

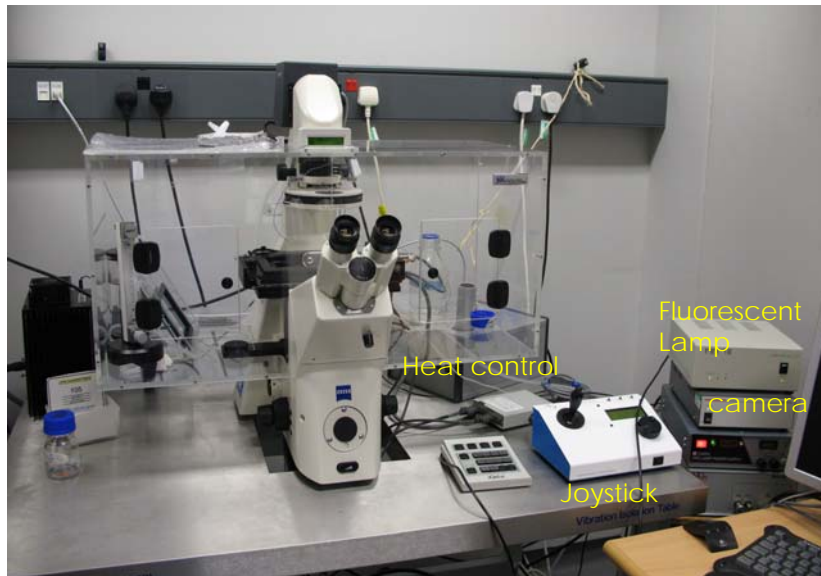
QUICKSTART GUIDE:
WF2 ZEISS
AXIOVERT 200M
+ SIMPLEPCI
(SAFB 409)



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Start up procedure



Turn on (in this order):

- switch on the Xenon fluorescence lamp
- MAIN switch on the wall
- computer, start SimplePCI software

White box = stage movement:

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

You have to switch to open the simplePCI software before starting any observing and using the microscope.

Adjusting brightfield Köhler illumination

- Make sure the LIGHT PATH (button on the left / bottom) is switched to 100% BINOCULAR) and you work with 10x objective.
- Start brightfield shutter with S1 (labelled 'BF' on the key pad)
- Adjust brightness with large black button on front / bottom of microscope)
- Make sure illumination iris is not completely closed (two buttons on top of illumination filter wheel)
- Focus on your sample:
- Observe sample from outside and coarse-focus (with oil lenses until it touches the oil)

- Look through the eyepiece and fine-focus
- If you can't find the focus, try the edge of the coverslip
- If it still doesn't work, try to insert the 10x lens (do not switch from oil lens!)
- If focus beeps, override the top limit by pushing the top focus button while turning the focus wheel
- 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 wheel)
- Centre iris (2 silver screws)
- Reopen field iris until the edge is just not visible anymore

Observing fluorescence through the eyepiece

- in SimplePCI, choose the fluorophore that you want to observe and click on LIVE
- Switch between brightfield and fluorescence with FL ON / OFF on the software.
- Turn fluorescence immediately off if not needed, to avoid bleaching
- Find your sample on the coverslip
- Switch back from fluorescence to brightfield (→ quickly check whether your cells are fit and healthy)

Acquiring images in the Simple PCI software

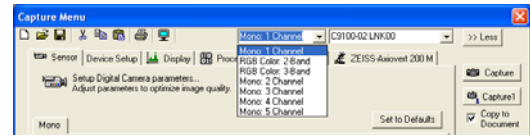
General informations

- All microscope parts can be controlled by the software (shutters, filters, focus, ...), except the light path (BINOCULAR / BASE PORT)

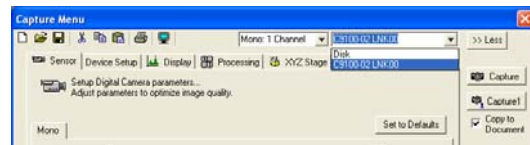
Acquiring the image

- Start the SimplePCI software (make sure the dongle is attached to a USB port)
- On the microscope stand, change the light path to BASEPORT.

Choose the required number of channels (colours)



Verify you work with the right camera setting (9100-02).

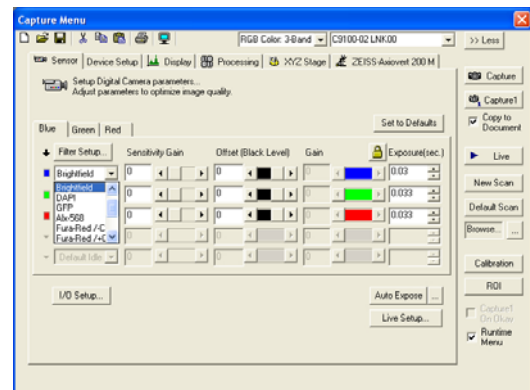


Assign a setting lifter to each channel: brightfield, DAPI, GFP/FITC, etc...

