

Observing Life As It Happens

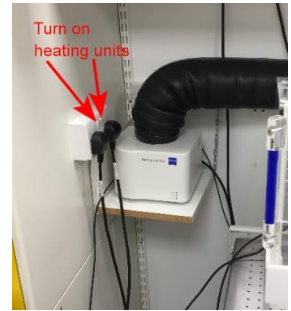
**QUICKSTART GUIDE:**  
**WIDEFIELD HWF1**  
**Zeiss Cell Observer**  
**Live Cell Imaging System**  
(HAMMERSMITH, L BLOCK, ROOM 314)



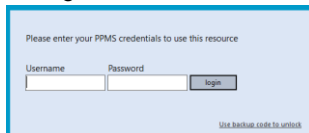
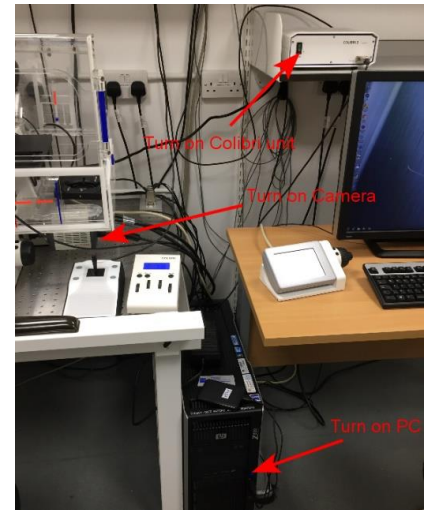
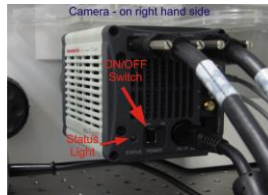
Start-up procedure .....	2
Viewing your sample .....	3
Adjusting brightfield Köhler illumination .....	3
Image Acquisition .....	4
Saving Images .....	5
Additional Methods .....	5
Software Autofocus .....	5
Z stack.....	5
Tile and Positions.....	6
Time series.....	6
Heating & CO <sub>2</sub> units.....	7
Shutdown procedure.....	7

# Start-up procedure

- Turn on the heating units at the wall plugs to the left of the microscope
- Switch on power units above microscope Right to Left



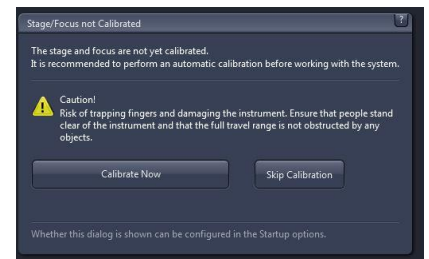
- Turn on Colibri unit above PC screen
- Turn on Camera (right hand side below chamber)
- Start microscope PC
- Check Laptop PC is on (on Shelf)
- **Check Laptop PC is on (on Shelf)**
- **Login to the Laptop using your college credentials**
- **A PPMS login screen will appear – logon on to this using your college credentials** (click Logon button if it does not respond at first)



- Logon to the microscope PC using:  
username: .HWF1  
Password: \$Microscope1
- Run Zen and click on “Zen Pro”

- If you are the first user after switching on – the stage will want to calibrate.

!! Important !! - Select the 5x objective first on the TFT controller and then click Calibrate Now



## 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:

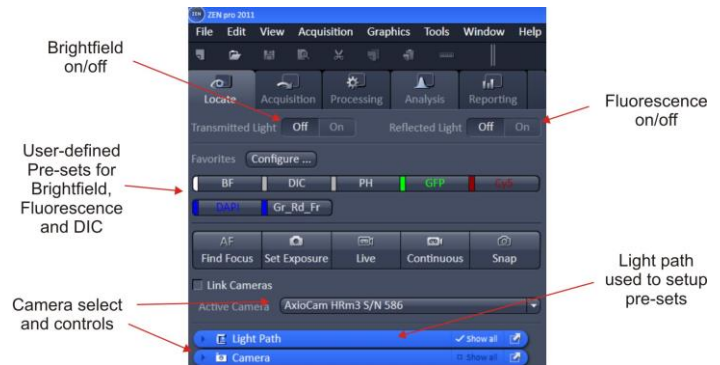
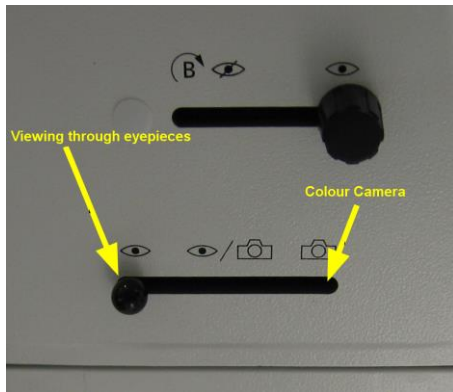
- Select objective
- Switch on/off Transmitted light
- Switch on/off Reflected light
- Select reflector cube
- Control heating and CO2
- Additional focussing control



