Imperial College London

Facility for Imaging by Light Microscopy

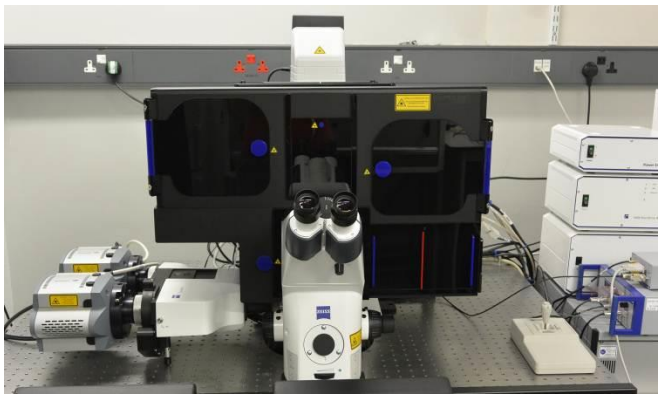
Observing Life As It Happens

QuickStart Guide: super-resolution SR1:

TIRF / PALM / STORM

Zeiss Elyra PS.1

(South Kensington, room 530)



Start-up procedure

NB: The cameras and the system (pc and software) need to be switched and started at least 30 mins before use

Turn on in this order:

1) Turn on 2 switches on left hand wall, the third switch LED is always on
2) Turn on the PC
3) Login to your account
4) **Important:** run macro (desktop shortcut) to select single-camera (2) or dual-camera mode (1), will be remembered from previous user

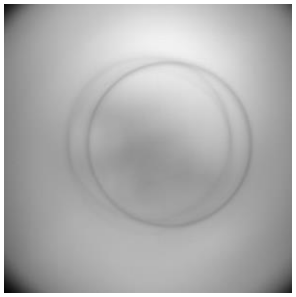
start batch file | select database | start ZEN

5) Start **ZEN black** on the desktop and run "**Start System**" when the boot status window pops up
6) Under Acquisition tab select “Laser” window and switch on required lasers

Aligning the sample holder

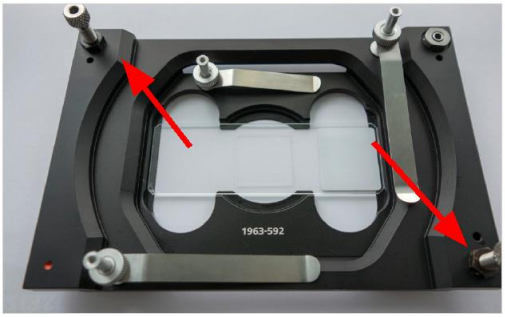
Universal holder, not mini – incubator, this is important for SIM imaging

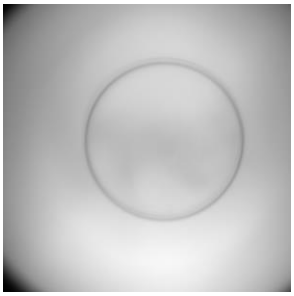
1) Without sample on stage, select 5x objective on TFT display (home/ microscope/ objectives) or in the Zen software
2) Remove sample holder and then the black cover from objective turret, there should be a glass slide instead of the objective in this position.
3) Put an empty glass slide on the sample holder and put the holder back in the stage
4) Open ‘alignment.czi’ file (on desktop or in ‘D:\users\’) and apply settings and go live in Zen



5) Switch on green LED with very, very low intensity (setting 2 on LED panel), select min/max in display settings.
6) You should see an image with two circles like in the image on the right

7) Align the two circles using the micrometer screws on the stage insert (red arrows on the right) until they overlap in the centre like in the picture below.





