adolphe merkle institute | UNIVERSITY OF FRIBOURG SWITZERLAND
excellence in pure and applied nanoscience

ZEISS Fluorescence microscope

Introduction

BASICS

Version 3 – June 2025

adolphe merkle institute | UNIVERSITY OF FRIBOURG SWITZERLAND
excellence in pure and applied nanoscience

D vanhecke | Adolphe Merkle Institute

Demonstration: Booking and information

Prerequisites:

A Switch edu-ID account

Registered at https://instruments.am-institute.swiss/

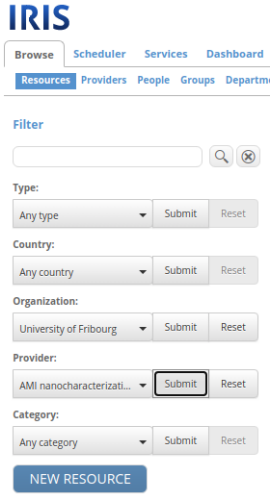
After the introduction, you will be added to the SEM users on the OpenIris platform

Instruments platform: **https://instruments.am-institute.swiss/lsm/**

Contains all resources, links, help, troubleshooting. Register and login

Booking platform: **https://openiris.io/**

1. Click the **Sign in / register button.** sign in with your **UNIFR credentials**
2. The SWITCHaai portal. Select:
   - Select your organization: University of Fribourg (and click select)
   - Then enter your Switch edu-ID credentials (and click login)
3. The OpenIris platform loads:



You are guided to the OpenIris platform. Under “Organization”, select “University of Fribourg”; Under “Provider”, you should find “AMI nanocharacterization platform”

In the list you should find the instruments you are eligible to use. For each instrument (below the info), you find 4 icons/buttons. From left to right

- Magnifying glass: Information about the instrument
- Clock: Booking of the instrument
- Pen: Submit an issue
- Letter: Contact admin

To book a session, either click the picture of the instrument or the green clock and add the details in the next screen

Please submit issues through OpenIris

Demonstration: Shutting down

Prerequisites:

Data recorded

Finishing your session

Please leave the instrument in good health for the next user.

Experiment: shutting down the system

Microscope

- remove you sample

- remove immersion oil, when applicable, by using the provided lens cleaning solution. Use Kim wipes of lens paper.

- Turn the lens turret to have the 10 X lens activated

- Close all doors on the incubater box (front and top)

Data

- Data is always saved on the D:\Data drive. There you will find a folder “data” where you can store your data in a folder with your name. Data that is not in these folders, ie not assigned to a person, will be deleted.

- Copy your data using a server of choice (switchdrive, Bigdata, …). Please **do not use USB based data disks** (USB drives, external harddiscs).

PC

- Close the Zen software and turn off the PC

System

Flip the master switch to off, as detailed on page 7.

Universal rules

Rule 1: don't touch a control if you are not sure of the outcome of that action

Rule 2: never, ever force anything beyond finger strength

Rule 3: if in doubt ask for help

Content

Demonstration: Experiment information

Prerequisites:

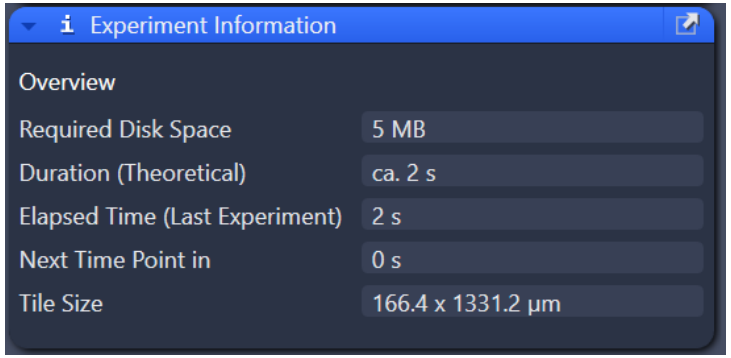
An lightpath is setup

Providing information on time and data size before a snap or experiment starts

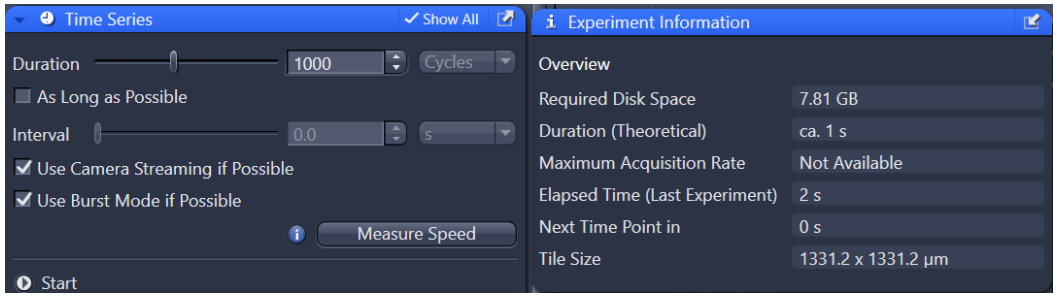
Located in the multidimensional Acquisition list, this window is purely reporting information.

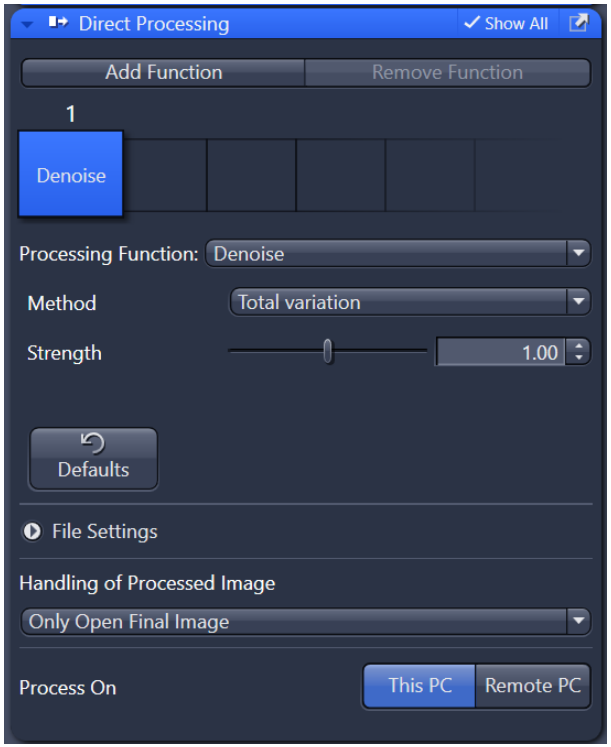
Examples:

a Z stack of 5 slices of 1024 x 1014x, 8 bit



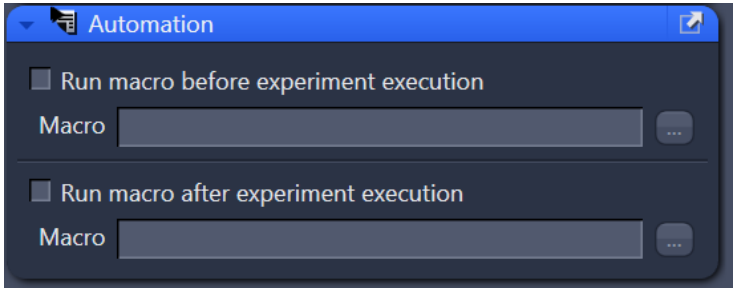
A burst mode time series of 1000 slices, 16 bit





