Imperial College London

Facility for Imaging by Light Microscopy

Observing Life As It Happens

QUICKSTART GUIDE:

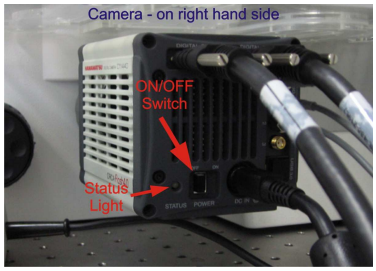
WIDEFIELD WF3

Zeiss Cell Observer

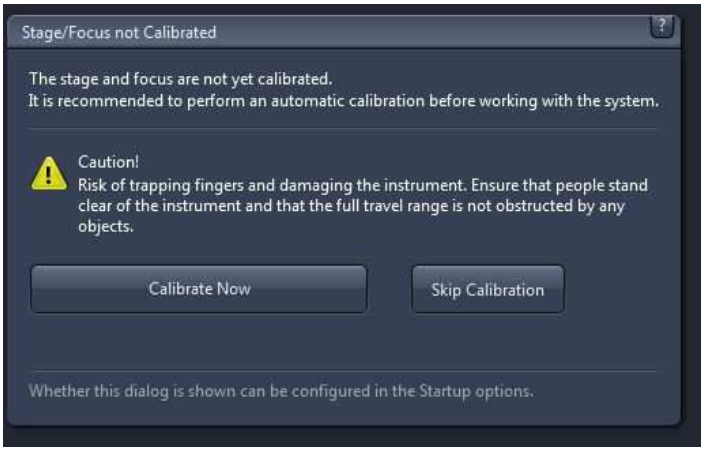
Live Cell Imaging System

(SAF, ROOM 409)

Startup procedure



- All switches on wall to on
- Check camera is on (left hand side)
- Start PC and login
- Run Zen blue and click on “Zen Pro”
- If you are the first user after switching on – the stage will want to calibrate. Select the 5x objective on the TFT controller and calibrate

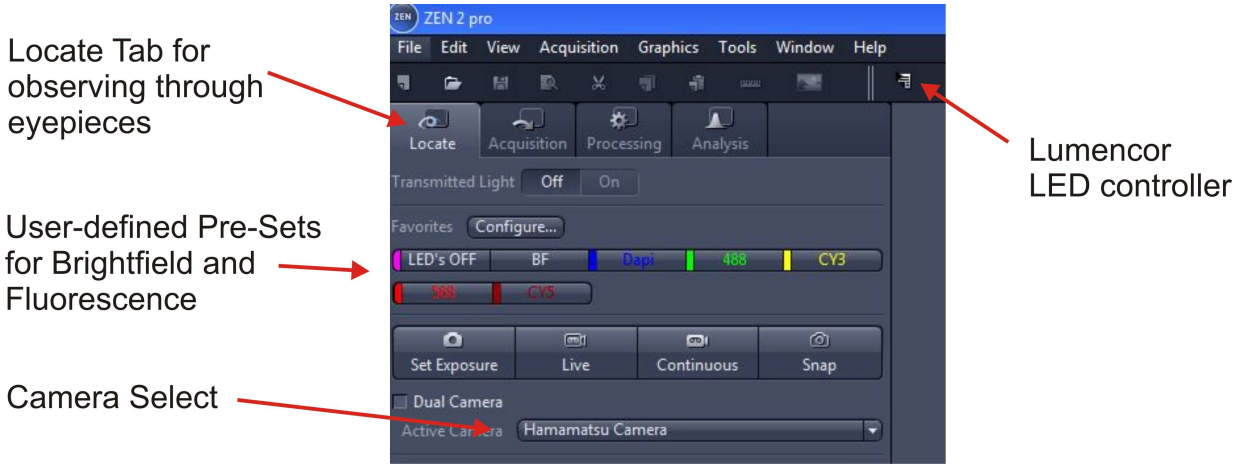


TFT touchscreen control
Can be used to:

- Select objective
- Switch on/off Transmitted light
- Switch on/off Reflected light
- Select reflector cube
- Control heating and CO2
- Additional focussing control
- Other microscope controls

Viewing your sample

- In the software select **locate** tab
- Select **BF** (brightfield)
- Turn on Transmitted light either in software or using the TFT touchscreen



