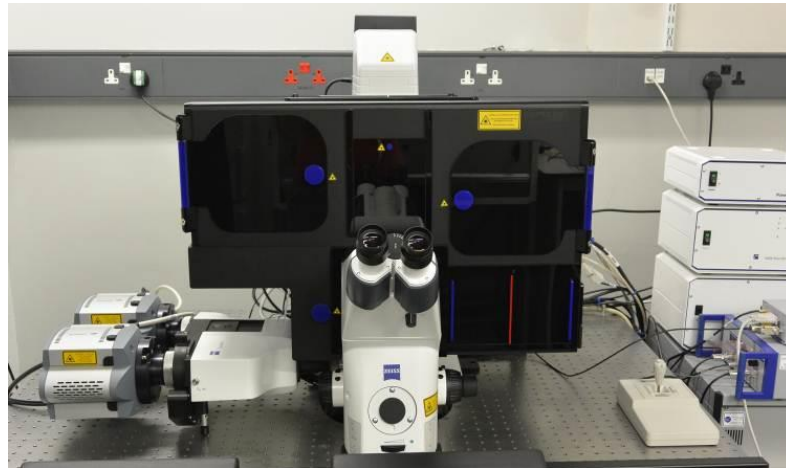


Observing Life As It Happens

Structured Illumination

Zeiss Elyra PS.1
(South Kensington, room 530)



SIM (Structured Illumination Microscopy mode)	2
Start-up procedure	2
Aligning the sample holder	2
Universal holder, not mini – incubator, this is important for SIM imaging	2
Finding your sample	3
Using the LED light source	4
Adjusting brightfield Köhler illumination	4
Setting the Acquisition Parameters	5
Capturing Images	6
Z-stack	7
Time series	7
Saving/ Exporting data	7
Definite Focus (Setting the Focus Strategy)	7
Heating & CO ₂ unit	8
Ending your session	9
Dual-camera mode	9
Optical path	9
Setting up dual-camera mode	10

SIM (Structured Illumination Microscopy mode)

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



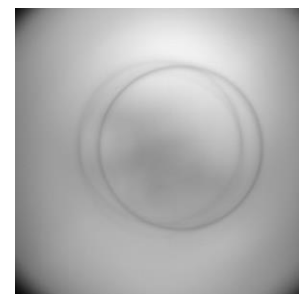
- 5) Start **ZEN black** on the desktop and run "**Start System**" when the boot status window pops up
- 6) Under Acquisition tab select "Laser" window and switch on required lasers



Aligning the sample holder

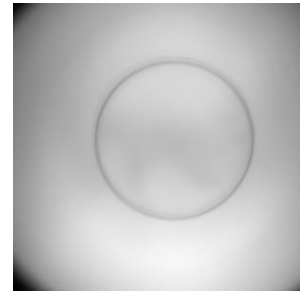
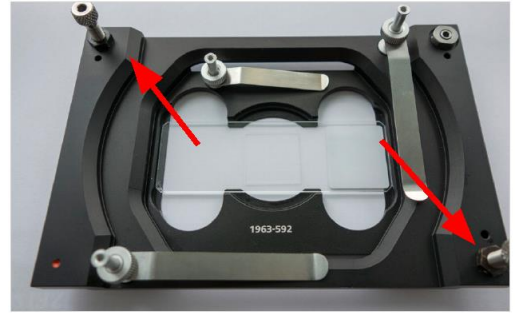
Universal holder, not mini – incubator, this is important for SIM imaging

- 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),
- “Fluo Green-Red” (for green/red wavelengths).
- “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 Locade tab

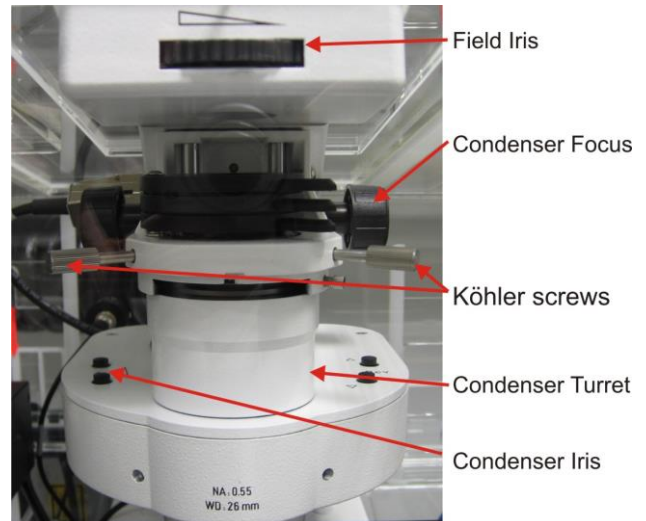
- Select **Multi** or **Blue**
- Select all or individual channels using the select buttons
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

