

Start system

Start the microscope system in the following order:

- Remove the blue dust cover and the protective cap from the eyepiece
 - Reset the eyepiece, rotate the ocular to the white dot at zero
- Fluorescent light source
 - Switch is located at the top of the lamp (#1)
 - The blue LED will blink when warming up
 - When the blue led stops blinking the lamp is ready for use



NOTE: Due to the heat the lamp needs to be on for at least one hour

- Microscope stand
 - Switch located at the left side of the stand (#2)
 - Check if the aperture wheel (A #3, right) is set at 3
- Camera
 - Switch located at the top of the camera, cooling will start in ~5sec
- PC and Zen software
 - Windows will log in to user account automatically
 - Start the program Zen blue
 - Select Zen Pro

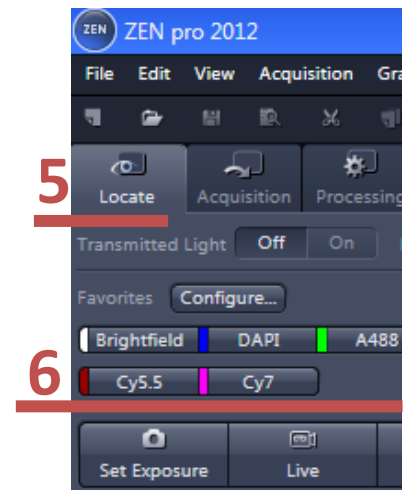


Focusing your sample

- Manually turn the 20x objective in place
 - Place your slide on the microscope
- NOTE:** Make sure no oil is present on the slide when using a dry objective
- Go to the tab Locate (#5) and select Bright field (#6)
 - Use the table to move your slide so that the edge of the slide or your tissue is exactly under the objective
 - Use this (glass) edge to find focus

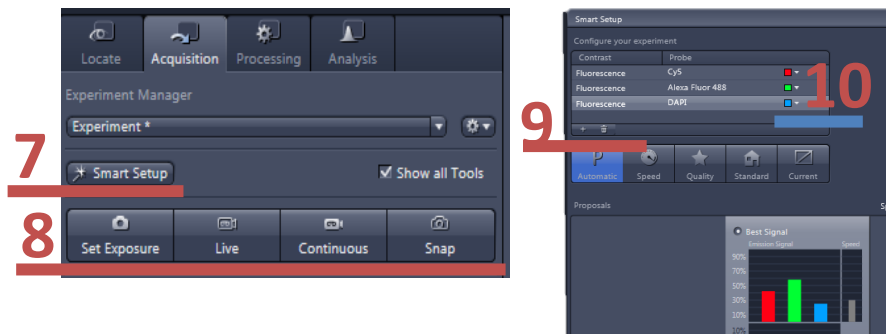
NOTE: This method will always work

- Move to the middle of your slide and check for fluorescence
 - Use one of the buttons to choose the correct filter for your staining (#6)
- Select the objective of choice
- Use these signals for fine focusing and/or finding your target
- Switch between eyepiece to camera with the metal pin
 - Located behind the eyepiece on the right side of the microscope (#4)
 - Pressed in = eyepiece
 - Pulled out = camera



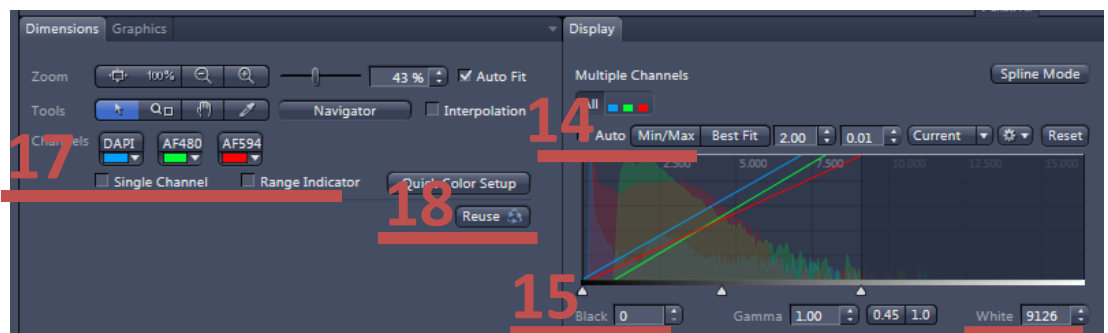
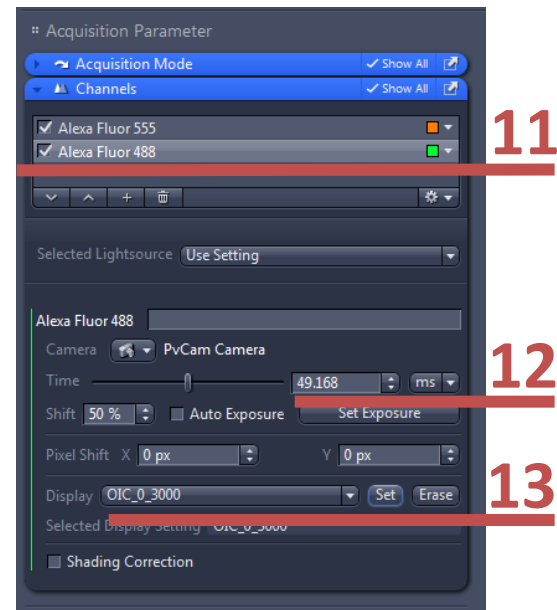
Setup the tracks

