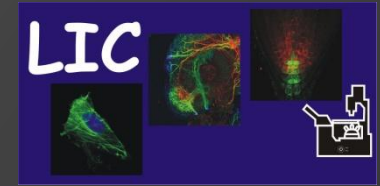
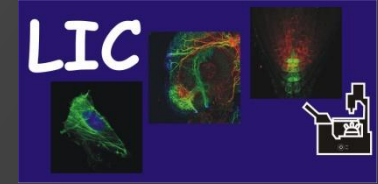


Topics

- How to calibrate the LSM scanner
- How to measure the PSF
- How to clean the microscope
- How to adjust the pinhole alignment
- How to adjust the Collimator





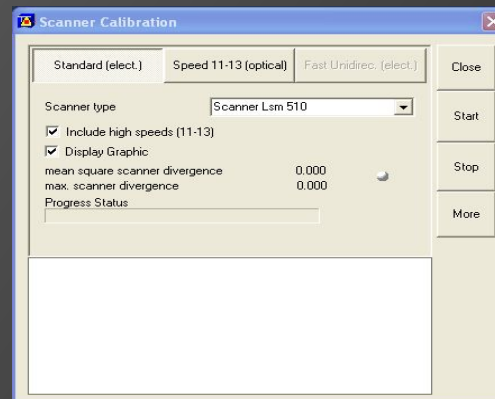
How to calibrate the LSM scanner

The scanner calibration is requested every month.

Therefore the microscope has to be switched on two hours before, to warm up. There is no need to switch the lasers on.

Start the Zen black software, then click start system.
In the upper line you will find under maintain [scanner calibration](#).

Open this window and activate
-Standard (elect.)
-Include high speeds (11-13)
-Display Graphic



Then start the calibration.
The new calibration will overwrite everything which is done before.
It will now take 5-10 minutes.

If the small dot in the middle of the window is grey or green, the calibration was successful and you can close the window.

If you also use the AIM 510 software, you have to do the same in that software tool.

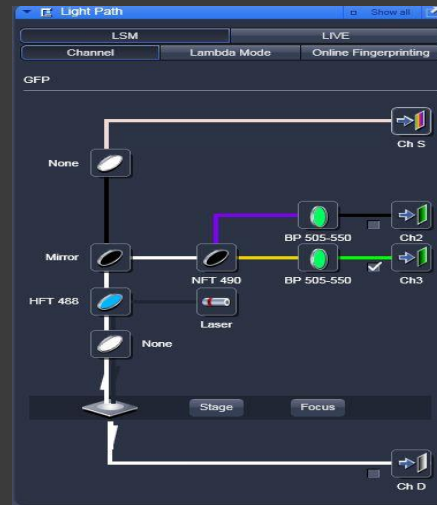
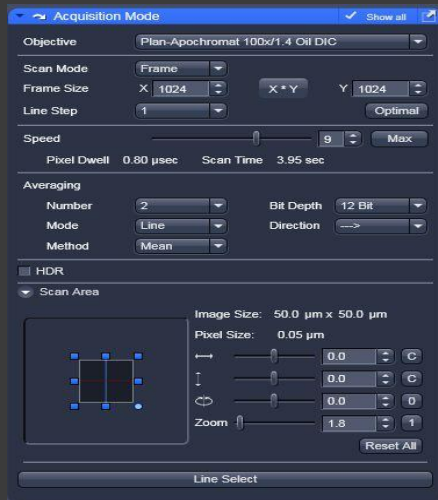


How to measure the PSF

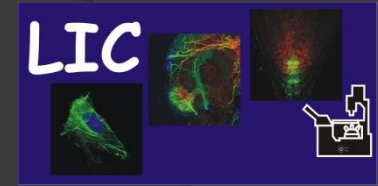
To measure the Point Spread Function you need a slide with 0,2µm objects - for example green fluorescent beads.

Start the ZEN software.

