

Observing Life As It Happens

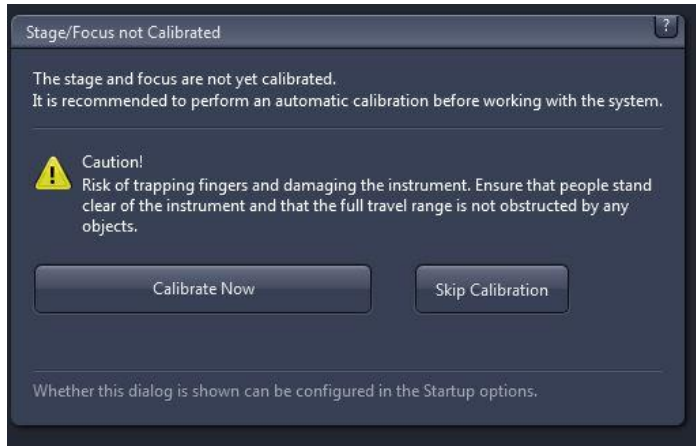
QUICKSTART GUIDE:
WIDEFIELD WF1
Zeiss Cell Observer
Live Cell Imaging System
(SAF, ROOM 409)



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Startup procedure

- Switch on at wall
- 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
- Select and control LED's
- Control heating and CO2
- Additional focussing control
- Other microscope controls



Viewing your sample

- In the software select **locate** tab
- Select **BF** (brightfield)
- Adjust lamp brightness on front of microscope



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

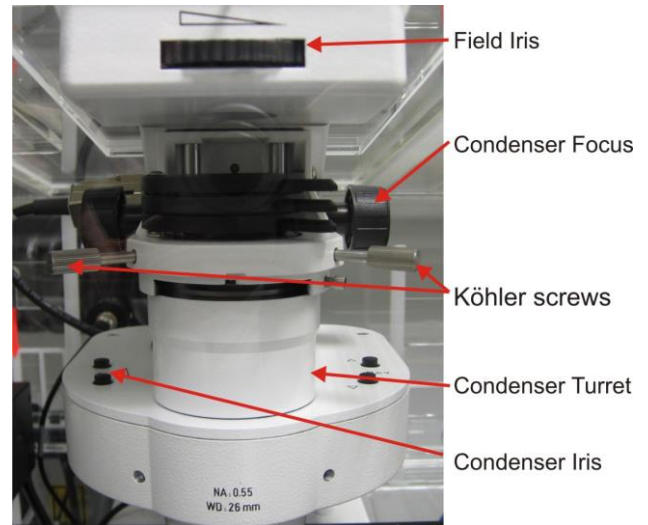


Image Acquisition

Select the Acquisition tab, then

Either

- load an existing image and reuse the settings (select the “reuse” button from below image window)

Or

- load saved configuration

Or

- Select Smart Setup
- Select camera
- Select required channels
- Choose appropriate method and click ok

Select the appropriate camera

Click + to add channels. Select fluorophores and/or brightfield as required from list. (repeat for all fluors present)

