

Observing Life As It Happens

QUICKSTART
GUIDE:
**WF2 ZEISS
AXIOVERT 200M
+ HCIMAGE**
(SAFB 409)



Start up procedure 2

 Turn on (in this order): 2

Adjusting brightfield Köhler illumination 3

General information 3

Setting up HCImage 4

 Observing fluorescence through the eyepiece 4

 Acquiring the image 5

 Additional notes on image capture 5

 Acquiring an image sequence (time, XY, Z) 6

 XYZT Sequence 7

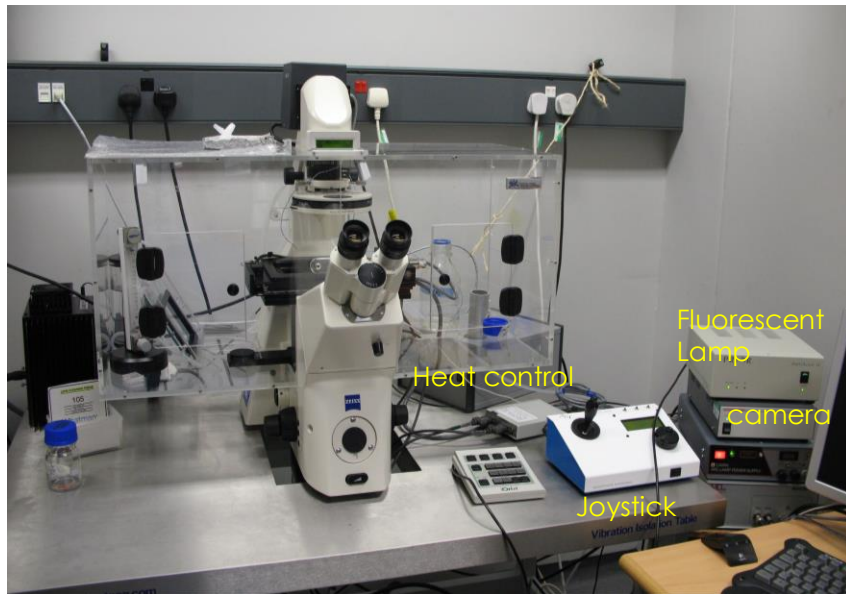
 File formats 3

Shutdown procedure 8

 If nobody is booked within two hours: 8

 If someone's booked within two hours: 8

Start up procedure



Turn on (in this order):

- All switches on the wall
- Switch on the Xenon **fluorescence lamp**
- Switch on computer (square button on front of PC)
- Check HClmage dongle is inserted
- Login using
Username: .WF2
Password: \$Microscope1
NB PC is no longer on the network
- Start HCmagel software (***You have to open the HClmage software before starting any observing and using the microscope***)
- Heating if required

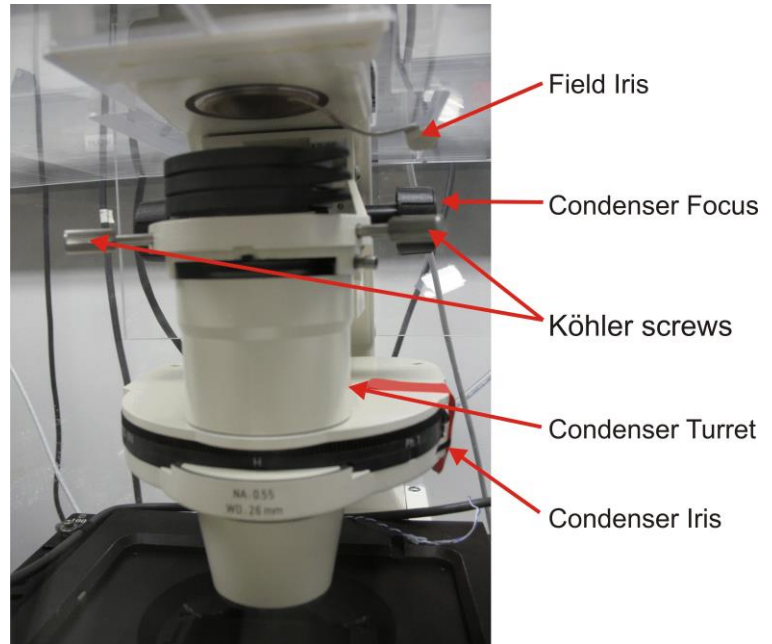
White box = stage movement:

- joystick: button on top toggles between fine / course movement

Adjusting brightfield Köhler illumination

- always recommended, essential for transmitted light image

- Select brightfield
- Switch to 10x objective
- Make sure the lightpath button on microscope stand (lower left of stand) is switched to the left.
- Select brightfield in HCLImage and go live
- adjust brightness with large black button on front / bottom of microscope)
- make sure condenser iris is not completely closed (side of condenser wheel)
- focus on your sample
- completely close field iris (top silver lever) (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
- readjust every time you switch to a different objective



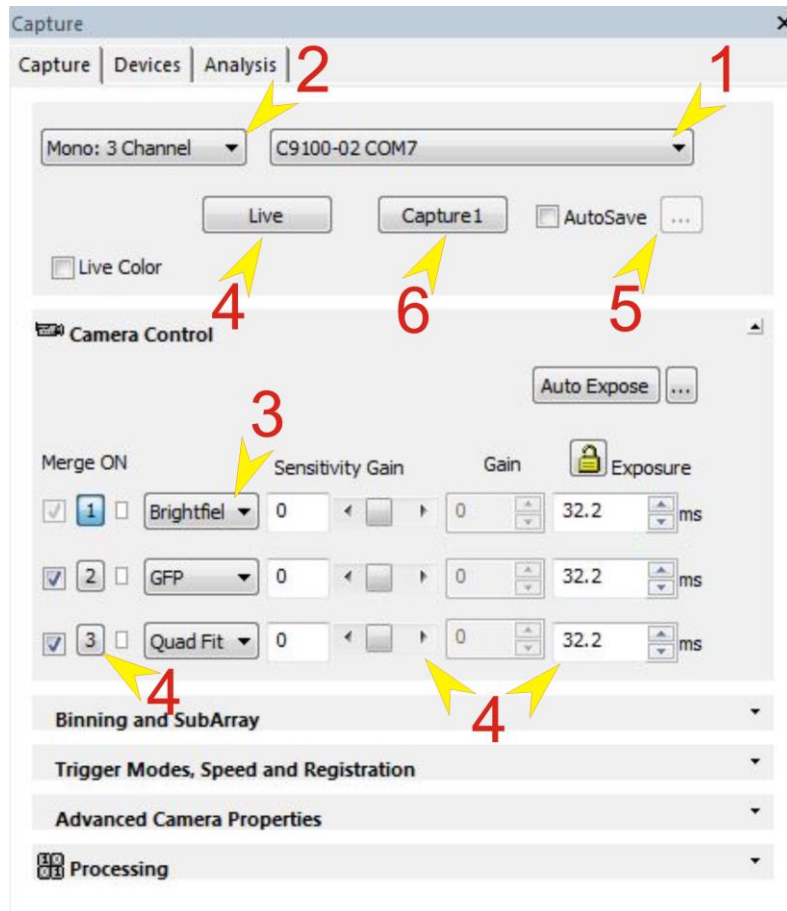
General information

- All microscope parts can be controlled by the software (shutters, filters, focus,), except for the light path. This is controlled button on microscope stand (lower left of stand). It has 3 positions: Left – lightpath to binocular, Right – lightpath to camera and Centre – 50/50.

File formats

Files can be opened in FIJI/ImageJ.

Setting up HClmage



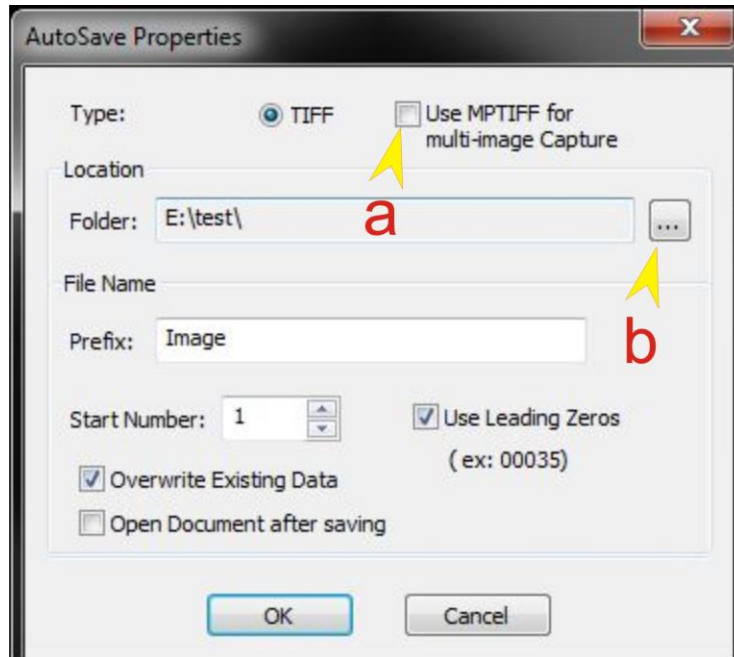
1. Verify the correct camera is selected (9100-02).
2. Select the number of required channels.
3. Assign filters to Channels