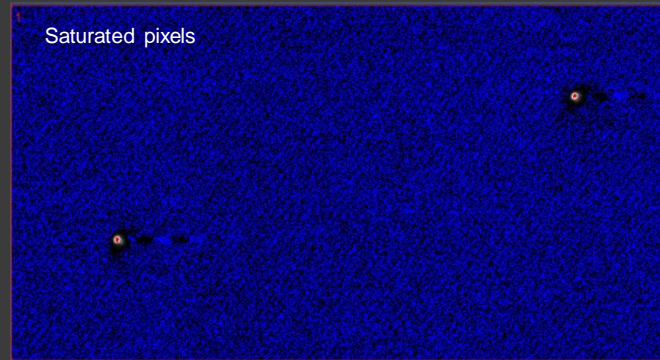
Under the **Light Path** window, set up the confocal for a green dye.



In the **Acquisition Mode** window set the **Frame** mode, **Frame Size** of 1024x1024 pixels, **Line Step** of 1. Scan **Speed** of 5-9, mean line averaging of 2-4 to reduce pixel noise, Bit Depth of 12 Bit, a Zoom factor of 3 and scan only one direction.



Find an area where you have 3 or 4 beads in a field of view but not overlapping.



The **Digital Offset** must be set above zero (~12) so that no pixels read zero intensities.

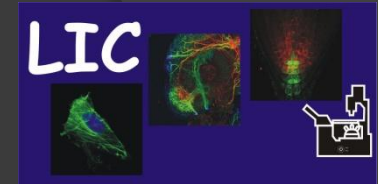
Choose the 488nm Laser which should be warmed up for 1h.
Set the **Laser power** not higher than 4% otherwise you may have saturated pixels.



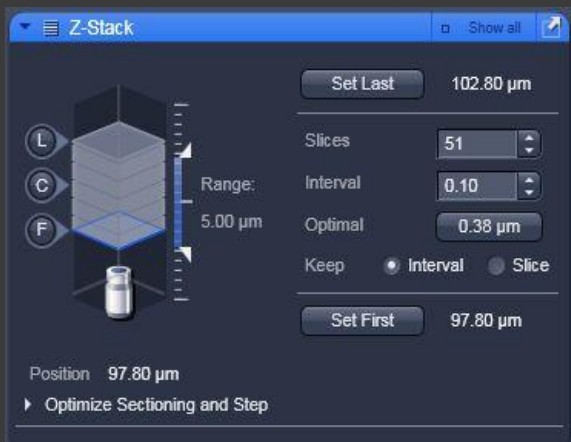
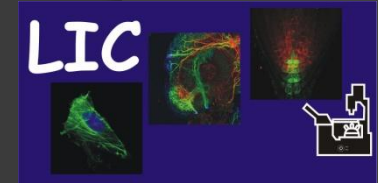
Set the **Pinhole** to 1 Airy unit. Take one overview image by pressing the **Snap** button.

Select the **Regions** and **Z-Stack** tab. Define a Z-stack over all these beads and make a snap.

In the **Ortho View** you can see if the beads are in the right orientation.



Select the **Regions** and **Z-Stack** tab.



Define a Z-stack over all these beads and start experiment.

Now you can open your data in the Huygens Software to calculate the PSF.

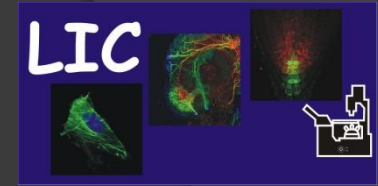
<http://cletus.lic.zbsa.privat/hrm/>

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How to clean the microscope

1. Check the correct position of the objectives (twice per week).
2. Clean the objectives and oculars with absolute ethanol (twice per week).
3. Special cleaning with special cleaning solution (weekly).
4. Check DIC slider (twice per week).
5. Check Filtercube positions (twice per week).
6. Check the motorized table (twice per week).
7. Check the syringes and tips for immersion medium (twice per week).
8. Check flasks abs.EtOH, water, oil, glycerin and wateroil (twice per week).
9. Check spray bottle dest. Water (twice per week).
10. Check spray bottle EtOH 70% (twice per week).
11. Check cotton wool and rod (twice per week).
12. Check Kimwipes (twice per week).
13. Check special table inserts (twice per week).
14. Check all wastes (twice per week).
15. Vacuum clean the room (weekly).
16. Wipe the room (weekly).
17. Check the aqua stop bottle (twice per week).