8) Remove the sample holder carefully without changing the alignment screws, take off the glass slide, put the black cover back and switch to you favourite objective and put the sample holder with sample back (red dot in lower left corner)

Finding your sample

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

- **"BF"** (Brightfield),
- **"Multi"** (Fluorescence).
- **"Blue"** (for Dapi wavelengths)

To switch light on/off

Brightfield:

- Transmitted Light Off / On buttons in ZEN
- Lamp brightness – use control on front of microscope

Fluorescence:

- Reflected Light Off / On buttons in ZEN
- Wavelength selection and brightness – use LED control box

TFT touchscreen control
Can be used to:

- Select objective
- Additional focussing control
- Operate definite focus
- Other microscope controls

Using the LED light source

(for viewing fluorescence down the eyepieces)

Zen **Locate** tab

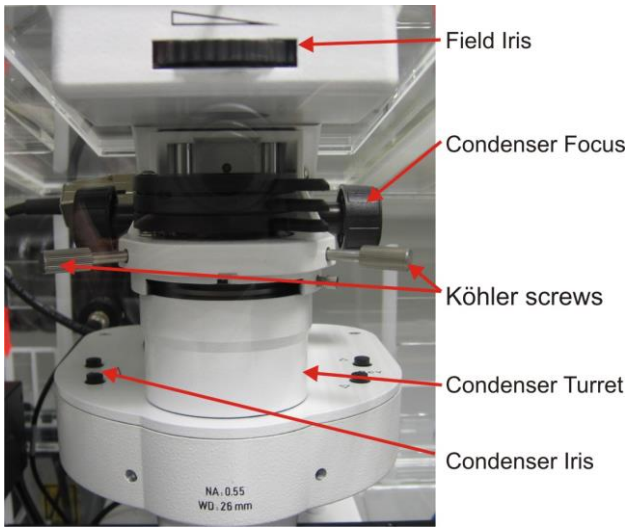
- Select **Multi** or **Blue**
- Select all or individual channels using the select buttons
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

!! Please remember to switch off after viewing and at the end of your session !!

Adjusting brightfield Köhler illumination

always recommended, essential for transmitted light confocal mode

- 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



TIRF – Total Internal Reflectance Mode

Setting the Acquisition Parameters

1) To create a track - select the mode, camera, laser, reflector cube and illumination mode
2) Give the track a name and select a look up table colour; and save the track configuration.
3) Repeat procedure for other tracks

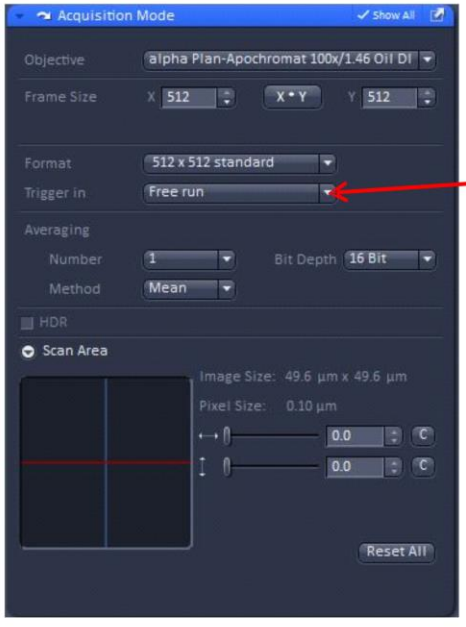
1) Select **Laser WF** mode

2) Select **Camera**

3) Select the **laser line** and power

4) Select additional magnification lens
1.6x → 60nm pixel size (default)
2.5x → 40 nm pixel size