5. Automation

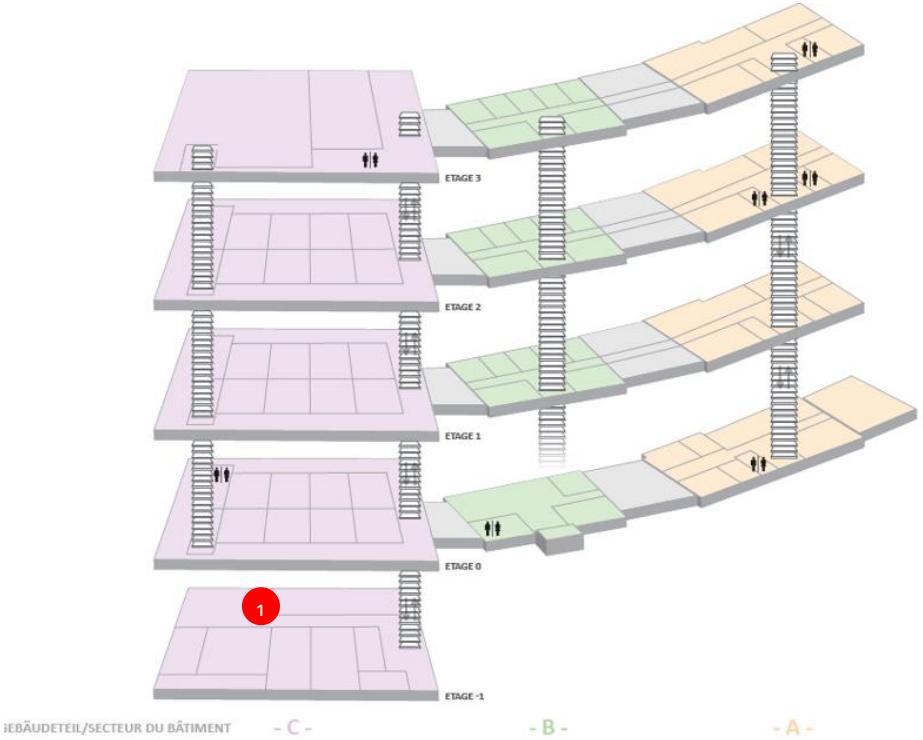
Here you can write scripts (macros) that run before or after an acquisition. Note that the macro IDE is not installed, so recording macros must be done by command line and text files.



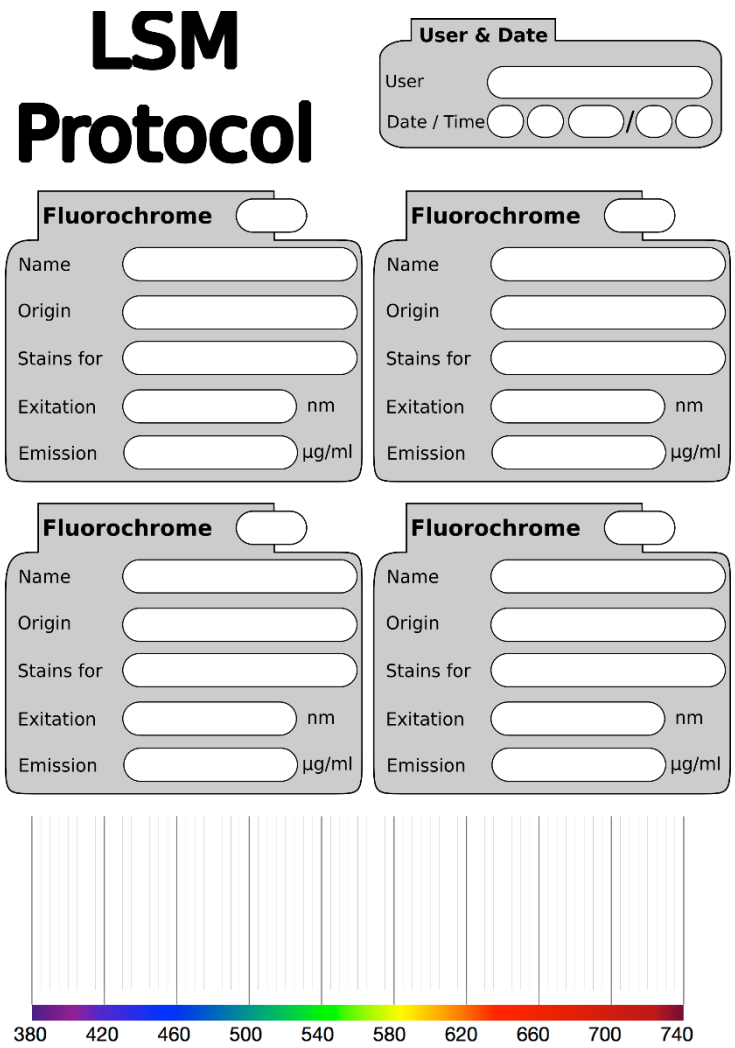
Locations

The instruments can be found here:

- Laser scanning microscope (room C0141)



Multi Fluorescence check



http://www.bdbiosciences.com/us/s/spectrumviewer

https://www.thermofisher.com/za/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

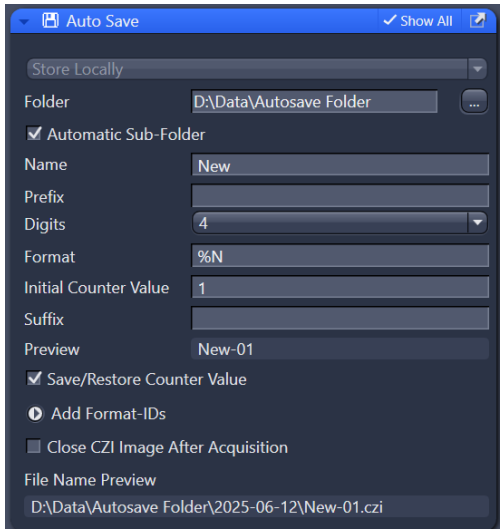
Demonstration: Switching on the system

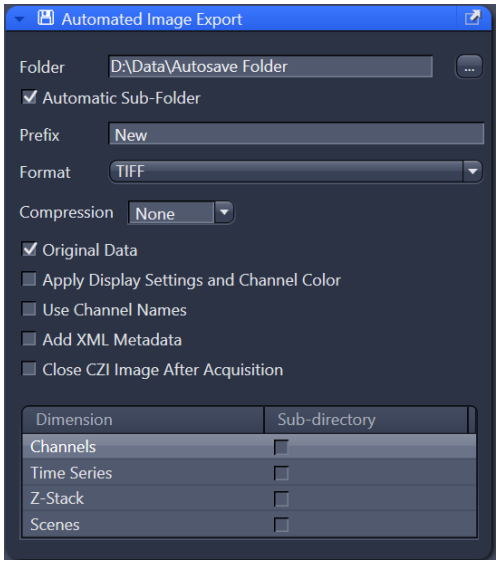
2. Autosave

To prevent data loss, you can write all the images directly to the hard disk.

3. Automated image export

Data is always saved as Zeiss proprietary CZI files. There are plugins for FIJI to open these files. However, you may want to export them to TIFF (never to JPEG!!).





4. Direct processing

Direct processing allows to run image processing tools immediately and automatically after acquisition: deblurring, deconvolution, denoising, extended depth of focus and unsharp mask are installed. It is possible to run more than one algorithm. Each algorithm comes with a set of variables.