18. Check the storage capacity of the Computer (weekly).
19. Delete separated user accounts (quarterly).



How to adjust the pinhole alignment

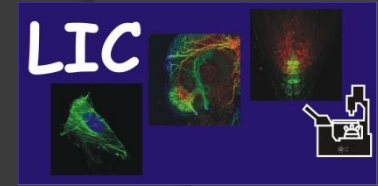
To align the pinhole means to find the correct position in the X-Y plane. To collect the emission light from the region of your sample you are exciting, the pinhole must be properly aligned with the focal point of the lens.

You should do this once a month to see the drift in the system.

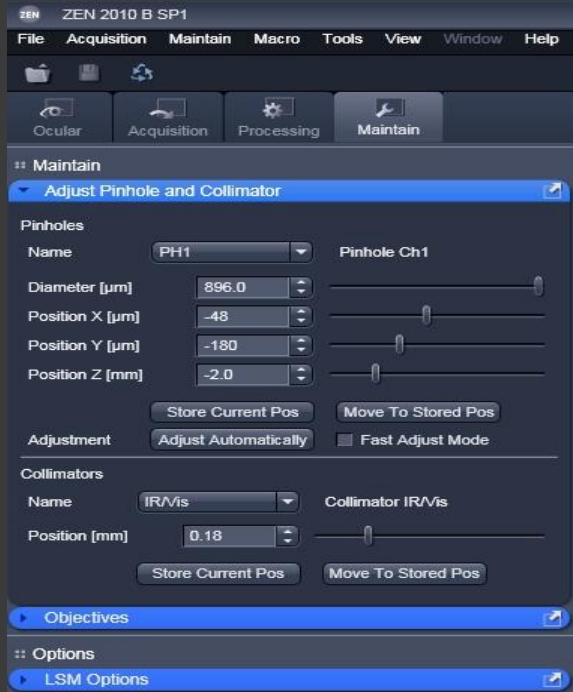
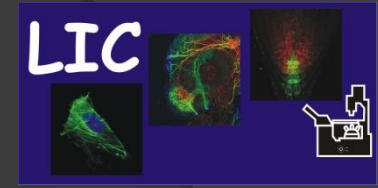
1. Use a *Convallaria* sample.
2. Click in the ZEN software on Maintain (right side on tool bar) and select Pinhole.
3. A new window is popping up, in this window you select PH1, PH2 or PH3 depending on the configuration you want to align.
4. Select one configuration and verify that the detector for the selected track is the same as in the pinhole window.
5. Find a good homogenous region in your sample.
6. Set the gain and the laser power such that the signal is not overexposed.
7. Press continue or fast scan and move the slides for x and y direction to the left or right side and store the position where you can see the strongest signal and the most precise image.
8. If the pinhole was incorrectly aligned you can see a noticeable difference.
9. The brighter the signal is the closer the pinhole is properly aligned.
10. Do not forget to press the store pinhole button, otherwise the system will move back to the previous alignment.

Each track for a configuration has a different pinhole setting. The alignment you save will be loaded when you click the Reuse button on an image to load the configuration.

The alignment may drift a little, so you have to manually adjust the pinhole every few months.



How to adjust the Collimator



Start the Zen software.

Under **Maintain** you find the **adjust Pinhole and Collimator** window.

Use a 15µm bead labeled in green with a blue ring on the outside.