5) Select the **reflector cube**
See annex for filters

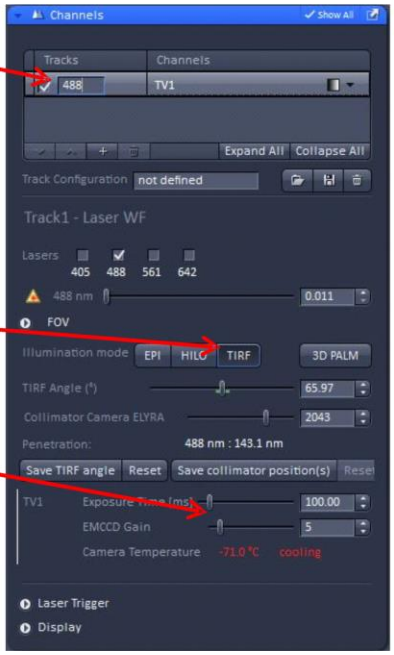


6) Rename Track

7) Set Trigger to **Free Run**

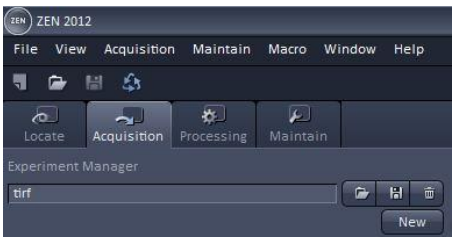
8) Set Illumination mode to TIRF

9) Initial Settings
- Exposure 100ms
- Gain 5



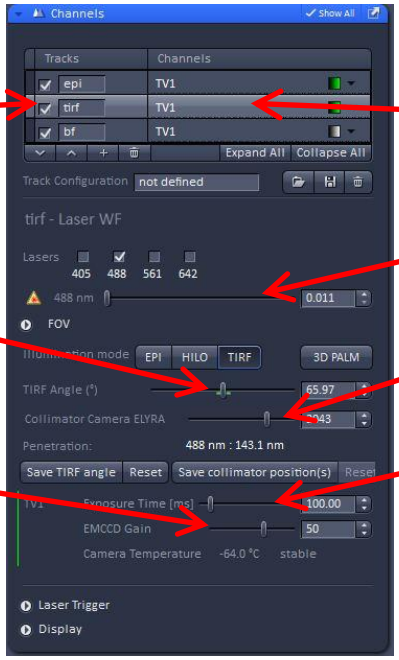
These tracks for Brightfield BF, Epifluorescence EPI and Total Internal Reflectance microscopy TIRF can be saved as a configuration setting (just below the "**Acquisition"** tab – top left in ZEN).

These can be re-loaded to restore configurations



Capturing Images TIRF

Channel window



Tick means Channel is active

Highlight Channel to display controls below for that channel

Laser Power

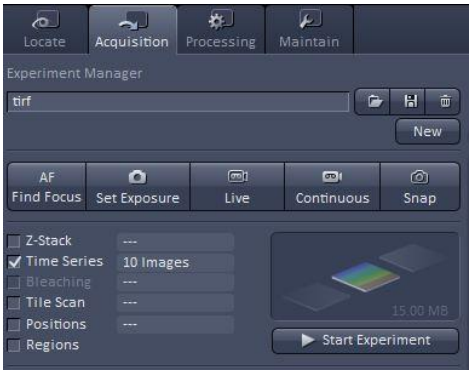
TIRF Angle

Collimator – controls flatness of field

Exposure time

Gain

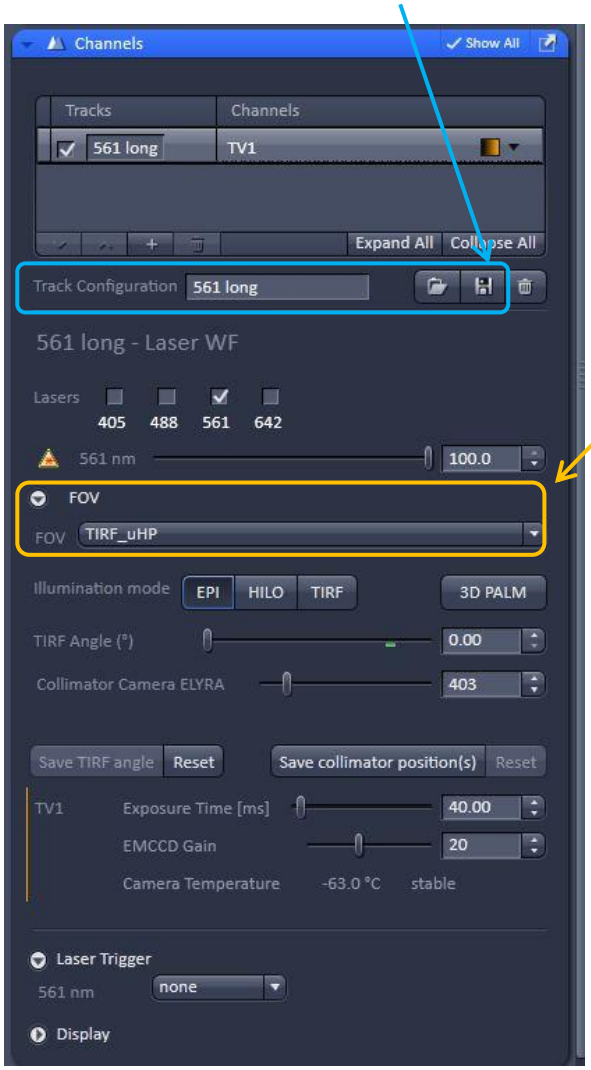
- Select Track
- Use "**Live**" or "**continuous**" in acquisition mode to optimally set exposure time, gain, laser power, TIRF angle etc. Use the range indicator ("dimensions" tab under the image) to ensure the signal is not saturated
- Use **"Snap"** (single image) or **"Start Experiment"** (time series) to capture image