The exposure time:

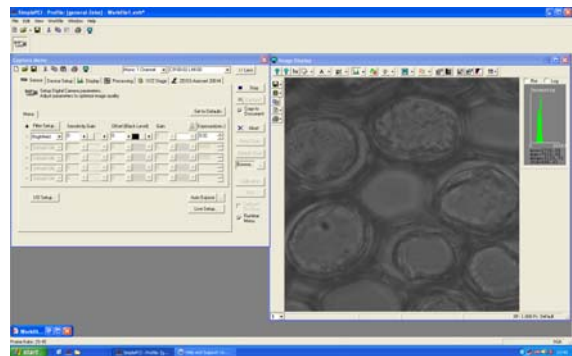
Click on **Live**:

- Adjust the right exposure time directly on the screen,
- Switch between channels by clicking in the respective row
- Then **Abort**



Tick the 'copy to the document' box

Click on **Capture1** to make a snapshot



Acquiring an image sequence (time, XY, Z)

Click on **New Scan**, choose the right option:

- Time scan or time lapse
- X or Y scan or position list to make a mosaic image
- Z scan or Z stacks
- X,Y and Z Scan with time scan
- Wavelength Scan

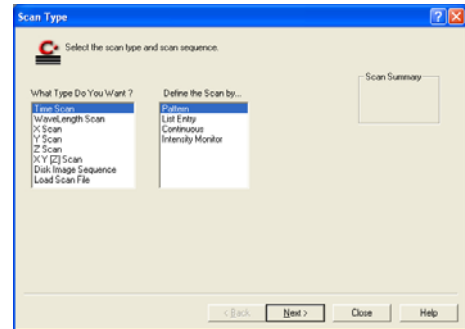
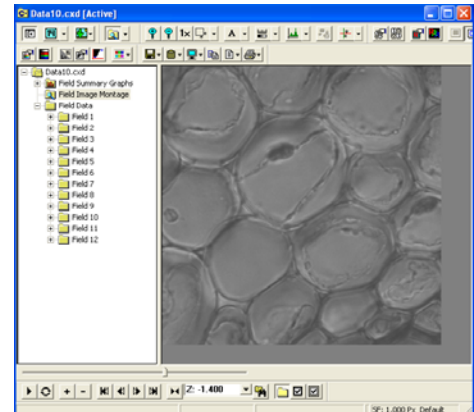


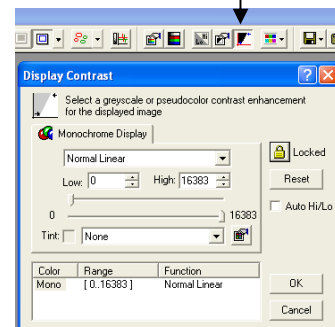
Image visualisation and Image processing

In the simplePCI window you have different icons in the toolbars.



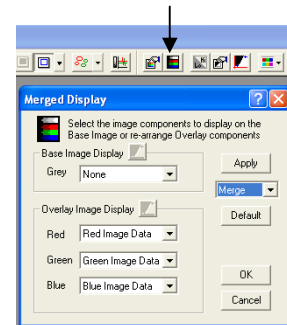
Contrast property/Toggle contrast on/off:

- Allows to adjust the contrast. Just move with the small bar in each extremity of the scale bar.
- To adjust the channels separately, unlock the settings
- Also used to change to pseudo colours



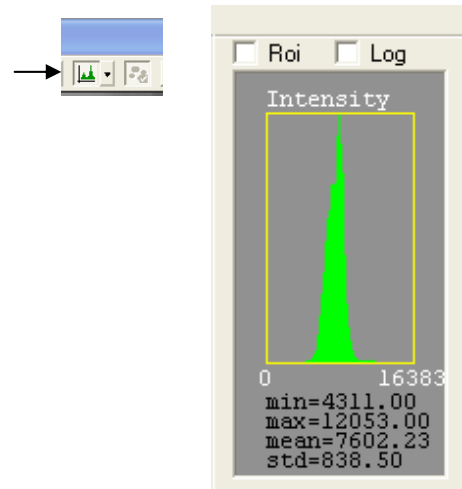
Merged display properties/ Toggle merged image on/off:

To define the brightfield (gray) channel and fluorescence (overlay) channels.



Display Intensity histogram/ Display Intensity Scattergram

- The histogram represent the intensity distribution of image pixels
- Useful to avoid saturation.



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

Additional useful information

Control buttons on the microscope:

- **Bottom button:**
 1. Eye
 2. Front port (empty)
 3. Base port (camera)
- **Middle button:**
 1. Sideport (empty)
 2. Eye / front / base (default)
- **Top: octavar in/out**

File formats

- → *File/Export/collection or Image sequence/...*
- **TIF :**
 - Saved in OME-TIF format
(<http://www.loci.wisc.edu/ome/index.html>)
 - All meta format are saved in the header of the file
 - Files can be opened in any imaging software.
- **MultipageTIF:**
 - Raw data (12 bit)
 - Loses all enhancements but keeps original data
 - **cxd:**
 - ♦ Original image file format for image sequence
 - ♦ Contains all imaging information
 - ♦ Contains all images in 1 file (real layers)

Photoshop shows only the first image.

- **Movie files:** PC animation avi