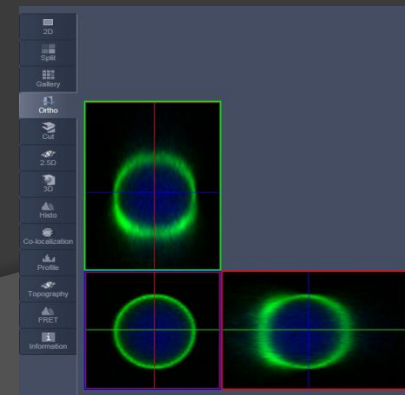
Set a Light Path for GFP and DAPI and define a z-stack over this bead.

You have to make sure, that the pinhole setting for GFP and DAPI is correct.

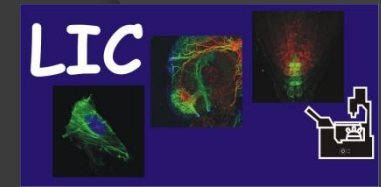
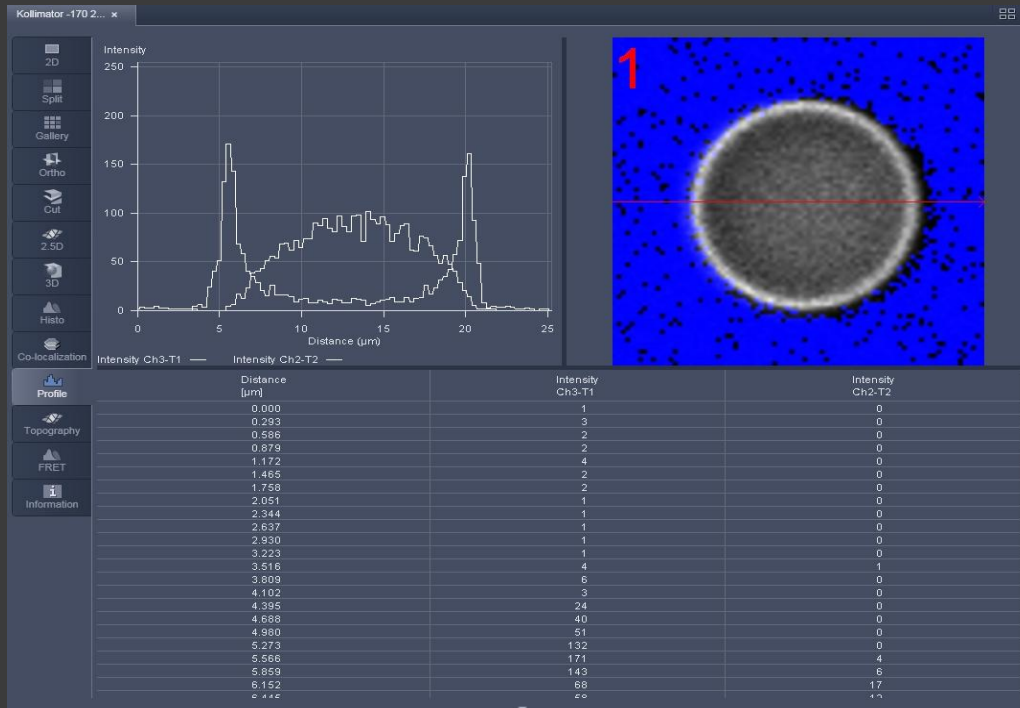
Now adjust each channel in the way that you have no red pixels and only a few blue pixels.

Start the z-stack with **start experiment** and look in the ortho view.

The blue signal should be in the middle and no overlapping with the green outer ring.



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When you look in the Profil view the GFP signal should be in the middle and two DAPI peaks left and right.

If the DAPI peak is not in the middle and you see a shift, you have to adjust the collimator.

Most you have two different Collimators, one is IR/VIS and the other UV/VIS.

When the IR/VIS is well aligned you can go step by step with the [position\(nm\)](#) cursor to the left or right as far as the blue signal is directly in the middle.