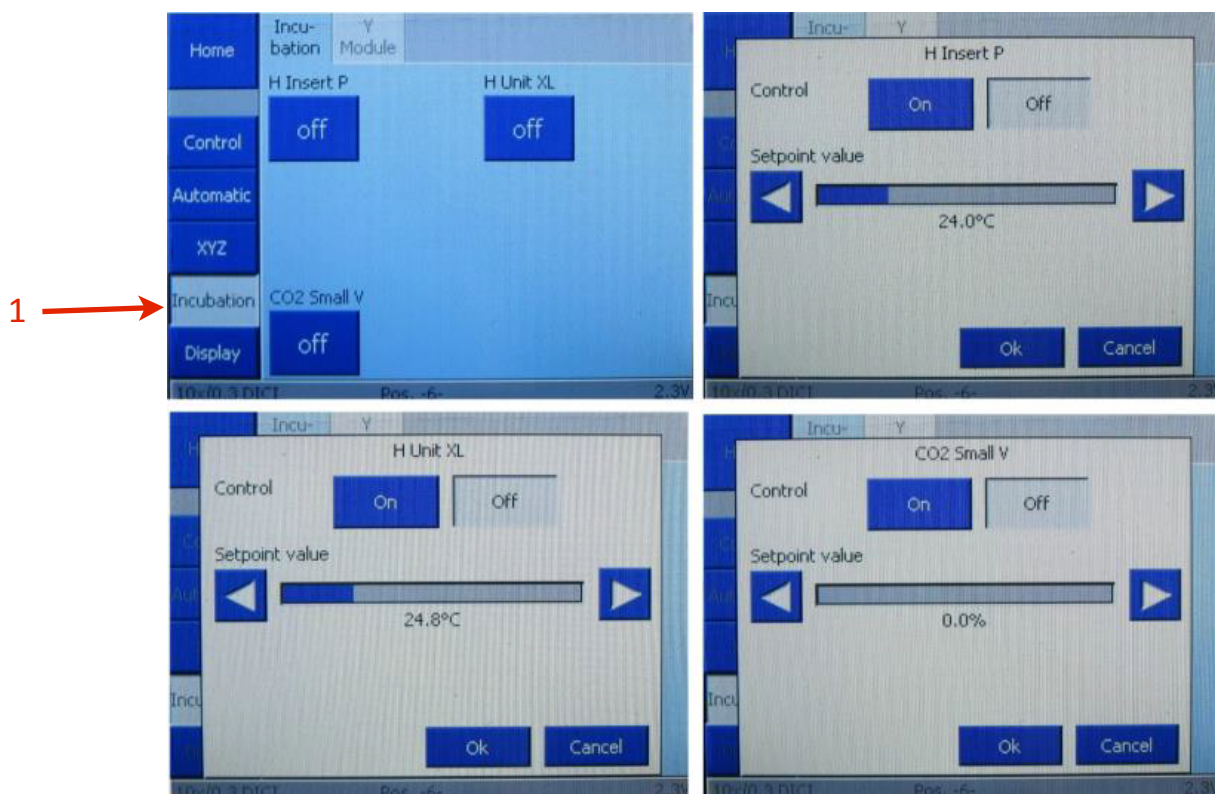
➢ If using software, start PC, login, run software, select Incubator tab and set conditions (Channel 1 is the stage insert, Channel 2 is the CO2, Channel 3 is the chamber). Logout and shutdown as above.

QBI Fluorescent Marker Guide

Filter Sets to be used with
HXP or HBO light source

FS#49 : DAPI
Excitation: 300 - 400
Emission: 420 - 470

FS#47 : CFP
Excitation: 426 - 446
Emission: 460 - 500

FS#38 : GFP
Excitation: 450 - 490
Emission: 500 - 550

FS#44 : FITC
Excitation: 455 - 495
Emission: 505 - 555

FS#46 : YFP
Excitation: 490 - 510
Emission: 520 - 550

FS#43 : Cy3
Excitation: 532 - 557
Emission: 570 - 640

Alexa 350
ex: 346 (320-370)
em: 442 (410-480)

DAPI
ex: 350 (320-390)
em: 470 (420-520)

CFP
ex: 434 (400-470)
em: 477 (460-540)

GFP
ex: 490 (450-505)
em: 510 (495-525)

Alexa 488
ex: 495 (480-515)
em: 519 (500-545)

FITC
ex: 490 (470-510)
em: 525 (500-545)

YFP
ex: 514 (495-525)
em: 527 (515-550)

Alexa 555
ex: 555 (530-565)
em: 565 (555-590)

Cy3
ex: 550 (530-565)
em: 570 (555-585)

mCherry
ex: 587 (535-605)
em: 610 (580-645)

Alexa 594
ex: 590 (565-610)
em: 617 (600-645)

Alexa 647
ex: 650 (630-665)
em: 668 (655-690)

Cy5
ex: 650 (630-665)
em: 670 (650-685)

Filter Sets to be used
with the Colibri LEDs

FS#62 : B/G/HR
for Colibri LEDs

Excitation: 350 - 390
Emission: 400 - 450
(use LED 365)

Excitation: 460 - 488
Emission: 500 - 560
(use LED 470)

Excitation: 570 - 600
Emission: 615+
(use LED 590)

FS#50 : Cy5
Excitation: 625 - 655
Emission: 665 - 715
(use LED 625)

WORKING WITH CONFOCAL AND TIRF 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 – Exposure 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 and laser light – Lasers and a xenon light source 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 lasers, laser light path, or laser modules in any way. Avoid direct exposure to the light.

Scope

This procedure details the method for using the microscopes equipped with laser light sources.

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.

In the event of a laser accident, do the following:

1. **Shut down the laser system.**

2. **Provide for the safety of personnel (first aid, evacuation, etc). If needed, provide further medical assistance for** <u>Eye Injuries</u> **by:**
   Proceed directly to: **Royal Brisbane and Women's Hospital at**
   Cnr Butterfield St and Bowen Bridge Rd
   HERSTON, QUEENSLAND AUSTRALIA 4029
   (07) 3636 8222

   Note: If a laser eye injury is suspected, have the injured person keep still and looking straight up to restrict bleeding in the eye. Laser eye injuries should be evaluated by a physician as soon as possible.

3. **Contact UQ Security Emergency on 336 5333.**

4. **Inform QBI's Laser Safety Officer, Rumelo Amor on 04 4907 8485, of the accident as soon as possible.**

5. **A UQ online incident report must be completed as soon as possible after the incident.**

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