Light-sheet fluorescence microscopy

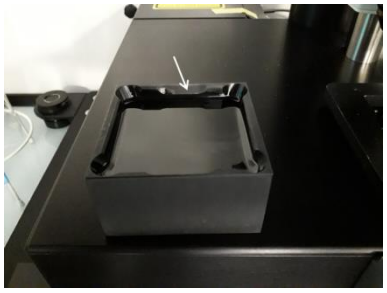
User Manual

Contents

How to Start

1. Switch on the microscope according to the startup procedure.

2. Fill the cuvette with clearing reagent. The maximum filling level is indicated via indentations on the inside of the cuvette (white arrow).

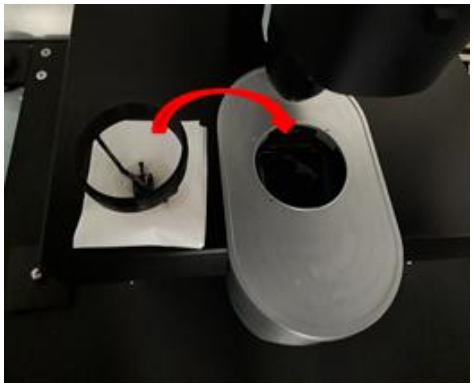


3. Remove the metal table. By using the special plunger, place the cuvette inside of the imaging chamber and put the metal table back to its original place.

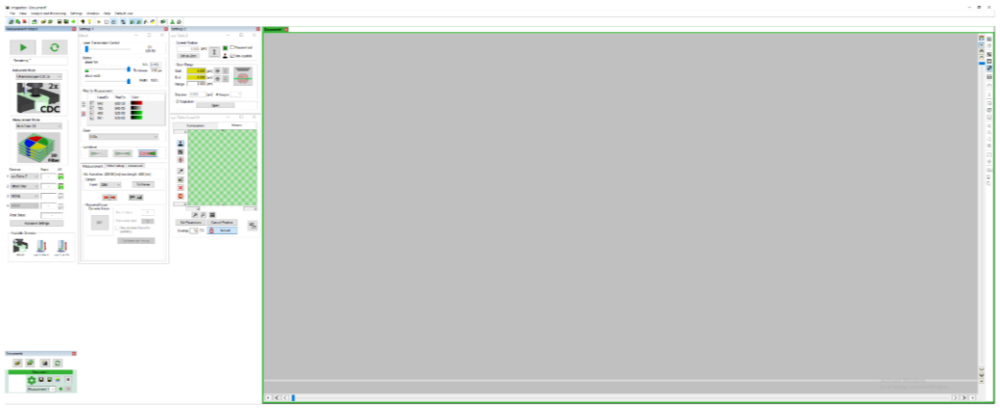
4. Select a sample holder for your measurement, fix your sample on it, place it into the sample mount, and lower this into the imaging chamber.