Single Molecule (PALM and STORM mode)

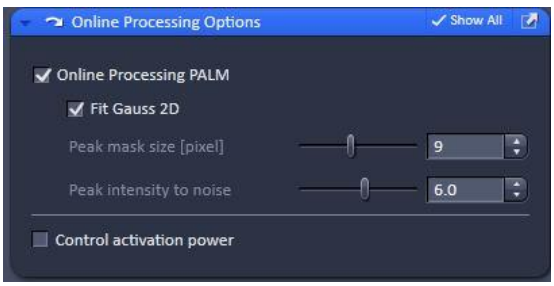
Setting the Light Path

- Select “Laser WF” mode
- Select EMCCD or CMOS camera and appropriate magnification.
  Note: for PALM/STORM experiments, pixel sizes of ~ 100-150nm are recommended.
- Select appropriate laser line emission filters (see “PALM/STORM Filters” on the wall)
- Save individual tracks



Setting the Acquisition Parameters / Channels

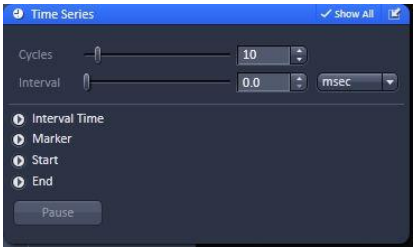
- Set laser power and additional FOV lens (TIRF, HP or uHP) depending on power needed to induce blinking/switching.
  Note: TIRF-uHP mode gives ~ 1.5 kW/cm2 at sample for 642nm on 100x.
- Select illumination mode (EPI / HILO / TIRF): HILO or TIRF are recommended for 3D PALM/STORM due to increased signal-to-noise ratio.
- Press “continuous” to adjust laser power / exposure time / gain etc prior to acquisition
- Activate “**Online Processing**” to get localizations “on the fly” of molecules (see below).
  Note: only works for 2D acquisitions



Setting the Multi-dimensional Acquisition Parameters

Time series

1. Select **Time Series**
2. Choose the number of time points (cycles) and the interval between them (0 for continuous)
3. Run **Start Experiment**



Multi-color acquisition

- Choose the appropriate filters for two-channels
- Select “Frame” or “frame fast” mode if the filters are on different cubes
- If using the same filter cube, sequential mode can be used.