Demonstration: Tools

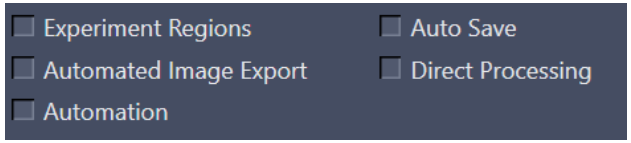
Prerequisites:

Brightfield source is properly set

Fluorescence channel(s) set up properly

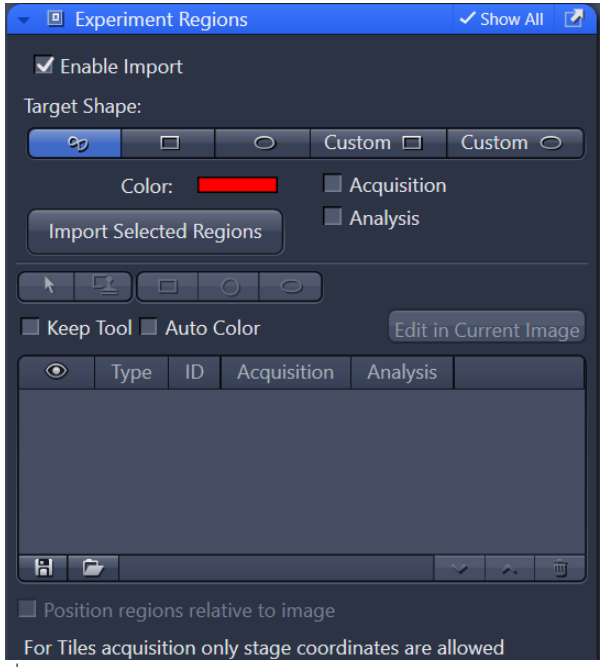
Useful tools for saving and post processing

Below the Experiments, you can opt for a number of possible useful tools. Tick the tools you want to use and a window will be added accordingly.



1. Experiment Regions

These regions allow to define specific areas of interest within your sample that you may want to image or analyze.



Prerequisites:

The system completely switched off

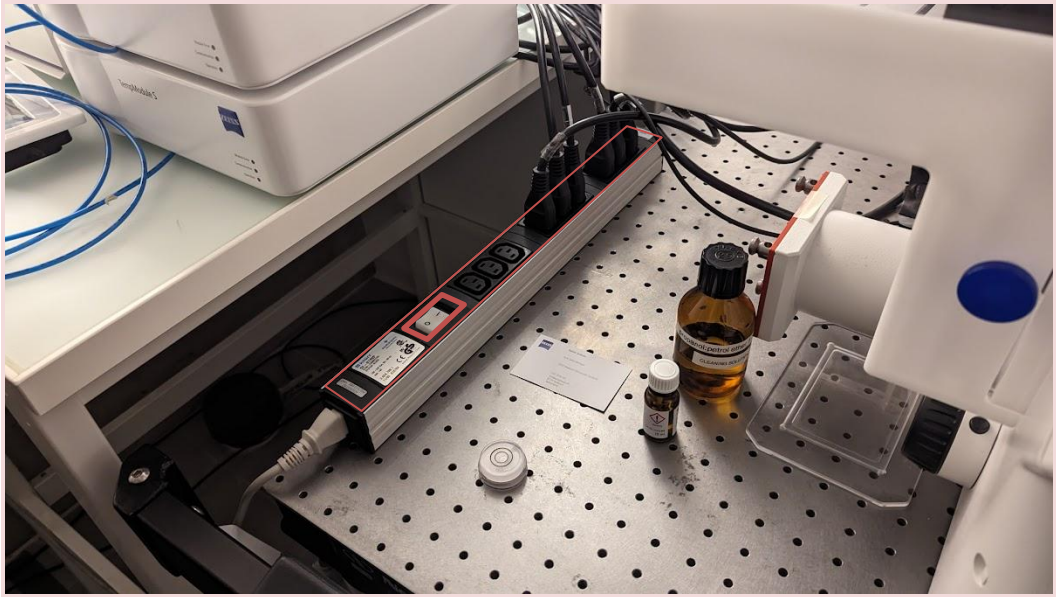
Action:

Switch on the system and the microscope PC

The PC and the system have separate power supplies

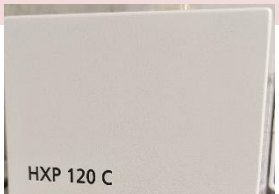
Switch on the system

On the left of the stand, find the master switch and turn it on.



The system starts up (check the Zeiss display). This takes about 30 seconds.

Assure the HXP is on

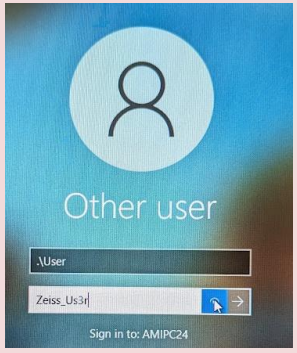


Located at the far left on the high bench. **If needed**, toggle the green power switch.

Switch on the PC

Located between the desk and the stand. Switch on the PC.

Login onto the PC



Use the following credentials:

Username **.\User**
Password **Zeiss_Us3r**

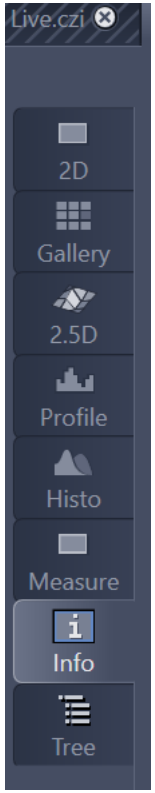
Note: the ".\" in the username is important. You must see the Infoline "Sign in to AMIPC24" below the password box.

Demonstration: Metadata

Prerequisites:

data recorded

Metadata are a love note to the future



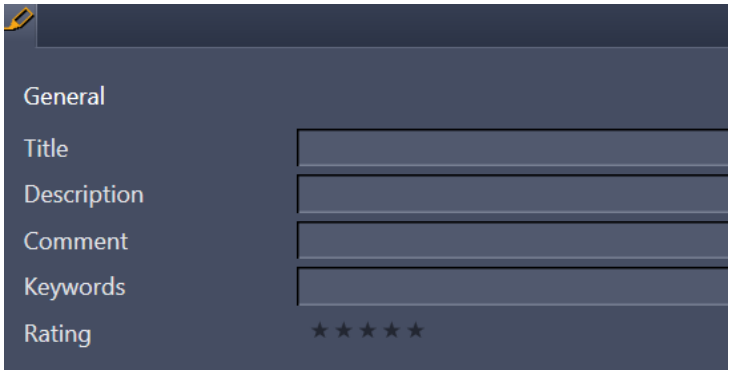
With Open data concepts, the use of metadata is extremely important. Tag your data with the relevant information. Key information that **must** be present in any dataset are:

- Who made this dataset
- When was it made
- What is it
- Why was it recorded / what was the protocol used to create this sample

Further information can be found by an online search for the F.A.I.R. principles

Experiment: complete your metadata

- With an image recorded, click the tab Info. Add relevant data in the general tab entries



3. Trigger control

The system does not have an input / output card, therefore external trigger control cannot be used. Please do not alter

4. Model specific

Please do not alter! However, if you encounter a mismatch between joystick and camera movements, check the tab “General > Orientation”. The value in the dropdown box must be: “Rotate 90 CCW”. If this is not the case, select this option. Do not alter anything else

Demonstration: Software startup

Prerequisites:

Running system and PC

Login window

Action:

On the PC screen: start the Zen software and login as User

There are separate profiles in the Zen software

Log in and select a profile

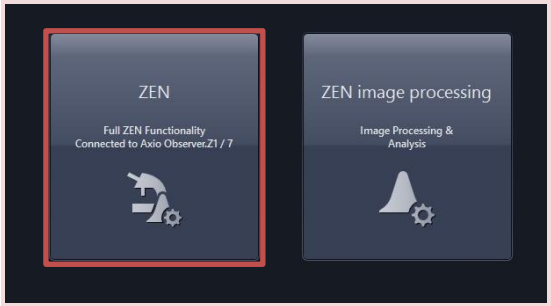
1. Log in

Double click the Zeiss ZEN icon. 

