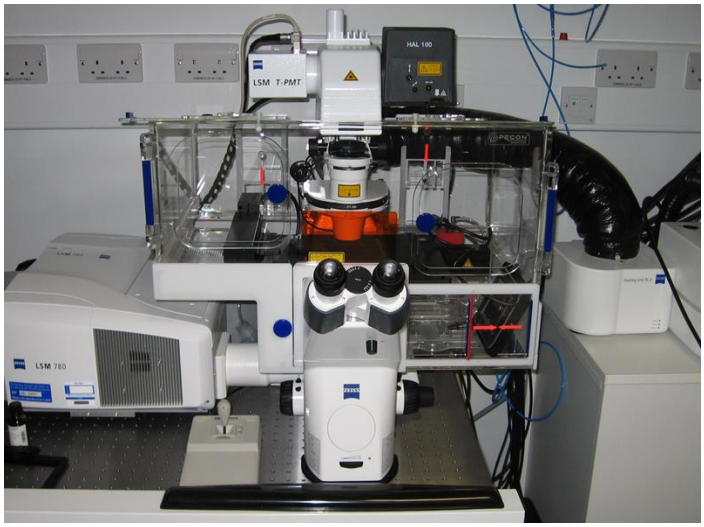
Imperial College London

Facility for Imaging by Light Microscopy

Observing Life As It Happens

QUICKSTART GUIDE:
CONFOCAL HCF1:
Zeiss LSM780 Confocal

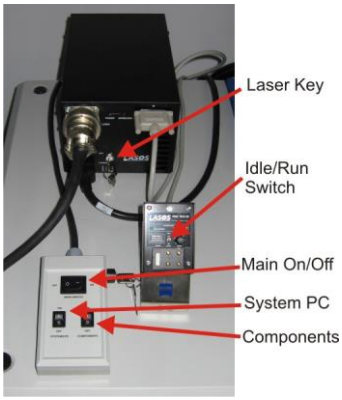
(HAMMERSMITH, L BLOCK, ROOM 314)



Start-up procedure

(If the system has been left on for you check the Argon laser is on and set the control box from "idle" to "Run").

Otherwise turn on in this order: (refer to diagram – right)



1. Turn on “Main” Switch (this may already be on if chamber is pre-heated)
2. Turn on “System PC” switch
3. Turn on the Components switch
4. If using the Argon laser - check that the Lasos laser switch is on and turn key to position 1
5. Set the laser control box from "idle" to "Run"
6. Turn on the PC
7. Login to your account
8. Double-click the **ZEN icon** on the desktop and choose "**Start System**" when the boot status window pops up

Finding your sample

Go to the **Locate tab** and choose one of the pre-sets for viewing:

- **“BF”** (Brightfield),
- **“DIC”**,
- **“Fluor”** (Fluorescence).

To switch light on/off

Either use software (Transmitted Light Off / On – Reflected Light Off / On) or use the TFT Touchscreen control (TL Illumination Off / On - RL Illumination Off / On)

Colibri controls

Screen – displays wavelengths and power

To switch on/off LEDs: press down on respective channel wheel.

These also control the power of each LED.

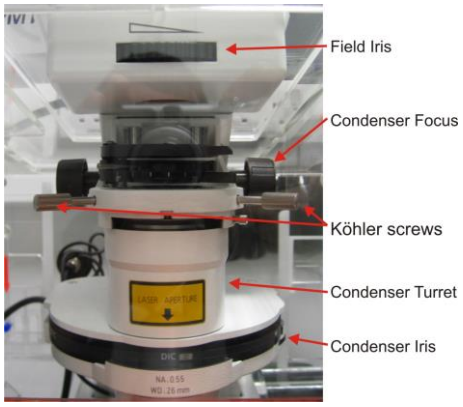
TFT touchscreen control
Can be used to:

- Select objective
- Select reflector cube
- Control heating and CO2
- Additional focussing control
- Other microscope controls

Adjusting brightfield Köhler illumination

NB. always recommended, <u>essential</u> for transmitted light confocal mode

- switch to 10x objective
- adjust brightness with large black wheel on front / bottom of microscope)
- make sure condenser iris is not completely closed (wheel on side of condenser)
- focus on your sample
- completely close field iris (top black wheel) (if the image turns completely black, reopen until you see some light, proceed to next step and close again after you have focussed the iris)
- focus iris (black condenser focus wheel)
- centre iris (2 silver Köhler screws)
- re-open field iris until the edge is just not visible anymore
- readjust field iris every time you switch to a different objective



Confocal Acquisition

Select the Acquisition tab then either:

- Select **Smart Setup**
- Select wavelengths
- Choose appropriate method (usually **Best Signal**) and select apply

or

- load saved configuration

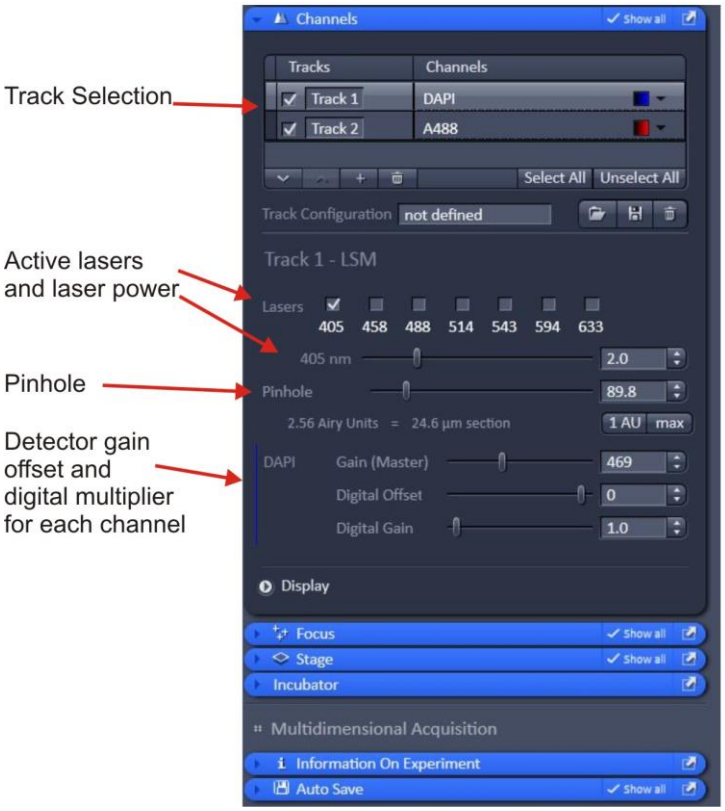
or

- load and existing image and click the "**re-use"** button below image window

or

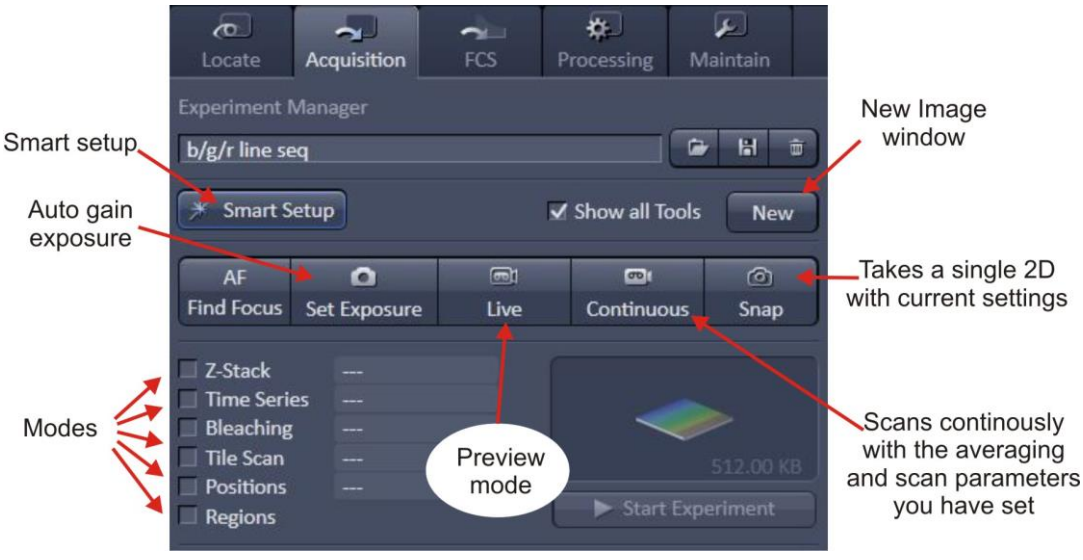
- setup configuration manually

Scanning and optimizing your image.