!! 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



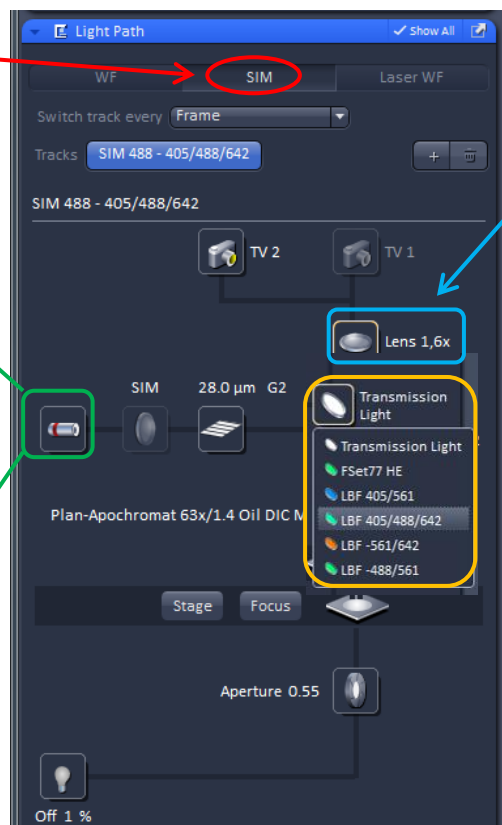
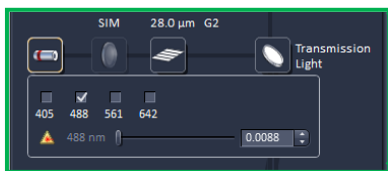
Setting the Acquisition Parameters

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

Setting the Light Path

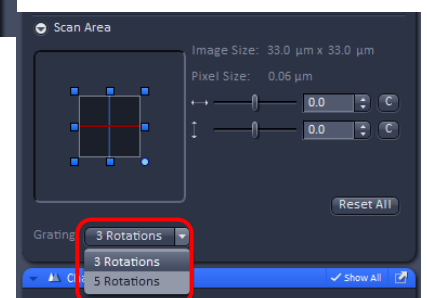
- 1) select the **SIM mode**;
the TV2 (sCMOS) will be selected by default.

- 3) Select the **laser line** and power
(the relevant grid will come into place depending on laser and objective)



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

- 4) Select the **reflector cube**
See annex for filters



Setting the Acquisition Parameters

- 5) Select the frame size (maximum 1280 x1280 pixels)
- 6) Select the number of rotations (3 or 5)
Note: 3 rotations are theoretically sufficient to reconstruct a SIM image; 5 rotations provide additional information and can give better reconstructions.
- 7) Give the track a name and select a look up table color; Save the track configuration.
- 8) Repeat procedure for **multi-channel acquisition** (as above), and select "Frame" or "Frame Fast" mode for acquisition.
Note: for multi-channel SIM, the grid will automatically be selected according to the longest
- 9) In **FOV**, set an exposure time, using the "Live" or "continuous" acquisition mode to adjust it. Use the range indicator ("dimensions" tab under the image) to ensure the signal is not saturated
- 10) Ensure the gridded pattern is clearly seen on the structure of interest – adjust the focus if necessary.

Note: In SIM mode, the grids are constantly rotating, making it difficult to assess the correct focus. It will be easier to focus on your sample using the Widefield mode, and switch back to SIM mode to set exposure time and start the acquisition.

- 11) For **Z-stacks**, activate tick box and set top-bottom – use optimal z-steps depending on magnification (100nm or 125nm)
- 12) “Start Experiment” to acquire SIM images

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

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

Gain

Exposure time

- 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

Z-stack

1) Check the **Z-stack** box and expand Z stack window

2) Click the **Live** button

3) Adjust the focus to the top of the specimen then **Set First**

4) Adjust the focus to the bottom of the specimen then **Set Last**

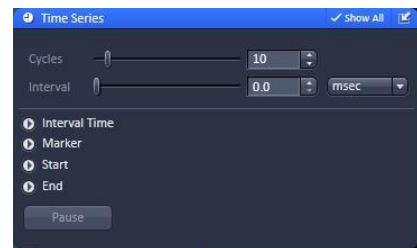
5) Click the **Live** (now Stop) button to stop scanning