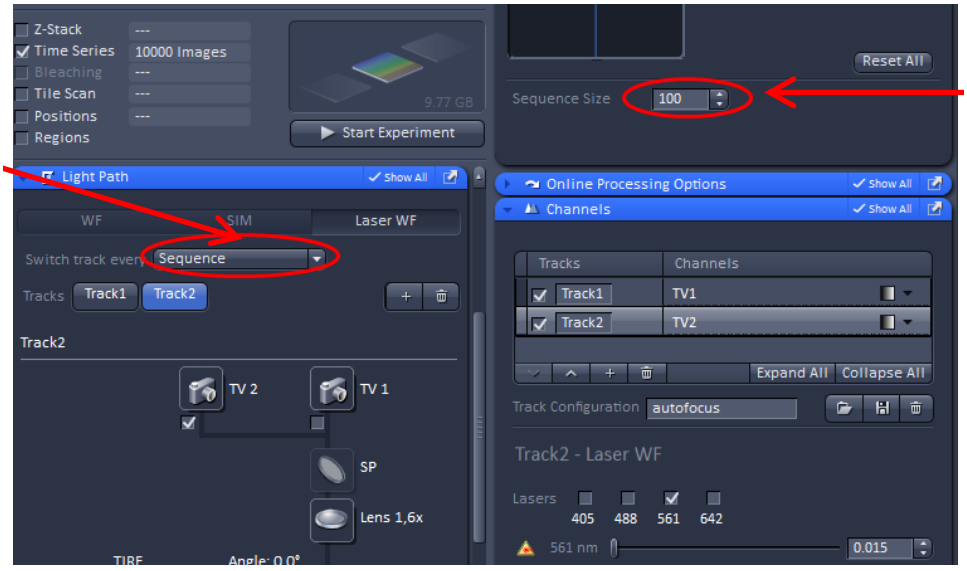
Sequential mode

Two-channel tracks can also be acquired in “sequence” where the lasers are switched on/off after a defined number of cycles or frames.

Note: also works in dual-camera mode

*Note: ensure the two channels are correctly set up first, with the **same multi-band filter***

Switch track every **Sequence**



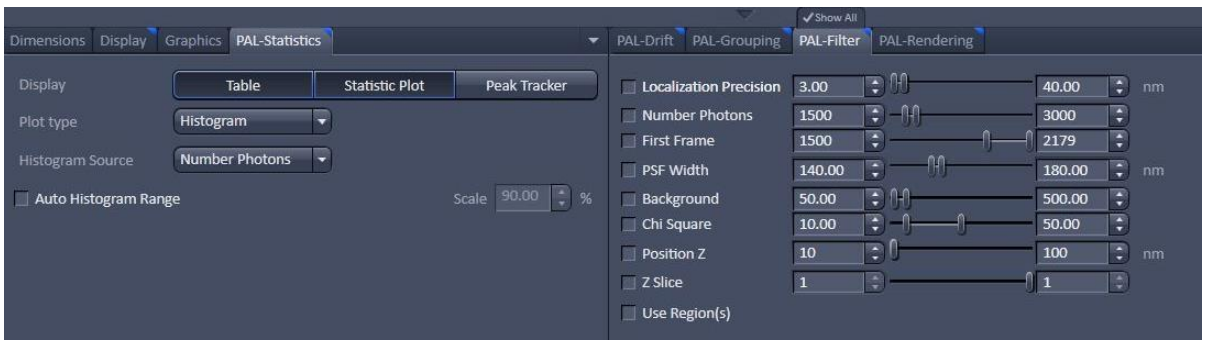
Acquisition panel:

Specify **size of sequence** ie # frames

Online Processing Tools

Note: the results of a PALM/STORM acquisition is a table of events (~ molecules) not an image.

7) Use **“PAL-Statistics**” to get histograms or plots of different features (e.g Number of photons, molecules per frame, etc).
8) Use “**PAL-Drift**” and “**PAL-Grouping**” to resp. correct image for drift and group molecules
9) Use “**PAL-Filter**” to sieve which molecules to use for the PALM image, e.g filter by localization precision, number of photons etc.
10) Use “**PAL-Render**” to convert the filtered events/molecules into an image.

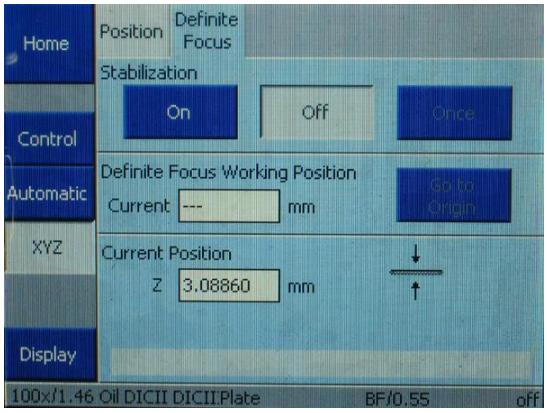


Saving/ Exporting data

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.