Start by selecting the first track in the Channels window (uncheck other tracks):

- Set the pinhole - click the 1 AU button
- Run **Live** from the main tabs
- Increase the **Gain** until an image is visible
- **Adjust the z-position** with the coarse/fine focus knob (notice these are on the touch screen pad as well as the microscope) to choose the plane of interest or if you are taking a z-stack generally choose the brightest plane to adjust the settings.
- Adjust the **Gain** and **Digital Offset** and **Laser power** sliders until a balance image is obtained (use the **Range indicator** – tick box below image window
- Click **Stop** and repeat with the other tracks



On the Acquisition Mode window, setup the image format by:

- Select the number of pixels under frame size (pre-set button x*y)
- Select Bit Depth (8, 12 or 16)
- Select Averaging
- Zoom - you can do this with the "Crop" button underneath the image window - resize the box that appears - or using the scan area controls in the Acquisition Mode panel.

To capture the image with all channels, select all tracks and click **Snap** and then **Save** the image.

NB: Snap will overwrite the current image if it is a single plane so make sure you save the previous image. Images are not saved automatically.

Additional information about setup

Laser powers - adjust the sliders (note they are non-linear) so you have reasonable excitation. The lowest value you can get away is best to minimize fluorophore saturation and phototoxicity.

Pinhole - adjust so a suitable optical slice is obtained. A trade-off between z-axis resolution and brightness. A pinhole of one Airy unit gives essentially gives you the best resolution, opening it slightly from there will allow you to collect more signal. (with each new setup/channel pinhole needs to be set)

Gain/offset optimization - adjust the gain (detector sensitivity) and offset (background level) for each channel. Selecting range indicator is helpful when doing this. In this mode saturated pixels are highlighted red and 0 intensity blue, a few red pixels and the background 50% blue is often a good point to aim for.

Averaging and scan speed - Averaging and slower scan rates improve signal to noise ratio (SNR) , choose a good balance for quality and speed.

Sampling rate - The number of pixels in the image can be selected. The optimal button selects the correct number for a particular objective, wavelength and zoom. This will give you a digital sampling rate adequate to fully sample the optical resolution.

The acquisition Mode panel also allows you to adjust between **unidirectional and bidirectional scanning** (faster but sometimes causes image degradation from interlacing), and the **bit depth** of the images (8-Bit is fine for standard imaging; 12-Bit and 16-Bit are better for quantification).

Saving/ Exporting data

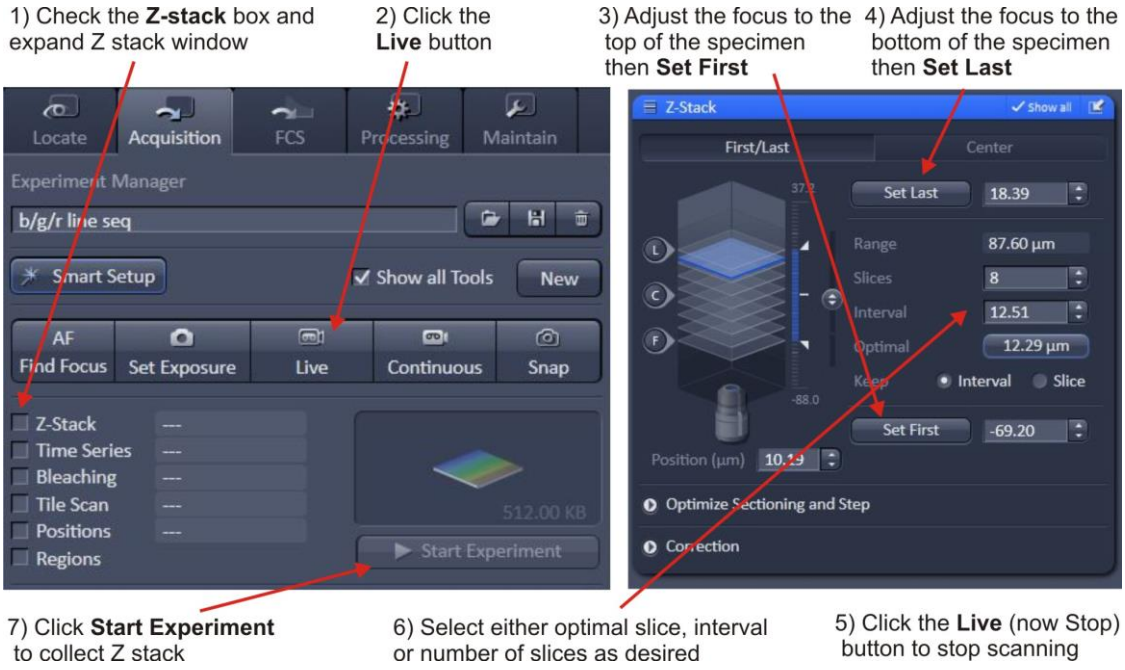
Note: Snap will overwrite the current image if it is a single plane so make sure you select new image before snap or save the previous image. Images are not saved automatically.