- Other microscope controls

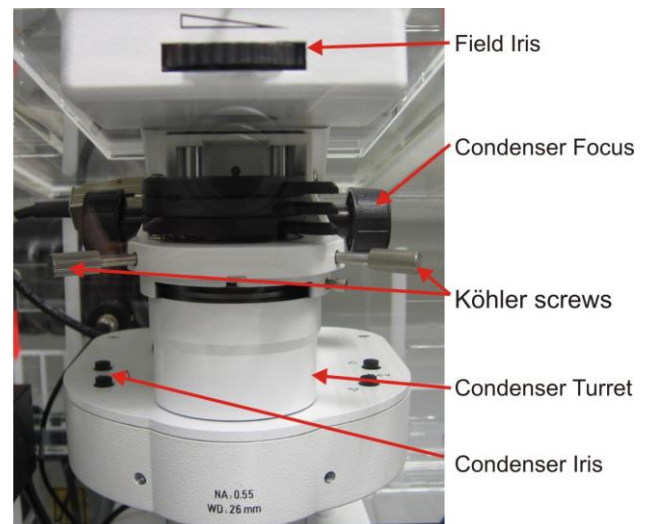
## Viewing your sample

- In the software select the **locate** tab
- Select a user-defined pre-set appropriate for the sample (DAPI, BF, etc)
- Find and focus you sample at low magnification (5x or 10x) first before selecting higher magnifications
- **!!! IMPORTANT !!!** if you can't see anything, check that the lightpath to the eyepieces is open (slider under eyepieces on binocular head)



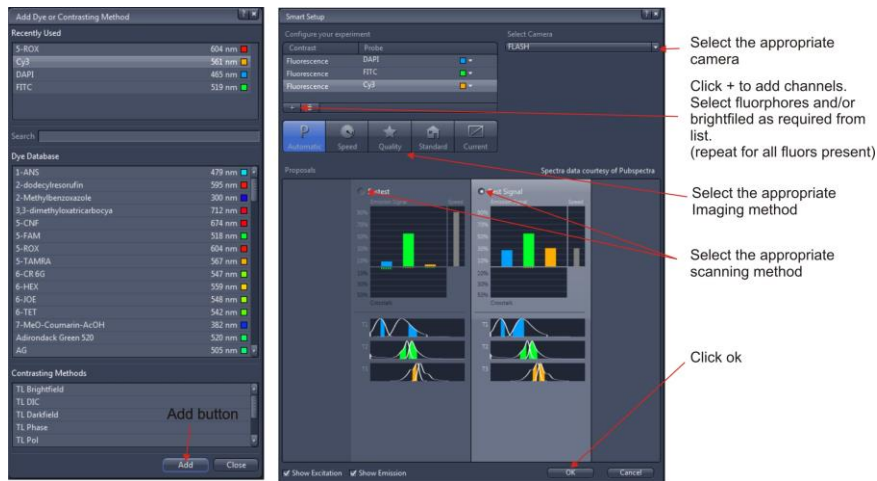
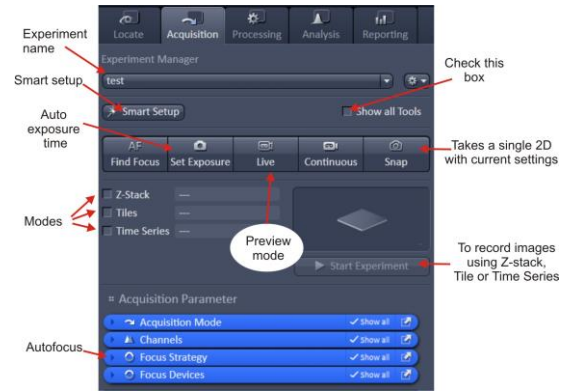
## Adjusting brightfield Köhler illumination