Definite Focus (Setting the Focus Strategy)



Use "**Definitive Focus**" (DF) to prevent focus drift and out-of-focus artefacts over time series

Note that DF only works for aqueous mountants – and is especially useful for live TIRF.

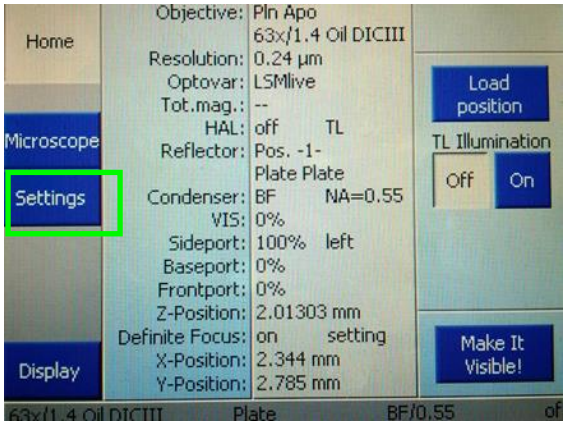
Operate definite focus from TFT touchscreen.

Select **XYZ** and **Definite focus**

Select best focus in **"Live"** mode and press **"On"**

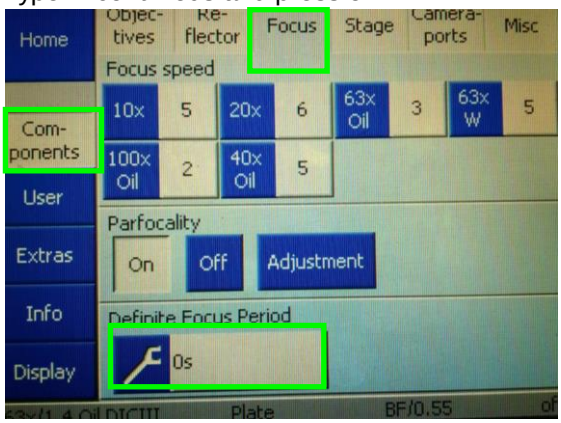
Changing the definite focus period:

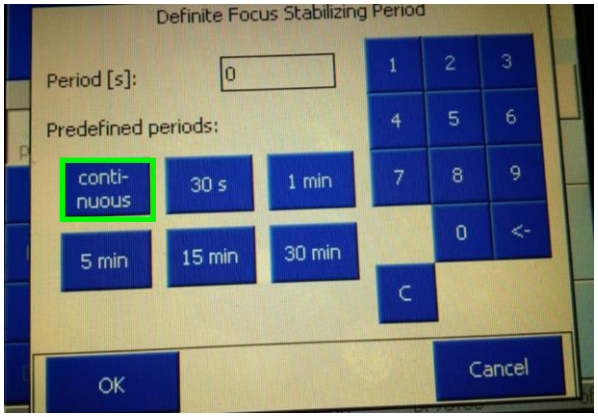
Under settings:



Select components, focus, definite focus period

Type in continious and press ok





Note: It is also possible to set the focus once every time point during a time series of z-stacks, to do this the definite focus needs to be switched off on the TFT display and selected in Zen under focus devices and strategies, this might affect the timing.