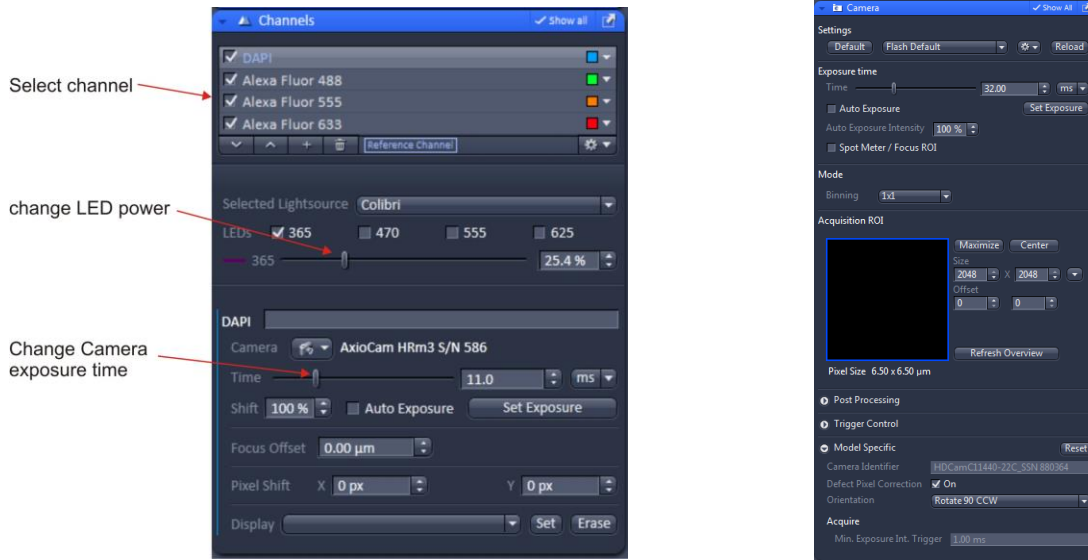
Select the appropriate Imaging method

Select the appropriate scanning method

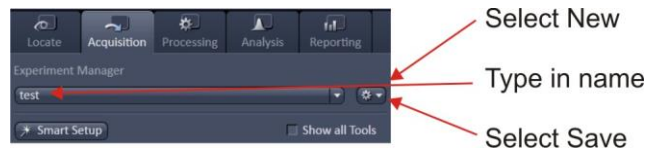
Click ok

Adjustments

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



- Settings can be saved for future use

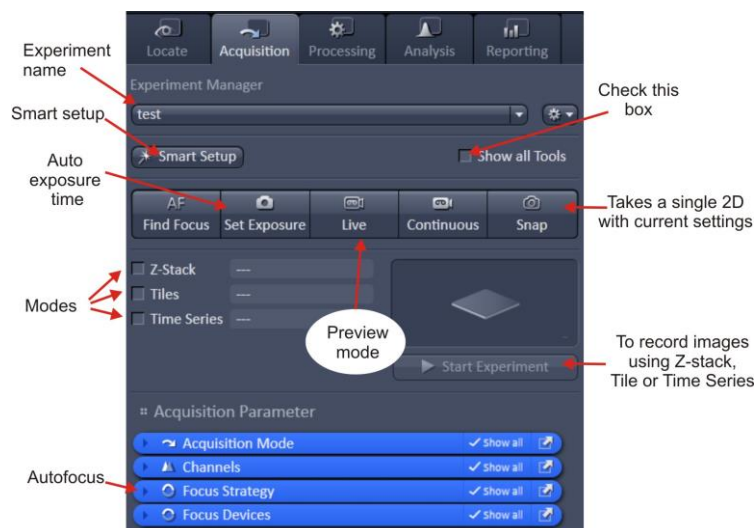


Saving

- 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

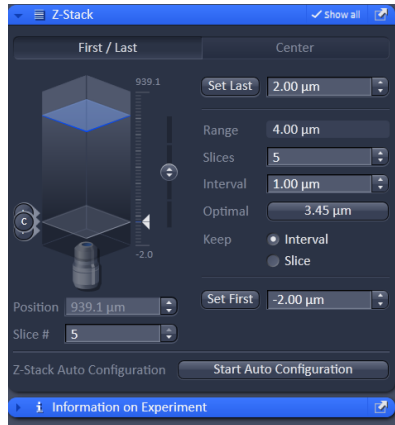
Select Focus Strategy tab

Options will depend on what other functions are selected eg Z stack and Tile

- Absolute fixed Z position
- Software autofocus
- Local focus

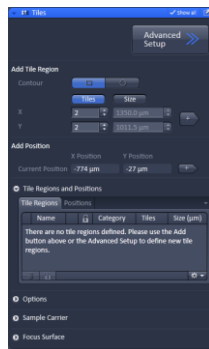
Z stack

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



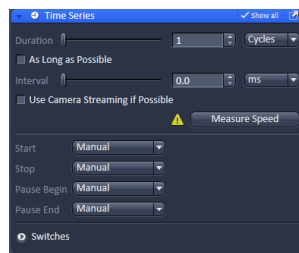
Tile and Positions

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



Time series

- Setup duration and cycles
- Start experiment



For very fast acquisition single channel

- Load WF1 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"

Multi-position and automatic plate formats

Ask FILM staff for help

Heating & CO₂ units

- Found to the left of the microscope
- Switch on heating at least 1 hour before required
- For CO₂ 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 PPMS
- secure your data (e.g. copy them to the server)
- shut down hardware in reverse order from start up

If someone's booked within two hours:

- update usage in PPMS
- 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