- Select **BF** (brightfield)
- Select 10x objective either with the TFT touch screen
- 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

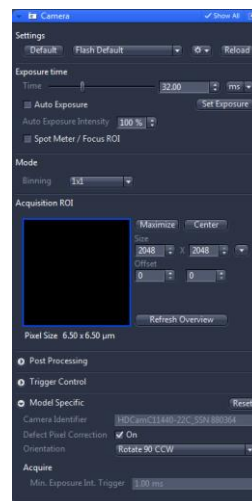
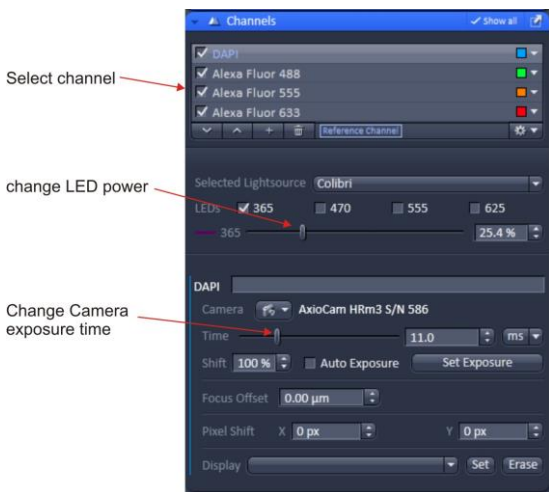


# Image Acquisition

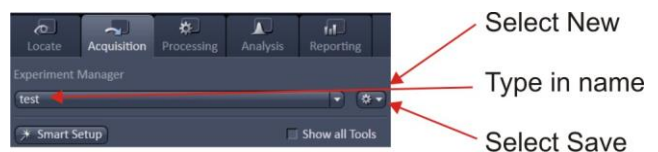
- Select the **Acquisition** tab (make sure “show all tools” checkbox is ticked)
- Either select **Smart Setup** or re-load saved configuration (or select the “reuse” button from a previous image – load image, reuse button appears below image window)
- For **Smart Setup**, select camera
- Add the required channels
- Select best signal



- Click the “Live” button and in the “Channels” tab, select each Channel in turn (click on the channel to highlight it) – adjusting LED power and exposure time to get a good image (use the range indicator button, below image window to help avoid saturation)
- Additional camera settings are available in the “Camera” tab (Frame size and binning)
- To capture the image, select required channels (tick box by each channel) and then click “Snap”



- Experiment settings can be saved for future use

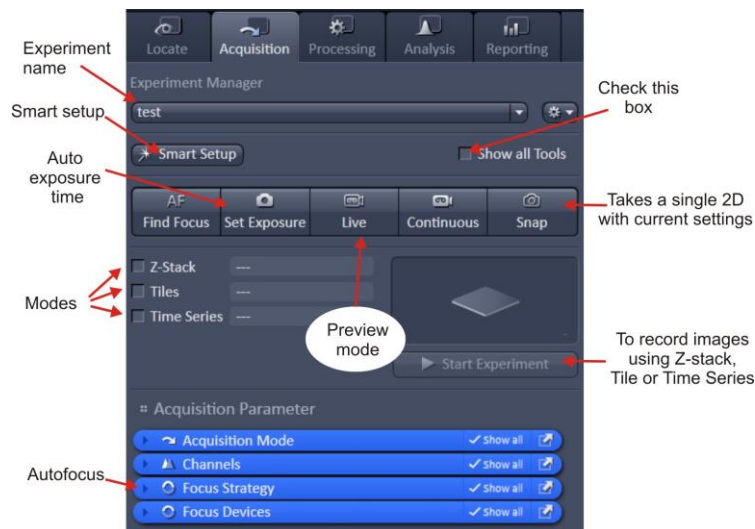


## Saving Images

- Right click on the image in the right-hand window that is to be saved and select “save selected” or “save selected as”.
- Select directory, rename and save.
- ! Warning ! – right clicking will also show a delete button. It is recommended that this is never used as it can delete even saved images