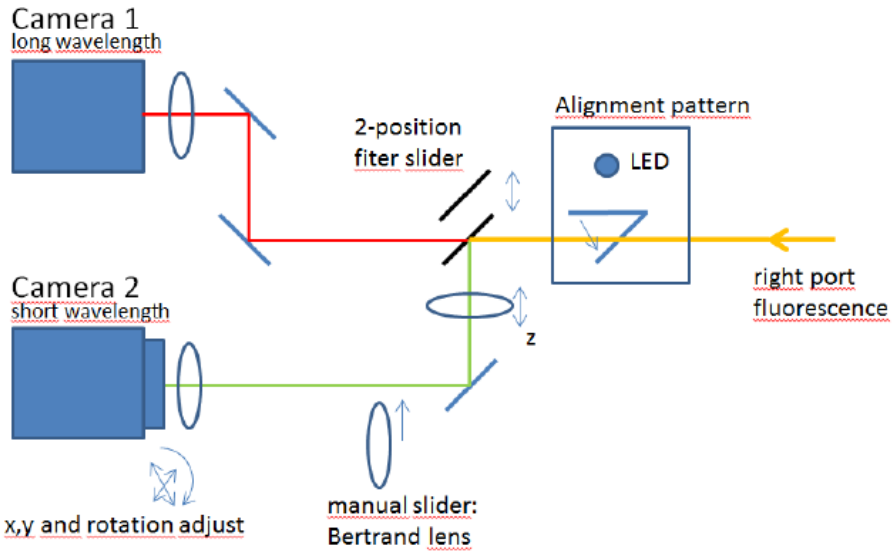
Heating & CO_2 unit

1. Switch on main button
2. If necessary adjust temperature
3. Switch on Gas power and adjust CO2 if required

Dual-camera mode

Optical path

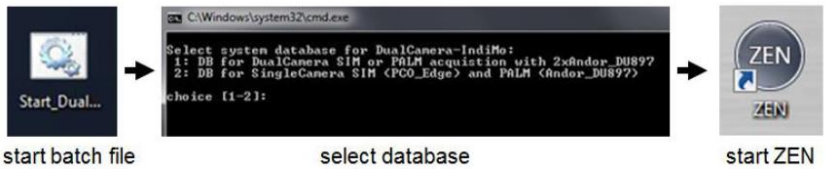
Make sure to select the appropriate excitation and emission filters (see annex)

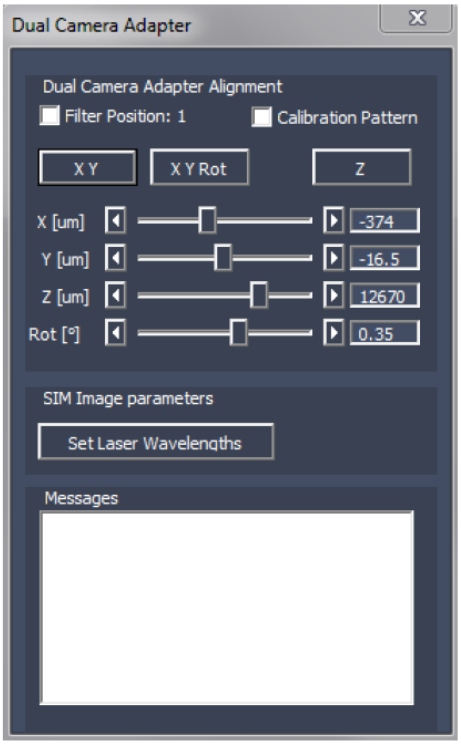


VIP: PRESS Set Laser Wavelength in DualCam Macro mode before saving

Setting up dual-camera mode

1) Start Macro “Dual-Camera” from desktop shortcut and select “1” for dual-EMCCD mode and start ZEN program





2) The Dual Camera (DC) adapter is controlled via a Macro. It can be found under the Tab “Macro” as **DuoLink SR** and is stored in “C:\ZEN\Macros\DualCameraIndimo.lvb”

3) Always perform channel alignements for each filter combination before acquisition

Ending your session

- Remove specimen **- if oil was used, clean objective with fresh lens tissue**
- check if anyone is booked after you within 2 h ours
- update the time in Sharepoint

If nobody is scheduled to use the confocal after you or within 2 hours, please power down the system

To power down:

1. Turn off lasers
2. exit Zen2011 software and Copy files to the server or to other storage device
3. Turn off the PC from the Windows Start Menu
4. Turn off LED
5. Turn off wall switches

If someone's booked within two hours:

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