Note: a free version is available for your laptop (image processing only): Zen lite.

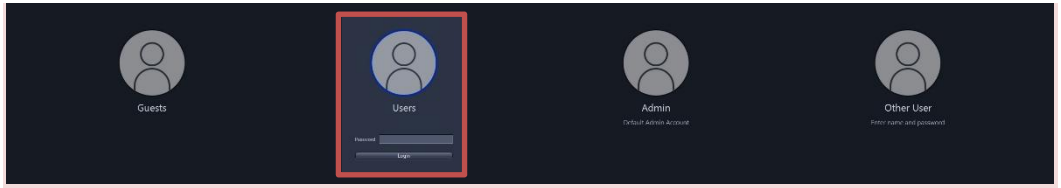
2. Select ZEN

Full ZEN functionality. Please perform Image processing not at the microscope. Use FIJI or Zen Lite on your laptop.



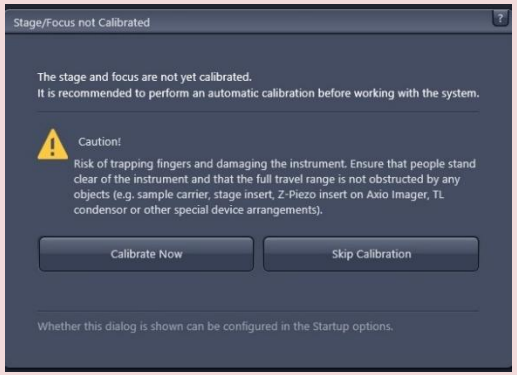
3. Choose the “Users” profile

Note: there is **NO password** (just press enter or click login)



Stage calibration

Hardware initiation takes about 30 seconds[1]. If this is the start of your session, you will be asked to calibrate the stage.



Make sure no sample is loaded! Click “Calibrate now”. The procedure takes about 20 seconds.

[1] Occasionally, an error occurs (camera initiation issues). In that case, restart the PC

Crop

allows to design of the cropping window on the current image/overview

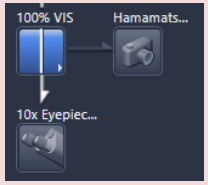
2. Post Processing

Black reference

This function compensates for the electronic background or dark current in the camera.

Experiment: Black reference

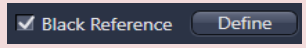
If longer exposure times are needed (> 5 s), individual bright pixels may become visible. Then, use the black reference these effects are measured and corrected. If needed, repeat the alignment, in particular if you are using long exposure times.



1. In the Imaging setup, close off the light path to the camera by switching the path to the eyepiece.

2. In Acquisition mode > Post-processing, click “Define“.

3. The scan will take < 1 minute. Afterwards, switch the lightpath back to the Hamamatsu camera



Noise filter (not recommended)

Filters the noise in the acquisition (not the live) image to an adjusted threshold. This can deal with sporadic bright events in single pixels on CMOS sensors, like blinking pixels in dark images or spurous cosmic ray events. **Not recommended**, but can be useful for high-gain settings or very dim signals.

Enable unsharp mask (not recommended)

Enhances contrast, probably through a linear local filter.

Demonstration: Acquisition mode

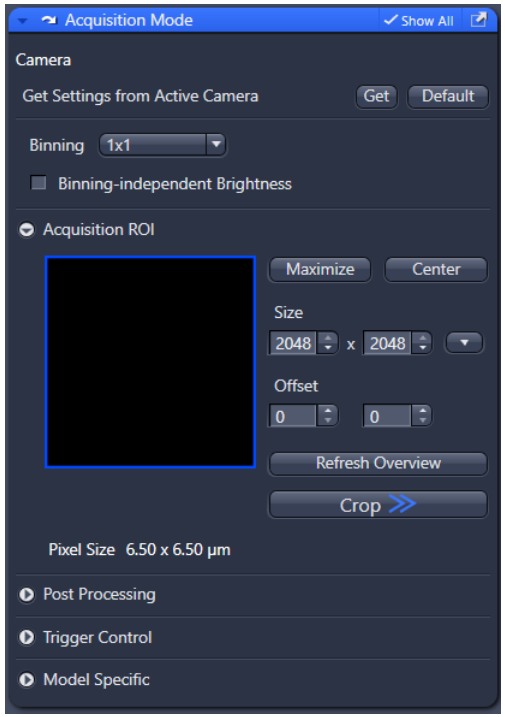
Prerequisites:

Brightfield source is properly set

Fluorescence channel(s) set up properly

Tweak camera settings

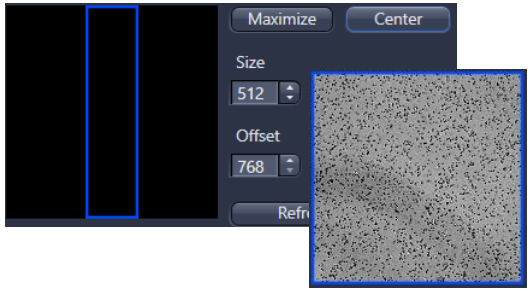
1. Acquisition ROI



Camera:
Get the current camera settings

Binning:
(note: CMOS camera!). Intensity is increased, but not signal-to-noise, at the cost of resolution.

Acquisition ROI:
Digital zoom in. To optimise recording speed, reduce X, but not Y



Refresh overview:
updates the Acquisition ROI with the current image.

Demonstration: Mounting a sample

Prerequisites:

System successfully started

Action:

Mounting your sample

The microscope is an inverted microscope! Mount your sample accordingly

Mounting a sample on the stage

1. Mount a sample. Note that the instrument is an inverted microscope; therefore, the sample should be **mounted upside down** (cover glass facing down).

2. Open the windows on the top of the environment chamber before tilting back the aperture arm.

3. Assure the corner marked with the red dot is clicked in on the bottom left of the stage. Mount your sample.

4. Return the arm and close all windows on the environment.

Note on the touchpad display

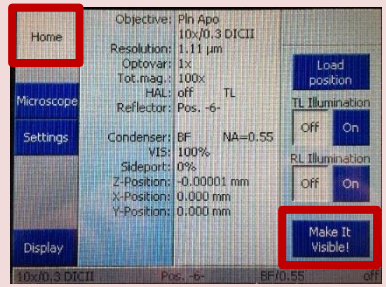
The software will be used to control the microscope; however, at setup, the touchpad display can be useful.

On the touchpad: touch top left 'Home'

Make it visible

Home > bottom left: Press when completely lost.

You will get a simple, brightfield illumination (ocular)



TL illumination

Transmitted light = bright field

Press 'ON' to get the bright field image

RL Illumination

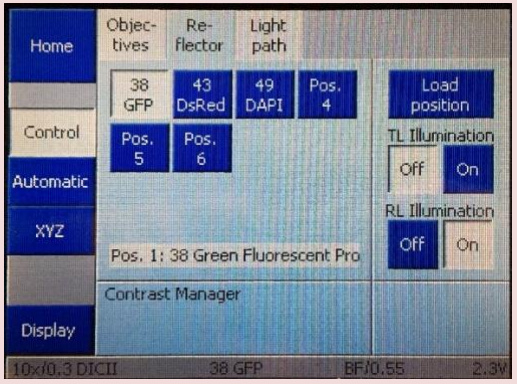
Reflected light = fluorescence

Press 'ON' to see the fluorescence signal

With the light source set up, we can choose the reflector (= filter cube).