Observing fluorescence through the eyepiece

- On the binocular head, change the light path to BINOCULAR(left)
- Select the channel that you want to observe
- Click on **Live**:
- Switch between channels by clicking in the respective row
- Then **Abort** to prevent photobleaching

Acquiring the image

- Setting exposure time
 - On the binocular head, change the light path to camera (right).
 - Click on **Live**:
 - Adjust the right exposure time (and sensitivity gain if necessary)
 - Switch between channels by clicking in the respective row
 - Then **Abort**
- Tick the **Autosave** box and put in the preferences



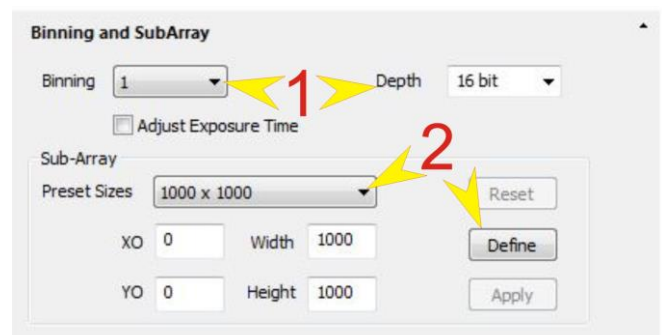
- Check use MPTIFF for multi-layered tif or TIFF for separate images
- Select output directory and any other criteria

- Click on **Capture1** to record image

Additional notes on image capture

Camera:

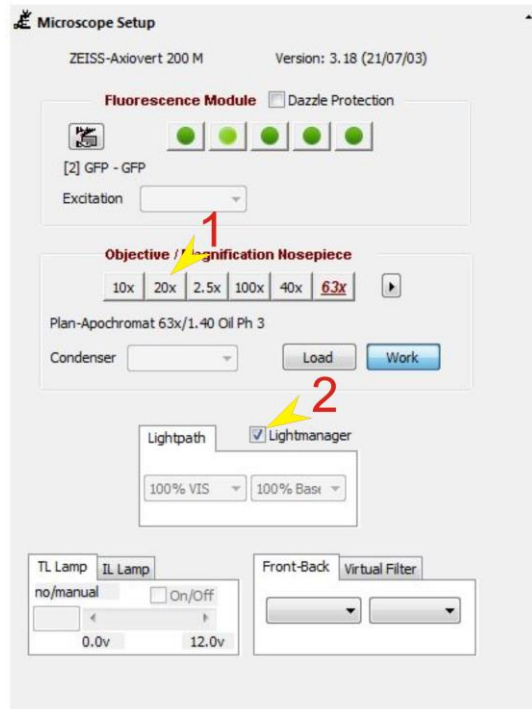
- Binning** – if the signal is weak or speed is needed then binning can be selected (2x2 binning will give 4 times the sensitivity but half the resolution)
- Crop (sub array). Either select from preset sizes or enter your own and then select **Define**



Mircroscope

Use this window to select:

1. Objective
2. Activate/deactivate Light Manager

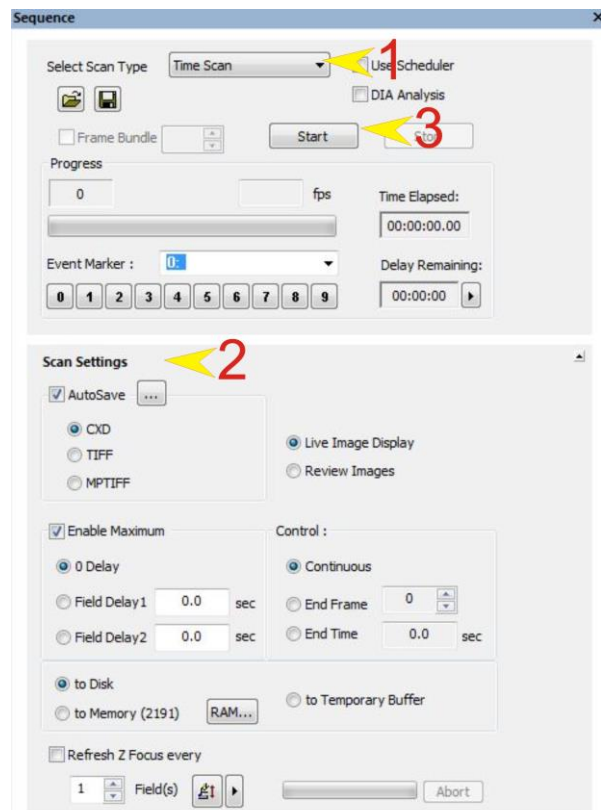


Acquiring an image sequence (time, XY, Z)

Time Scan

Select **Sequence** window

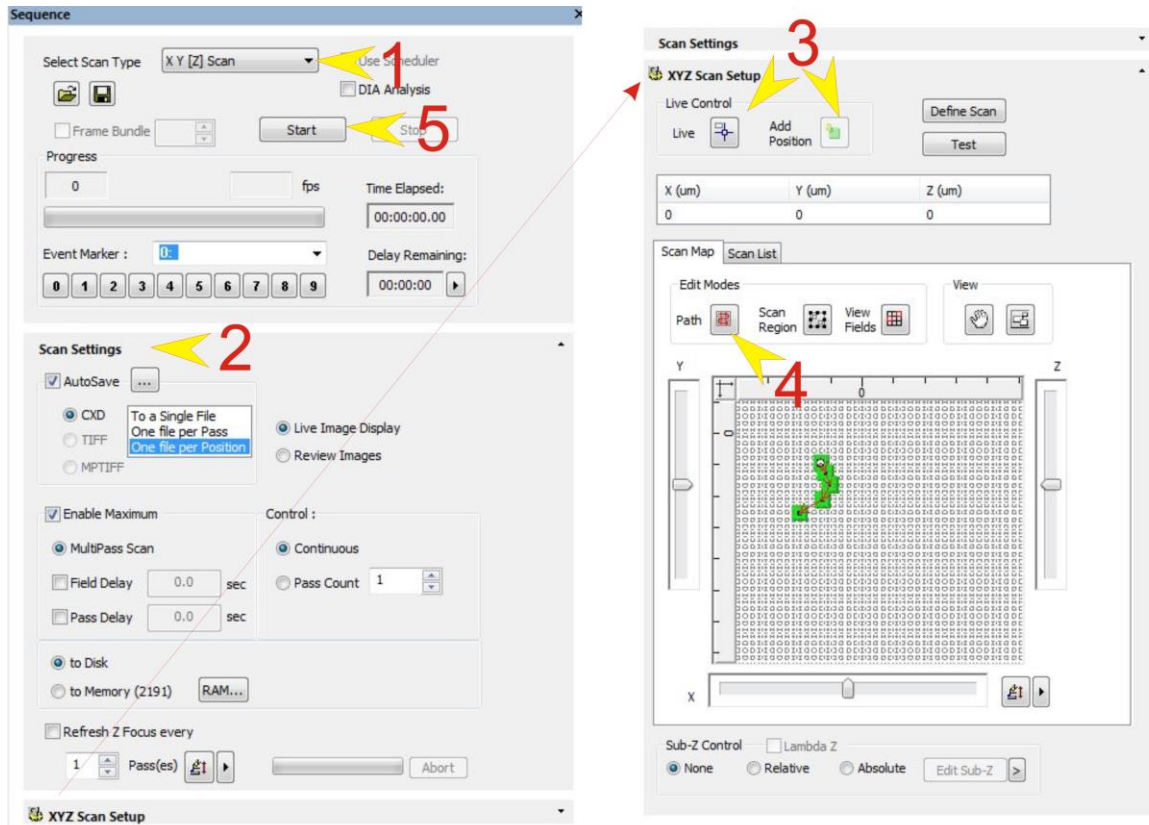
1. Select time scan
2. Set Scan Settings
3. Click Start



XYZT Sequence

Select **Sequence** window

1. Select X Y [Z] Scan
2. Set Scan Settings
3. Add positions
4. Set imaging path
5. Click Start



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