## Additional Methods

There are check boxes for Z stack, Tile and Time Series images. Selecting these will activate the respective control tabs  
To capture images using these methods use the **Start Experiment** button rather than **snap**



## Software Autofocus

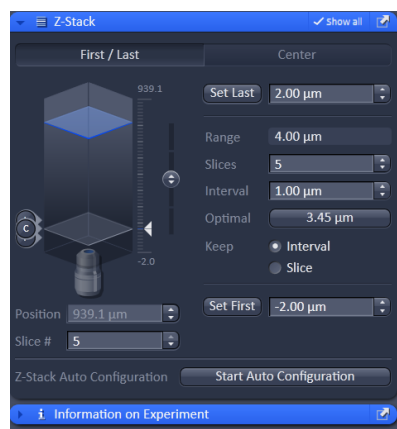
Select Focus Strategy tab

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

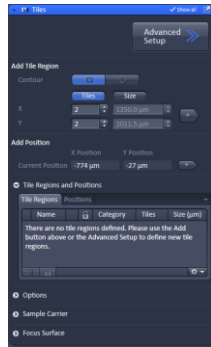
- Set First and Last positions
- Set interval
- Start experiment





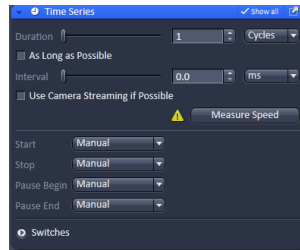
## Tile and Positions

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



## Time series

- Setup duration and cycles
- Start experiment

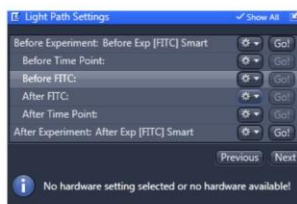


## For very fast acquisition single channel

- In “smart setup”
  - 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”



Set to none



Select

Setup light path and camera

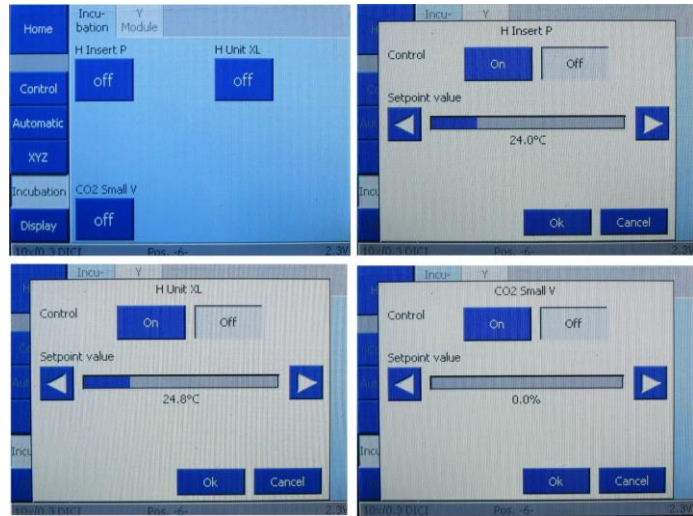
# Heating & CO<sub>2</sub> units

The heating & CO<sub>2</sub> 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:

- Switch on wall plugs
- Turn on “Power supply unit 232”
- On the TFT Touchpad
  - Select “Incubation”
  - Select unit to control

(H insert P – small environmental stage insert,  
H Unit XL – Chamber,  
CO<sub>2</sub> Small V – lid for insert.)

1. Set required environmental conditions
  2. Set to “on”
- Turn off “Power supply unit 232”
  - Leave left hand set of wall plugs 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

# Shutdown procedure

On the laptop PC

- Check if anyone is booked after you within 2 hours
- Copy files onto the server. There are links to the D and R data drives of the Microscope PC and also to the Scratch drive
- **Very Important - log off Laptop (sign out from the start menu) - If the lock screen has appeared because the screen has gone to sleep then log on again and then sign out.**

## If nobody is booked within two hours:

- remove specimen
- clean oil objective lenses with lens tissue
- Important: close incubation chamber completely (→ protects from dust)
- 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:

- remove your samples
- clean objective lenses with fresh lens tissue and close incubation chamber
- clear up the desk
- leave logged on