Experiment: choosing the filter cube (reflector) and lens (objectives)

On the touchpad: touch the '**Microscope**' button in the left menu (below 'Home') and then '**Control**'



Objectives

Cycle through the installed objectives

Reflector

3 Cubes are installed:

GFP: for fluorophores excited at 488 nm

DsRed: for fluorophores excited at 561 nm

DAPI: for fluorophores excited at 405 nm

The other 3 positions are empty

Light path

Shows graphically the light path through the microscope. Send it either to the Eyepiece/VIS (=ocular) or Sideport R (=Hamamatsu camera).

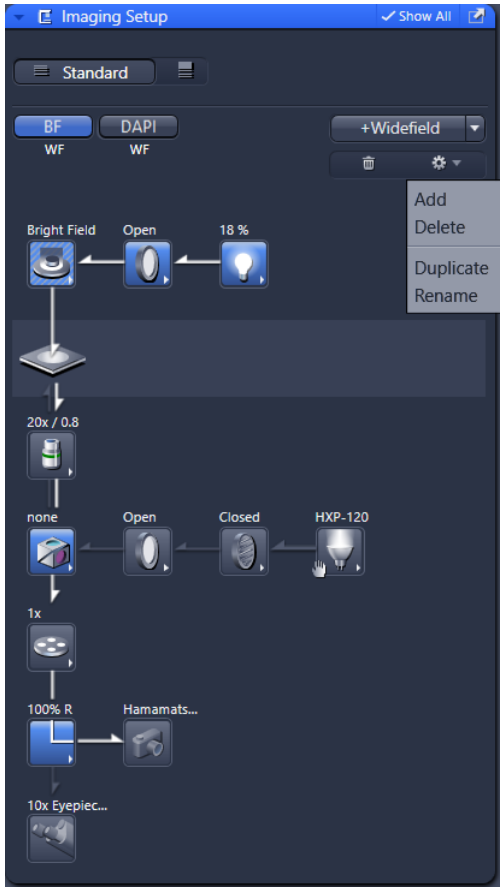
Demonstration: Imaging setup

Prerequisites:

Brightfield source properly set

Fluorescence channel(s) setup properly

Full control over all lightpath settings



Standard

Keep. If you switch to advanced, do not change any settings!

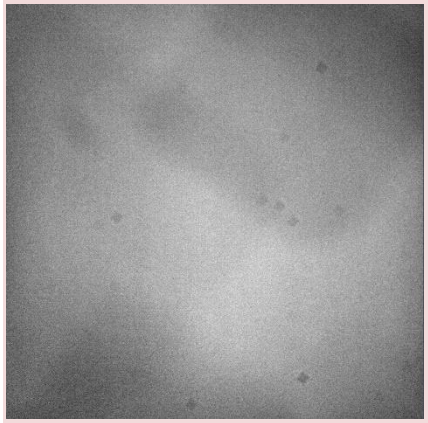
Widefield, Add/delete

Add channels will call Dye database dialog explained before)

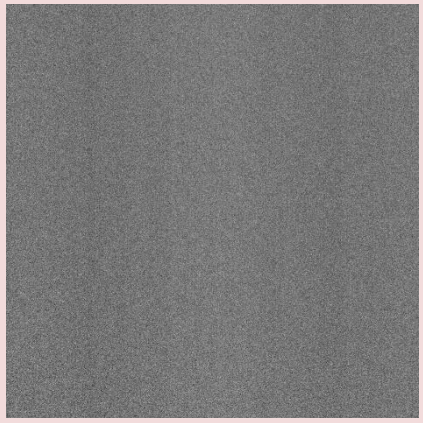
Channels will be added to the channels dialog. Click the channel (e.g. BF, DAPI) to see the exact lightpaths.

Experiment: record shading correction reference

1. Move outside your sample (and support)
2. Setup a brightfield path only
3. Use the global method and click Define



Live image before shading correction

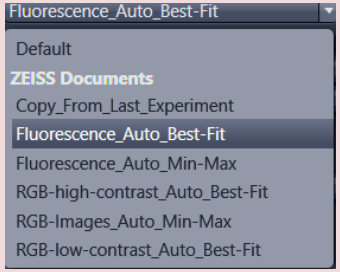


Live image after shading correction (global)

Experiment: display setting

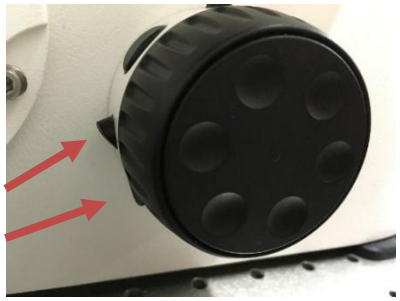
In the window Channels > Display setting. This allows to set a postprocessing agorithm

Only applies to the “snap” recording, not to the live recording!



Tip

For easy cycling through the filter cubes while using the ocular, there are shortcut buttons at the back of the left focus wheel:

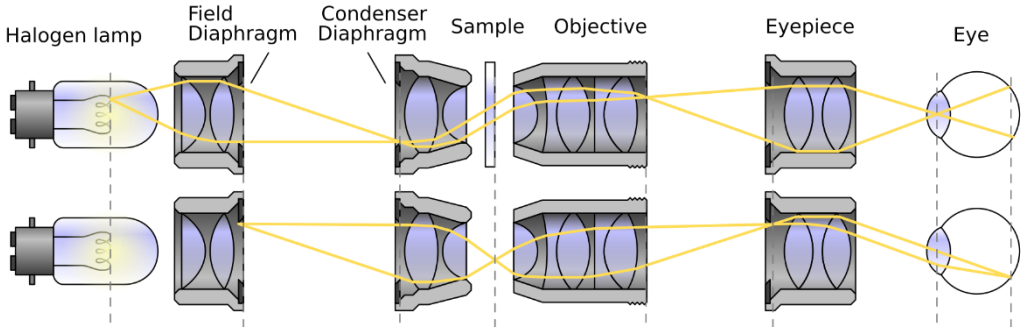


MICROSCOPE FRONT ➔

Background theory: Köhler illumination

Why: uniform illumination of the sample

When: when using the ocular



Top: Conjugated planes of the illumination beam path

Bottom: Conjugated planes imaging beam path.

The condenser lens projects the light through the sample, ensuring **the image of the light source is perfectly defocused** in the sample plane. Two sets of conjugate image planes are created: one with the light source image and one with the specimen.

Demonstration: Köhler illumination and focusing

Prerequisites:

Specimen mounted

10X magnification (lowest)

Pathway through the ocular

Action:

Setup Köhler illumination for brightfield imaging

Perform the Köhler illumination[2]

Illumination of the specimen is the most important variable in achieving high-quality images in microscopy.

Experiment: Focusing and finding the region of interest

Tip: Use a fluorescent signal (e.g. DAPI), which makes focusing relatively easy.

1. Use a 10X lens

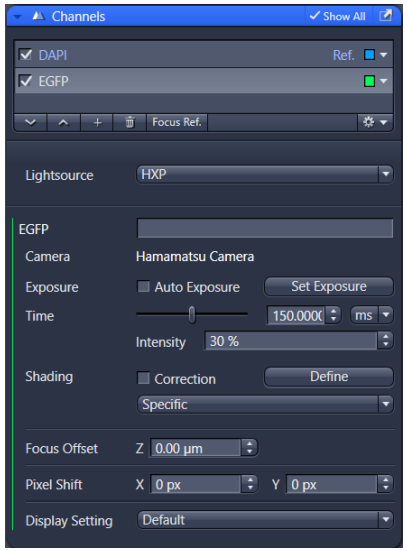
2. Move the sample as close as possible to the lens using the focus wheels (move UP = turning the focus know away from you) on both sides of the microscope or using the focus wheel at the touchscreen. Don't touch the sample with the lens.

3. Look through the ocular while **moving the lens down** (= turning the focus knobs towards you) from the lens until your object appears in focus.

