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Startup procedure

- All switches on wall
- Control units on right-hand side from top to bottom
- Turn on Camera (right hand side below chamber)
- Start PC and login
- Run Zen2012 Acquisition 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

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 locate tab
- Select BF (brightfield)
- Turn on Transmitted light either in software or using the TFT touchscreen
- !!! IMPORTANT !!! Check that the lightpath to the eyepieces is open (slider under eyepieces on binocular head)
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

Setting user-defined pre-sets
(for looking down the microscope)

- open Light Path tab
- select Settings Editor button
- Setup required light path
- Select save as and give it a name
- Go to “configure” on main window
- Add new button, rename it, and drag your new setting across to the Hardware setting Ref.
- Customise its appearance
- New button should be visible and ready to use
- Close configure
Image Acquisition

- Select the Acquisition tab (make sure “show all tools” checkbox is ticked)
- Select Smart Setup or load saved configuration (or select the “reuse” button from a previous image – load image, reuse button appears below image window)
- Select camera
- Select required channels
- Choose appropriate method and click ok

- “Set Exposure” will give approximate recording settings
- 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 Camera tab
- To capture image click Snap

- Settings can be saved for future use
Saving

- To select file location and auto naming goto "Tools" on the menu
- Next select "options"
- Under the "saving" tab
- Set path to D:users\yourname
- You can also select the auto-naming format

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
Method 1: (using the “movie recorder” under the locate tab)
Under the locate tab:
- Set light path or select fluorochrome from button
- Set the camera settings as required
- Go “Live” and set exposure and focus
- Start movie to record, stop to end recording

Method 2: (using “acquisition tab)
- 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”
Heating & CO₂ units

The heating & CO₂ 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,
CO₂ 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

- 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
- save files onto the server or on a mobile harddrive
- log off