Adjusting brightfield Köhler illumination

- Select 10x objective either with the TFT touch screen or software
- you can move your specimen in xy-direction with the joystick, holding the button on top of the joystick makes the stage moving faster
- focus on your sample
- adjust brightness with large black wheel on front / bottom of microscope)
- make sure condenser iris is not completely closed (two buttons on left top of condenser turret)
- 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)
- reopen field iris until the edge is just not visible anymore

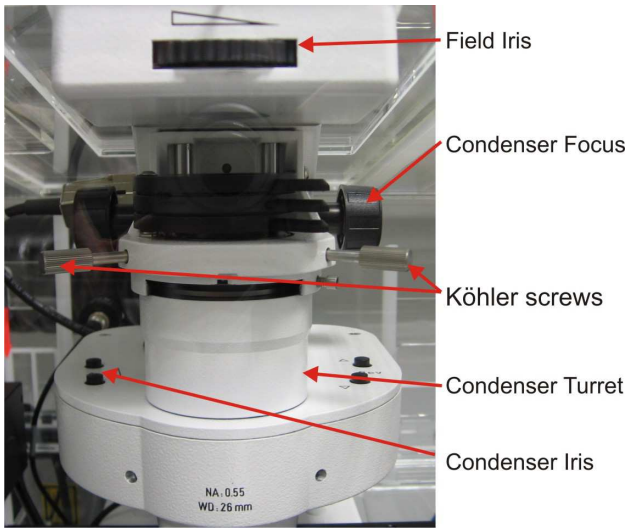


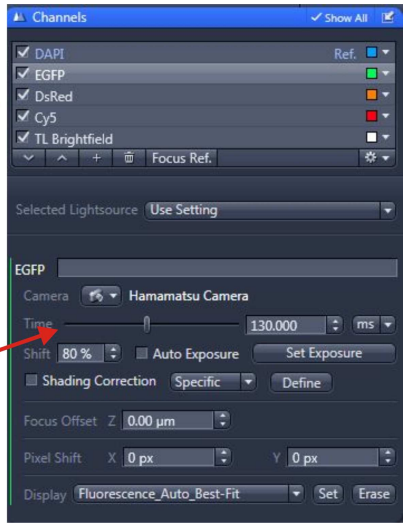
Image Acquisition

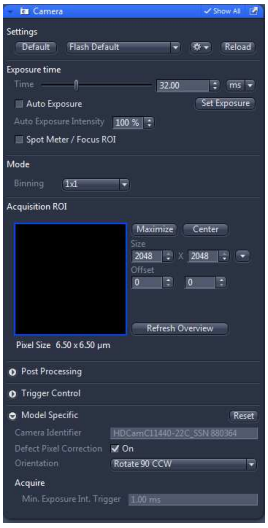
- Select the Acquisition tab (make sure “show all tools” checkbox is ticked)
- Either open and image and “Re-Use” or load one of the saved experiment setups (just below the Acquisition tab – Experiment Manager)
  - WF3 Basic 5 channel (Dapi, GFP,TRITC/Texas Red, CY5, Brightfield)
  - WF3 Quad (Fast acquisition - Brightfield, Dapi, GFP,TRITC/Texas Red, CY5)
  - WF3 Colour Camera
  - WF3 Flash BF (Flash camera brightfield only – live cell imaging)
  - WF3 CFP/FRET/YFP (request filter set)

- To adjust Channels use the “Channels” tab
- Click “Live”
- Select each Channel in turn – adjusting LED power (using the Lumencor button) and exposure time (range indicator button is below image window)
- Additional camera setting are in Camera tab
- To capture image click Snap