3. Once in focus, use the joystick to move around

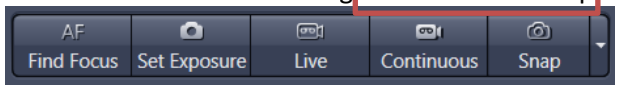
4. The button on top switched between coarse and fine sensitivity.

[2] https://www.zeiss.com/microscopy/int/solutions/reference/all-tutorials/basic-microscopy/microscope-alignment-for-koehler-illumination.html

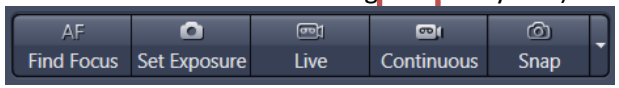


1. Channels list

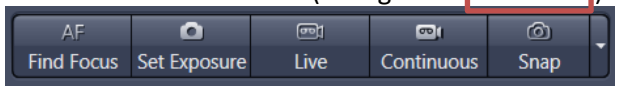
Ticked = active when starting Continuous or Snap



Selected = active when starting live (=only one)



Ref. = used for autofocus (change with Focus Ref.)



2. Lightsource

The lightsource for the selected channel (TL lamp for brightfield, HXP for fluorescence).

3. Channel settings

Click on the channel in the list to get the individual settings.

Exposure:
set the exposure time with the slider

Shading:
Can correct minor differences in illumination (e.g. dirt on camera or lens)

Pixel shift and focus offset:
Defaults are 0

Display setting:
automatically applied display setting after acquisition of the image (see next page)

Demonstration: Set up multiple channels

Prerequisites:

Brightfield source properly set

One fluorescence channel setup properly

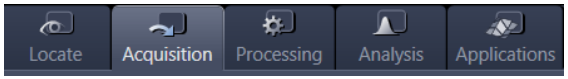
Action:

Use the Acquire tab

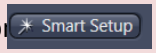
Choose the correct beam splitter settings

By now, you should see your object in brightfield and one channel through the camera.

Select the tab “Acquisition”



Experiment: select multiple channels

- In the tab “Acquisition”, find the button 

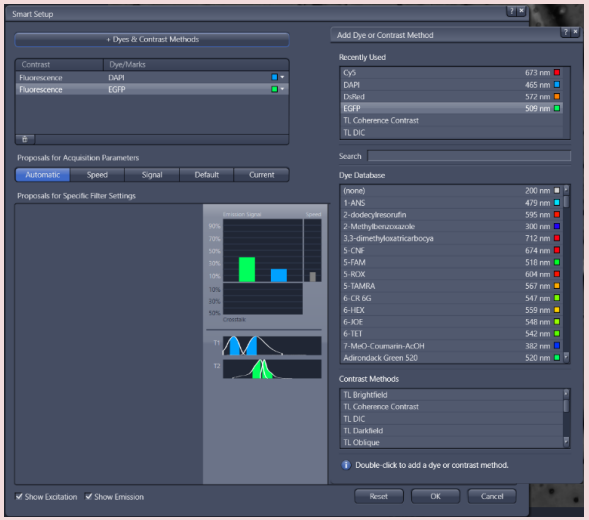
- From the smart setup:

Select the **dyes** from the Database.

You can also **Transmitted light** contrast modes

Use the Automatic Parameter (=2 channels)

You can now set the exposure time for each channel separately

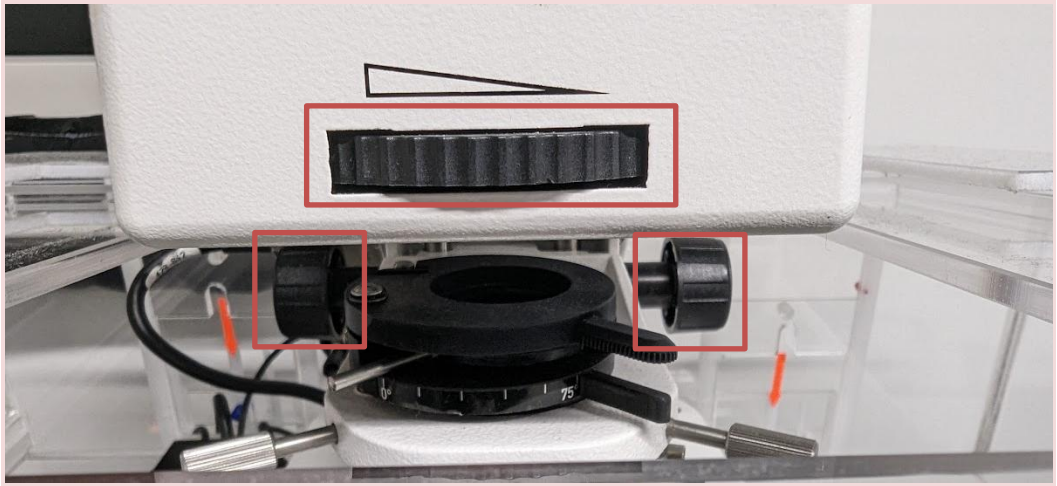


You cannot record two channels simultaneously

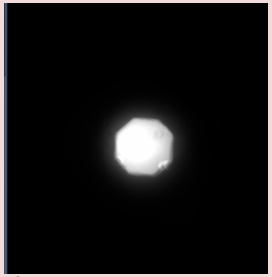
Experiment: Koehler illumination

1. Use the 10X lens, press ‘Make it visible’ (see above) and focus (see above). Remove any filter cube from the path (reflector position 4,5, or 6). TL = on, RL = off.

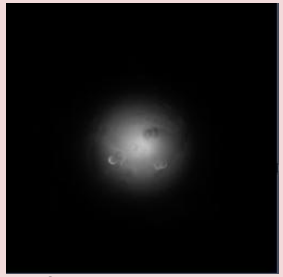
2. Turn the condenser aperture diaphragm wheel to the right. In the ocular, a spot of light will appear.



3. Adjust the height of the condensor with the knobs left or right of the condenser to **focus the edges** of the spot (look through the ocular).
4. Open the condensor aperture diaphragm (turn the wheel to the left) until no edges of the diaphragm are visible.



After alignment = good



Before Alignment=bad

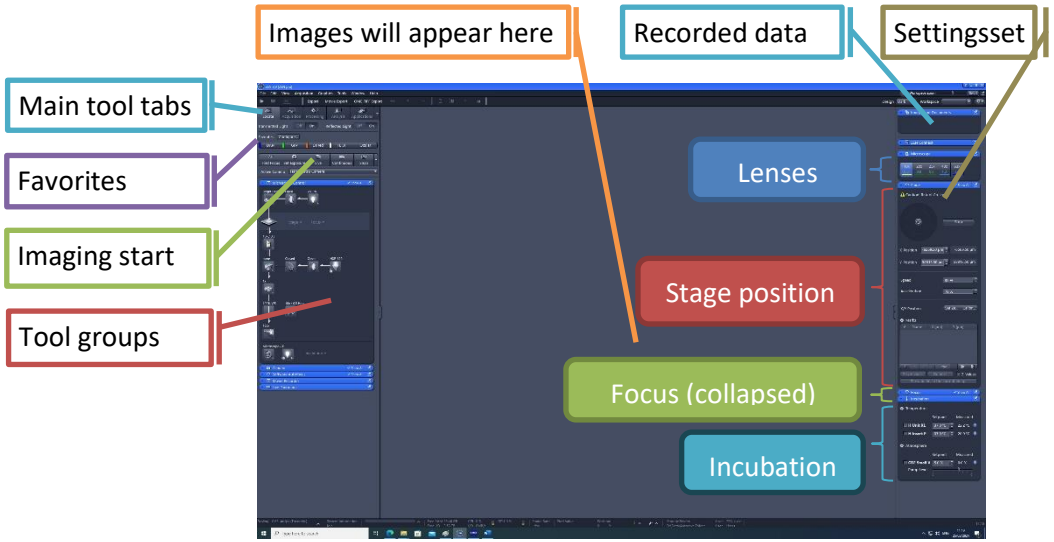
Demonstration: The Zen workspace

Prerequisites:

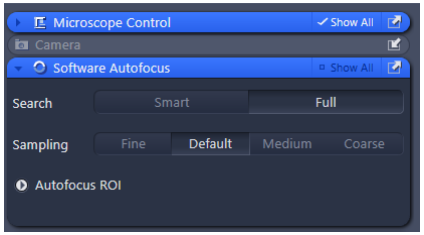
System successfully started and logged in

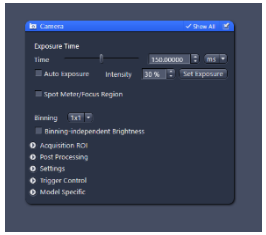
Köhler illumination

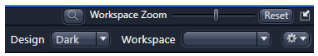
Get accustomed with the Zen 2010 workspace



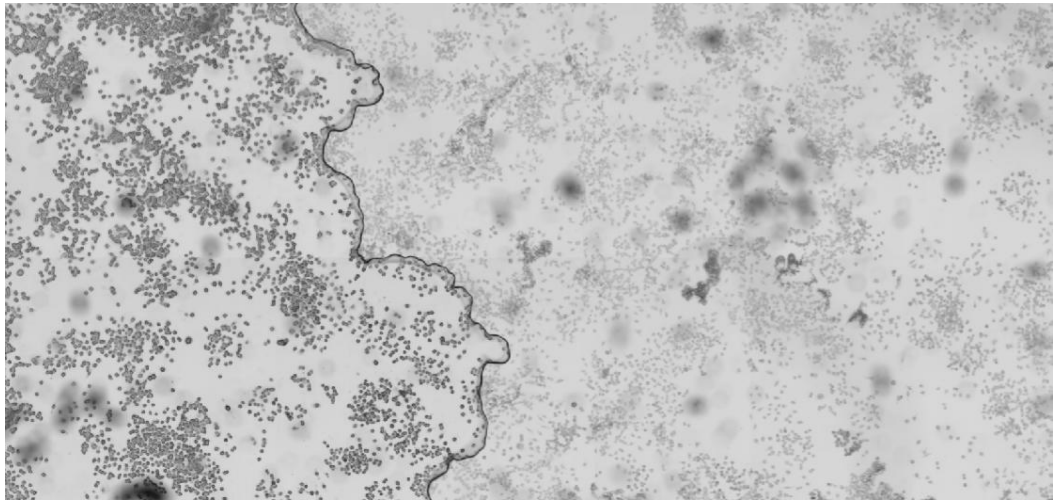
Collapsed, expanded and undocked tool groups







Top right: change zoom and appearance of the workspace. Save as personal settings.



Demonstration: Live panorama

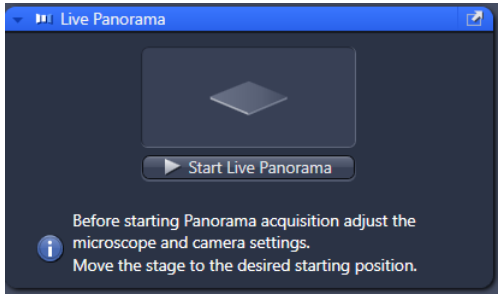
Prerequisites:

Brightfield source properly set, or fluorescence channel(s) setup properly.

Action:

Create a panorama with the live view and the joystick

The live panaroma will stitch movements with the joystick on the fly.



Your image now has a green border.

Live panorama

- Press the button on top of the joystick (you hear a beep), to select the fine movement

- Slowly move around and see how the panaroma is formed. Assure the border of your image remains green.

- If it turns red, you are moving too fast, slow down the joystick movement

When finished, click stop. You will be asked to generate a Image Pyramid. Click yes: this will greatly speed up how you can deal with this (large) data.

Ca. 5.0 mm by 3.0 mm image, collected in 30 seconds:

Demonstration: Set up the brightfield source

Prerequisites:

System successfully started and logged in

Köhler illumination, sample in focus in the ocular

Action:

Set up the light source

Avoid over- and under-illumination while maintaining a fast imaging speed

Under to tab “Locate”, find the following tools:

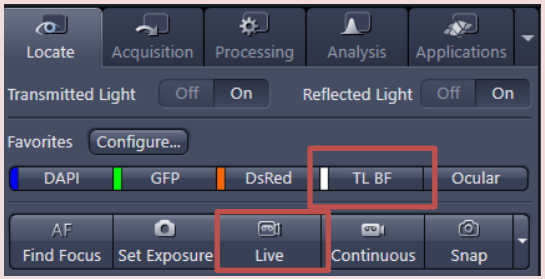
- Microscope Control
- Camera

Assure you can see the “display” (=histogram) at the bottom of the image

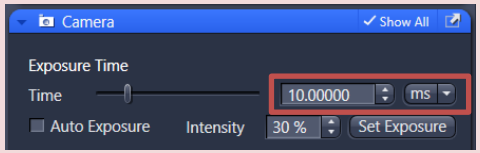
Experiment: Set up the light source

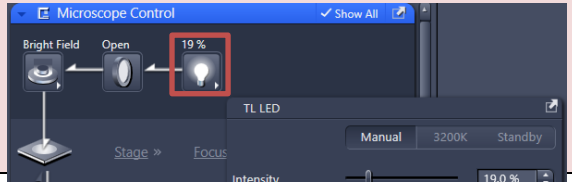
In the tab Locate, under favourites, click:
- TL BF (transmitted light, Brightfield)
- and then the button “Live”

You should now see an image in the centre of the workspace (or white)



- In the toolbox “Camera”, set the Exposure time to 10 ms.[3]
- In the toolbox “Microscope Control”, adjust the TL LED intensity to get good brightness

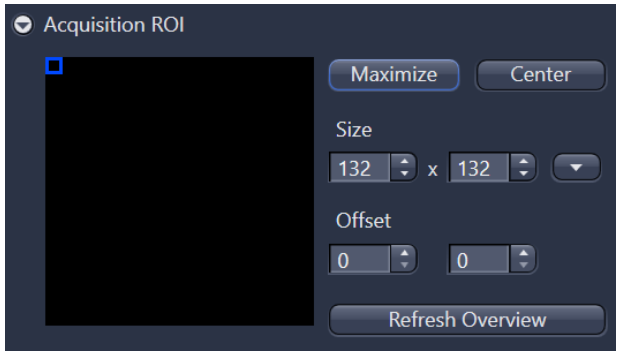


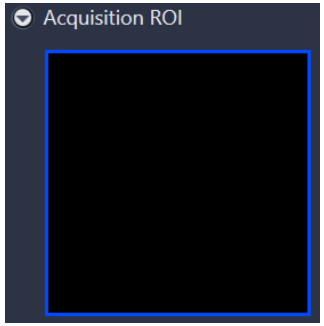


[3] This is not dwell time but total image acquisition time!

Notes:

1. You can use Auto Exposure if you are not sure what you are doing, however, better is to understand what you are doing.
2. I do not see anything or the image is blurry (and I focused it).
   In camera > Acquisition ROI (epand) > check that the entire field of view is used (click Maximize)





3. Exposure times under 5 ms will increasingly produce wavey patterns, as the camera readout cannot cope with the data being produced. 1 ms is the fastest scan time.