Select image from view in right-hand window, select save icon, save to disk. Open images that are not yet saved have a warning icon next to them. Images are saved as ZEN format (.czi) files or can be exported as TIFF. Generally it is best to save to the local D:/User Data drive and after your session move the data to a server, USB drive etc after you have closed the software.

Z-stacks, Time Series and Advanced techniques

- These techniques are found on the main tab window
- Ticking the check box of the required technique gives access to the relevant window for the process.
- Select the requires settings in the window and then use the “Start Experiment” button to record the image
- Note “Start Experiment” always creates a new image – it never overwrites an image

Z-stack



Time series

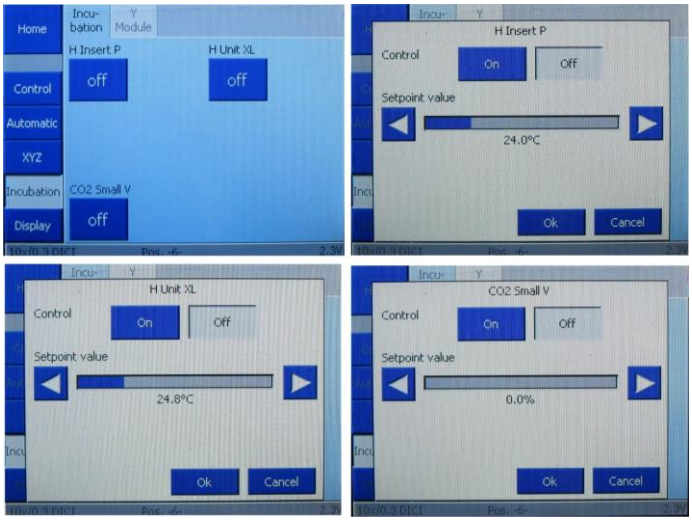
1. Select the **Time Series** box underneath the Z-series box of above
2. Choose the number of time points and the interval between them
3. Press start experiment

Heating & CO_2 units

The heating & CO_2 units can be controlled either by the TFT touchpad or through the software.

If it is required to pre-heat the chamber prior to a booking then:

- Turn on “Main” Switch
- Turn on “System PC” switch
- Turn on the Components switch
- On the TFT Touchpad
    1. Select “Incubation”
    2. Select unit to control



(H insert P – small environmental stage insert, H Unit XL – Chamber, CO_2 Small V – lid for insert.)

    3. Set required environmental conditions
    4. Set to “on”
- Turn off the Components switch
- Turn off “System PC” switch
- **Leave “Main” Switch ON**

If using software, start pc, login, run software, select Incubator tab and set conditions (channel 1 is the stage insert, Channel 3 is the chamber). Logout and shutdown as above

Ending your session

- Remove specimen **- if oil was used, clean objective with fresh lens tissue**
- check if anyone is booked after you within 2 hours

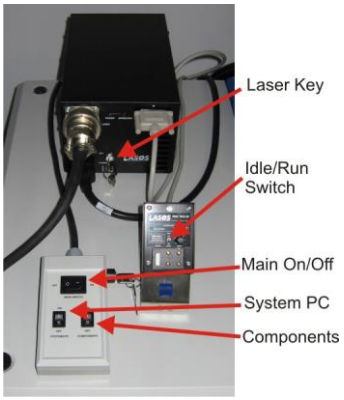
If someone's booked after you within two hours (check PPMS):

1. remove your samples
2. clean objective lenses with fresh lens tissue and close incubation chamber
3. clear up the desk
4. save files onto the server or on a mobile hard drive
5. **JUST** log off

DO NOT TURN LASERS OFF

If you are the last user or the gap is greater than 2 hours – full shutdown:

1. Flick the small switch on Lasos controller board to idle.
2. Turn the key on the Lasos Laser power supply (large black box) to position 0 – Laser fan will automatically shut off after about 3 minutes.
3. In the meantime, exit Zen2011 software and Copy files to the server or to other storage device
4. Shut down the PC from the Windows Start Menu – wait 30 seconds for windows to fully shutdown
5. Turn off the Components switch
6. Turn off "System PC" switch
7. **If laser fan has stopped** then turn off "Main" Switch (leave on if chamber is to be kept heated) IMPORTANT - do not turn off this switch if laser fan is still running



DO NOT TURN OFF THE LASER POWER SUPPLY (Button to the left of the laser key)

Offline image viewer for Zeiss LSM files

ZEN Lite - Freely available, see link on FILM homepage

FIJI (Fiji is just ImageJ) – Free, opens original files