6) Select either optimal slice, interval or number of slices as desired

7) Click **Start Experiment** to collect Z stack

Time series

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



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 (.dzi) 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)

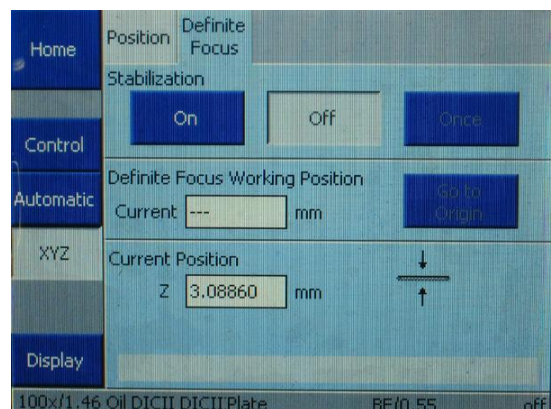
Use “**Definitive Focus**” (DF) to prevent focus drift and out-of-focus artefacts over time series

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

Operate definite focus from TFT touchscreen.

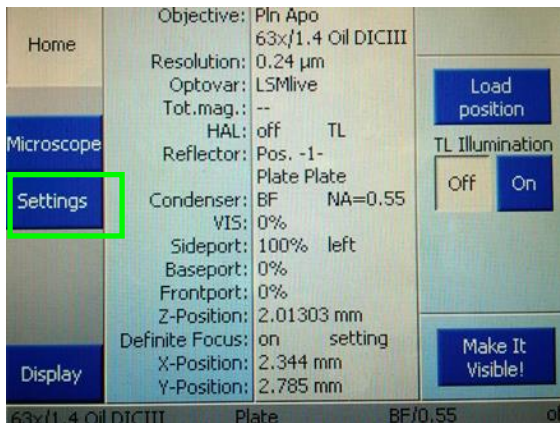
Select **XYZ** and **Definite focus**

Select best focus in “**Live**” mode and press “**On**”



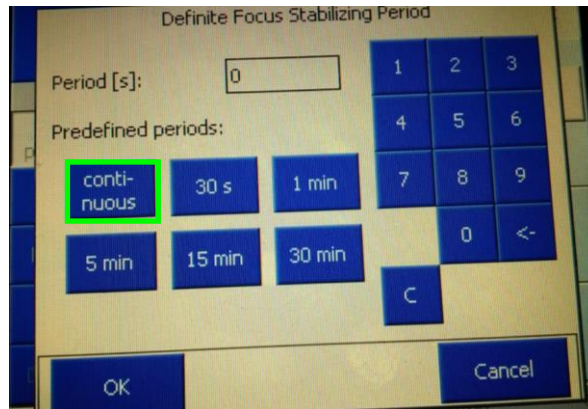
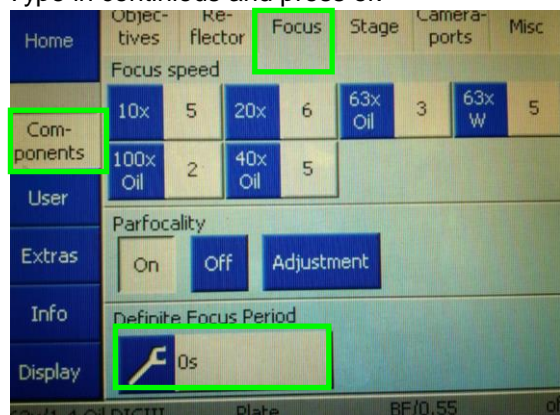
Changing the definite focus period:

Under settings:



Select components, focus, definite focus period

Type in continuous 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 & CO₂ unit

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



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
- 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:

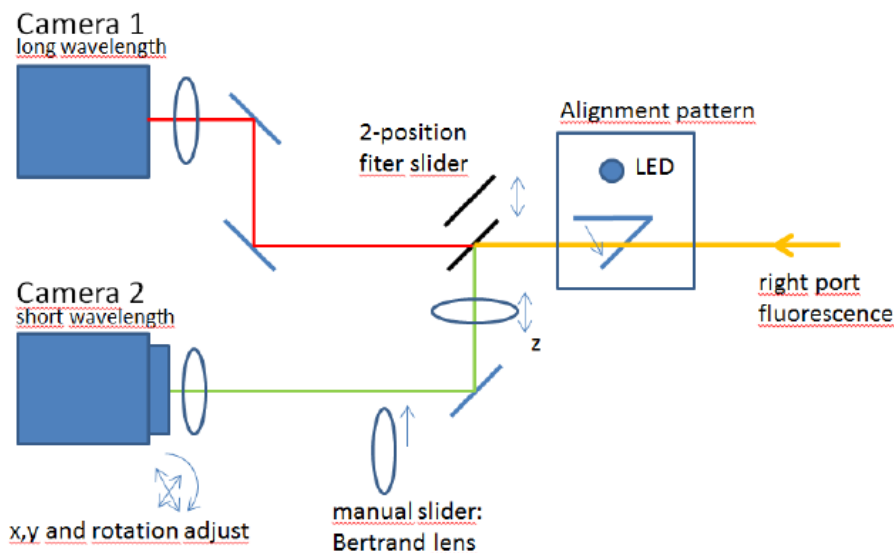
1. update usage in Sharepoint
2. remove your samples
3. clean objective lenses with fresh lens tissue and close incubation chamber
4. clear up the desk
5. save files onto the server or on a mobile hard drive
6. log off

Note: if used on subsequent days, the system can be left ON over the week so it is kept at temperature – only close the ZEN software. Ask FILM staff if unsure.

Dual-camera mode

Optical path

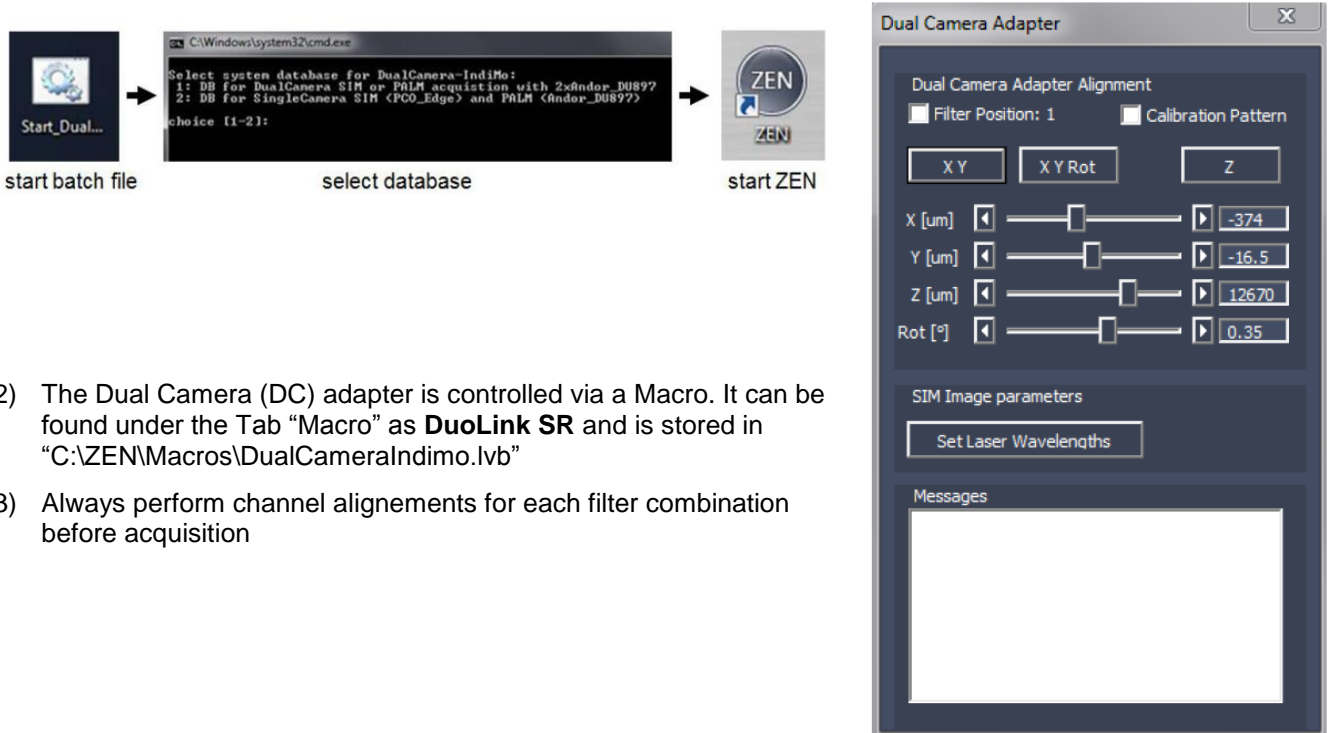
Make sure to select the appropriate excitation and emission filters (see annex)



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 alignments for each filter combination before acquisition