Experiment: a nice histogram

- Switch on “Auto” in the histogram
- Adjust the LED until your histogram spans almost the entire range

The histogram to the right is a good Histogram.

Demonstration: Record movie (1 fluorophore)

Prerequisites:

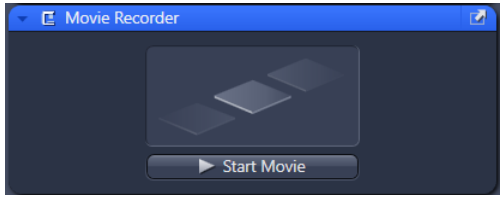
Brightfield source properly set, or fluorescence channel setup properly

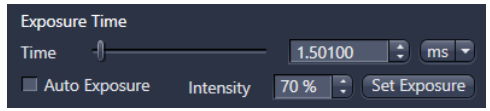
Action:

Record video

Recording time-lapse data

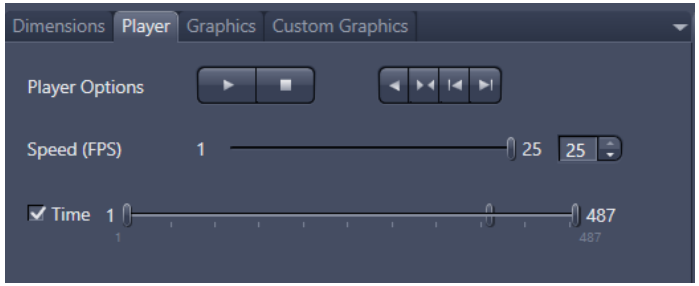
Note: the recording will use the exposure time set in “Camera” (see page 17). Autoexposure is not allowed (as the time must be a constant value).

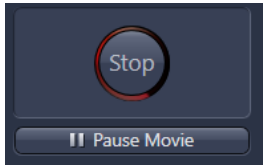




This will yield 666 images per second

The video can be played (at standard 24 frames/s) using the player in the central pane.





During recording, you have the possibility to pause the recording while waiting for an event to happen.

Demonstration: Software autofocusing

Prerequisites:

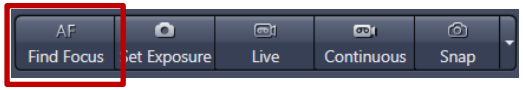
Brightfield source properly set

One fluorescence channel setup properly

Action:

Use autofocus

Autofocus can objectively set the focus, but may fail on thick samples



Mode: Use **AUTO**[4]

Quality: Use **Default**[5]

Search:

- Smart: use bidirectional scanning, finding a local maximum
- Full: unidirectional scanning, finding the maximum

Sampling (=step size):

Default: $d_z = \frac{2 \cdot n \cdot \lambda}{\sqrt{2} \cdot NA}$

Fine: $0.5 \cdot d_z$

Medium: $2 \cdot d_z$

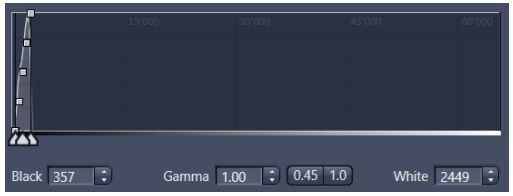
Coarse: $4 \cdot d_z$

Use relative range (assuming you have set the focus before through the ocular)

Press: "Find focus", and check with "Live"

[4] This will be the "contrast" method. Intensity method is useful on spinning disk systems.

[5] Low signal is useful when your sample covers only a small area (e.g. beads), or is noisy



Underillumination

- The auto brightness/contrast may still provide an acceptable image
- Flickering may occur

Remedy:
- Increase the TL LED setting
- If not possible: increase the exposure time

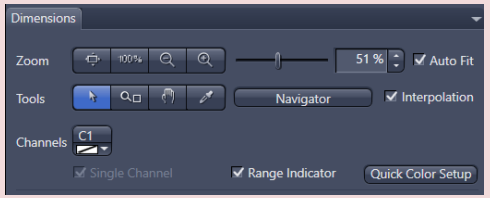
Overillumination

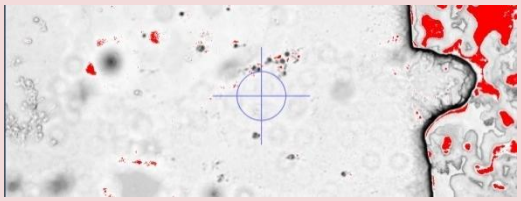
- Many red pixels as seen in the range indicator (see below)
- a peak at white value 65535,

Remedy:
- Decrease the TL LED setting
- If not possible: decrease the exposure time

Experiment: Range indicator

- At the bottom in the tab Dimensions
- Tick the range indicator
- Overilluminated pixels will turn red





Demonstration: Set up the fluorescence

Prerequisites:

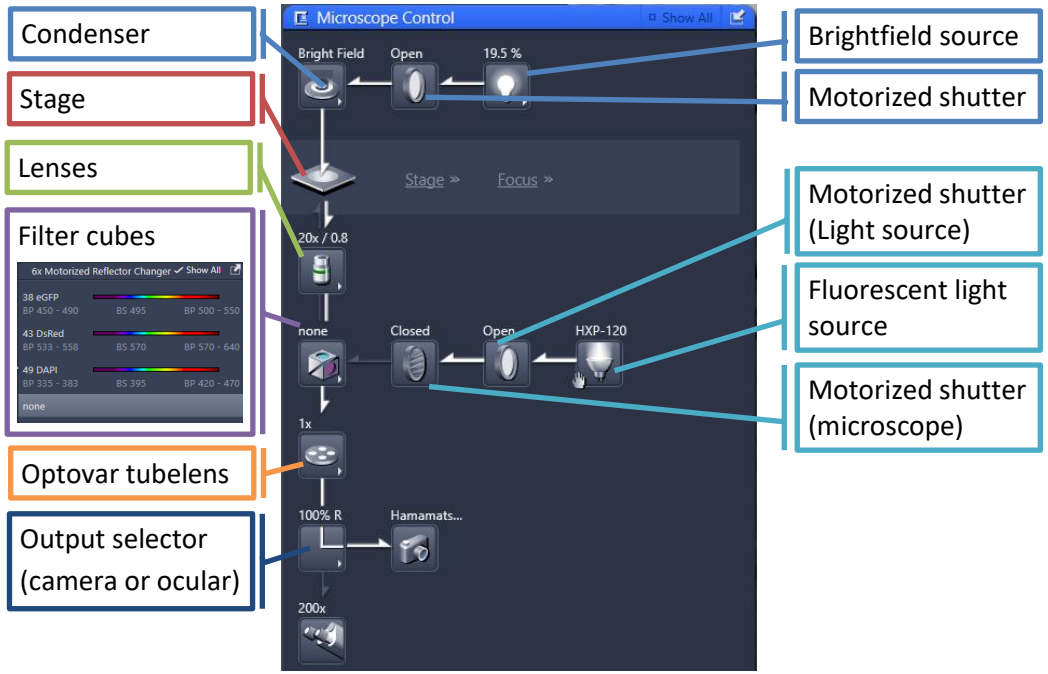
Köhler illumination, sample in focus in the ocular

Brightfield source properly set

Action:

Setup the fluorescence light source

White light and fluorescence light have different sources



Experiment: setup lightpath

- Make sure the shutters are open. The intensity on the HXP-120 is **not adjustable**
- Assure the correct filter cube is selected for your labelling
- Is the light going to the camera?

Exicting light	UV	Blue	Green
Emitted light	Blue	Green	Orange

Note: sequential channels will be added in the tab “Acquire”.

Experiment: Optimizing the histogram

The intensity on the HXP-120 is **not adjustable**.

Optimize the histogram (if possible) by increasing the exposure time