- Use smart setup to load the desired tracks (#7)
 - Use the + button to add tracks (#9)
 - Double click to add the dye to the list of tracks



Find optimal exposure time per track

- Select a track (#11)
 - If needed change the color of the track (#11)
- Set the exposure time to 300ms, for NIR dyes 1 second (#12)
- Start imaging with the button Live (#8)
- Check if you pulled the camera pin out and confirm the message about this manual component
- Inspect the histogram
 - Use the display setting: min/max (#14)
 - Look at minimum and maximum value below the histogram (#15)
 - Images are in 14bit which contain 16.384 (2^{14}) grayscale values
 - 2-5 times the background is sufficient for image analysis
- Adjust the exposure time to meet the required intensities
 - Exposure time of NIR dyes could be increased up to 30 seconds (or more)
- If the incremented exposure time is not sufficient for the required intensities change the aperture wheel (#3)
 - Lower aperture value equals more light at your sample
 - Range 1-6
 - Keep in mind that the light of all tracks is increased, adjust the other channels accordingly
- When acquiring an image press Snap (#8)
 - Make sure you've stopped Live mode, otherwise the first channel will not be acquired
 - All checked channels will be acquired in one multichannel image (#11)



Shut down system

- Bring the stage to its lowest position and remove your sample
- When you've used the 63x oil objective, **clean it!**
 - Use a tissue with ethanol to remove the oil
- Turn the 10x objective in place
- If changed, set the aperture wheel back to 3 (#3)
- Save your images
 - Save images in '.czi' format
 - Save images to the network drive O:\[your name]\

NOTE: No USB-drive can be connected with the PC

- Close the software
- Check the online scheduler if a user booked the microscope following your session.
If the next user starts within 1 hour: leave the system on, otherwise:
- Turn off the system in the following order:
 - PC and monitor
 - Camera
 - Microscope stand
 - Lamp; The blue LED will blink during the cooling down

NOTE: The lamp needs to cool down, it should be off for at least one hour.

- Bring the camera pin to the inwards positions
- Cover the eyepiece with the protective cap
- Cover the microscope stand with the blue dust cover

NOTE: Do not cover the lamp, only the microscope

- Write your session in the log, located next to the PC

Contact

For problems during your imaging session we urge you to contact us.

Also when you have questions about setting up an imaging experiment please feel free to contact us:

- Gert-Jan Kremers Be-346 tel. 43578
- Martijn de Gruiter Be-343 tel. 31105

Extra info

Display channels separately

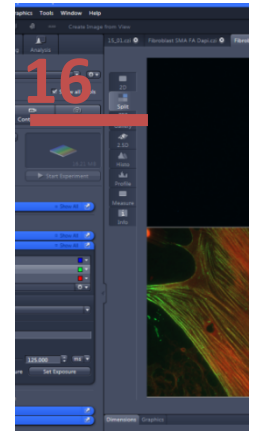
- Use the split mode (#16) to assess all channels and overlay separately at the same time.

Equal pre-defined scaling per track

- For equal comparison between tracks it is possible to auto-scale your histogram in a predefined manner
- Set range for every channel individually (#13) or (#14 & #15)
 - Most common choices are: 0-3000 or 0-5000

Display saturation

- To visualize the lowest and brightest areas in an image you can use the range indicator (#17)
- To visualize only one channel at the time, check Single channel (#17) and click on the channel buttons above
- Blue represents values below lowest auto-scale threshold
- Red represents values higher than the dynamic range of the camera, they are saturated
 - Pixels or objects which are saturated can't be analyzed, because the values are unknown and may over represent the size of the object, due to the locally high amount of light on the camera.



Use settings from previous acquired image

When repeating an experiment and you want to use the same settings without doing this manually you need to load that image acquired with this microscope

- Use the button Reuse (#18)
With this method all the tracks and exposure times used to acquire the image are loaded in the software.

Automatically save your acquired images

- Zen offers an AutoSave option
- Open the module and setup a folder and base name for the images
- Select a folder directly on the OIC network drive
- Every image acquired with Snap will be saved as a (multichannel) .czi (TIF format) file.