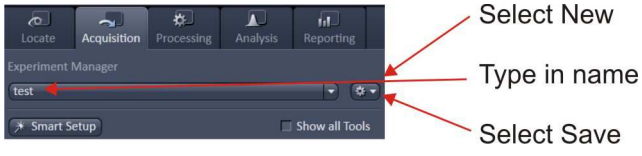
Select Channel

Change Camera exposure time



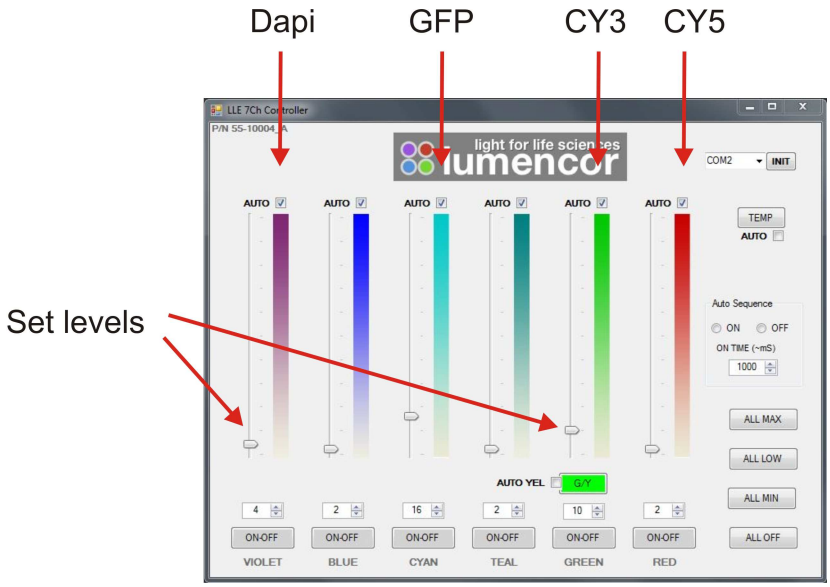


- Settings can be saved for future use



Lumencor LED's

Click the button above the image window and this will open the LED intensity controller



These settings are not stored with the image so need to be remembered (use the windows snipping tool to capture and save the settings

Saving

- Images are stored in your temporary folder until you "save" them
- To save the image – select it and press the save icon.
- Select location and name

Additional Methods

Select check box for Z stack, Tile and Time Series. This will activate the respective tabs
To capture images using these methods use the Start Experiment button

Software Autofocus

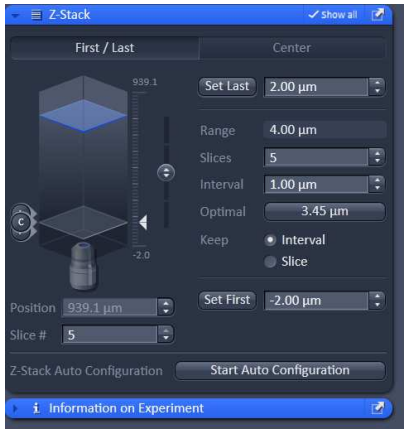
Select Focus Strategy tab
Options will depend on what other functions are selected eg Z stack and Tile

- Absolute fixed Z position
- Software autofocus
- Definite focus

- Software autofocus as a reference for Definite focus
- Definite focus as a reference for Software autofocus
- Local focus
- Local focus for software autofocus

Z stack

- Set First and Last positions
- Set interval
- Start experiment

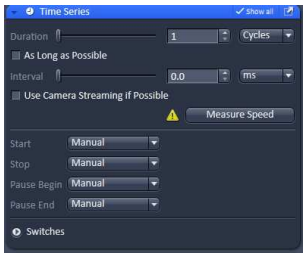


Tile and Positions

- Setup tile or positions
- Start experiment
- (ask for more instruction)

Time series

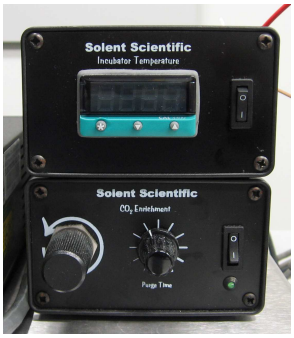
- Setup duration and cycles
- Start experiment



For very fast acquisition single channel

- Load WF3 Flash BF protocol
  - Select fluorochrome
  - Select “Speed”
- Set the camera settings as required
- Go “Live” and set exposure and focus
- Setup duration under “Time Series” and tick “use Camera streaming if possible”
- Run “Start experiment”

Heating & CO_2 units



- Found to the right of the microscope
- Switch on heatingat least 1 hour before required
- For CO_2 open cylinder in room 408
- Unclip hoseclips and adjust flow

Shutdown procedure

- check if anyone is booked after you within 2 hours

If nobody is booked within two hours:

- remove specimen
- clean oil objective lenses with lens tissue
- Important: close incubation chamber completely (→ protects from dust)
- update time in Sharepoint
- secure your data (e.g. copy them to the server)
- shut down hardware in reverse order from startup

If someone's booked within two hours:

- update usage in Sharepoint
- remove your samples
- clean objective lenses with fresh lens tissue and close incubation chamber
- clear up the desk
- secure your data (e.g. copy them to the server)
- log off