Sample holders

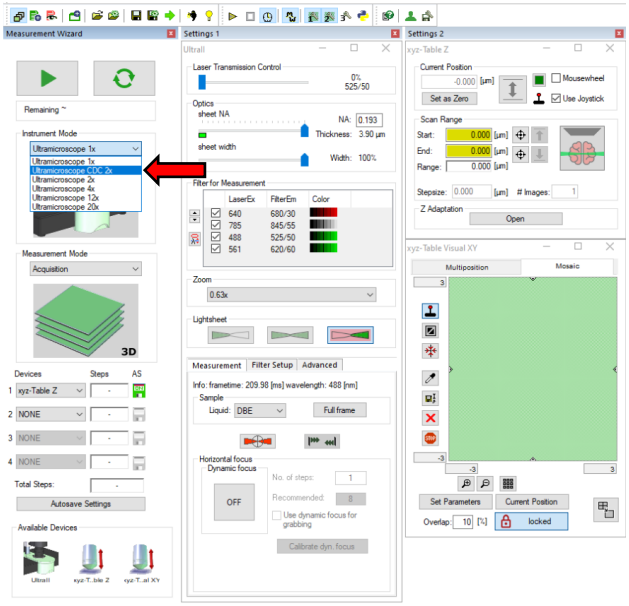
Sample mount



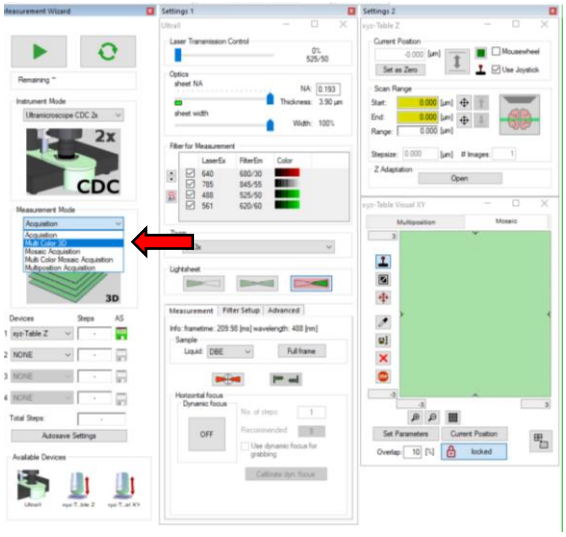
5. Start the ImspectorPro Software



6. Choose the correct camera (CDC 2x) in the “Instrument mode” dropdown (red arrow)

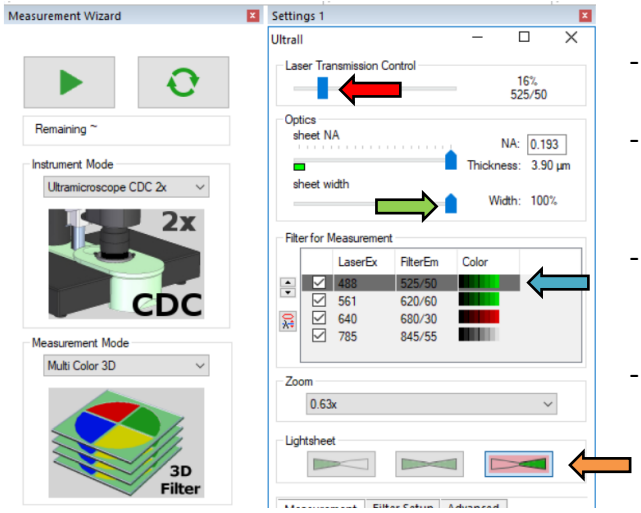


7. Activate the Measurement Mode “Multi color 3D” (red arrow)



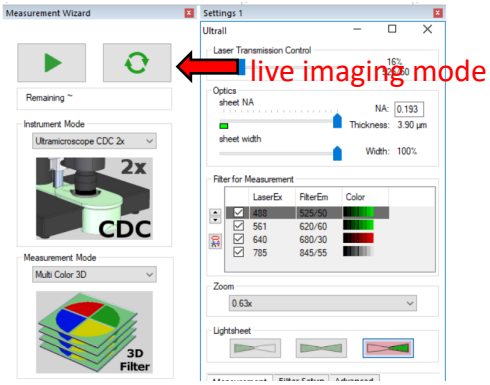
8. Select a zoom factor at the microscope and corresponding one in the software (red arrows).

9. Define the excitation settings in the ImSpector software:

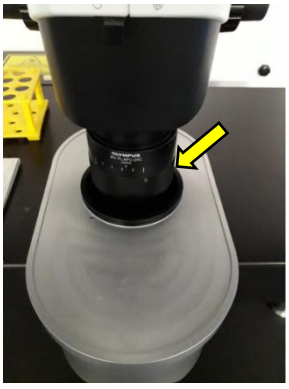


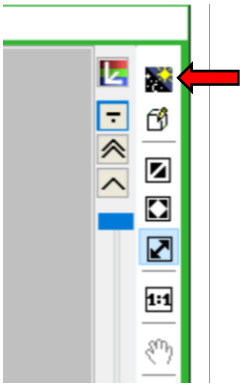
- set the laser power to 10%
- Increase "sheet width" to maximum (100%)
- select 488 nm as an excitation wavelength
- activate the right-side illumination button

10. For live imaging start the "live imaging mode" (red arrow) in the "Measurement Wizard" dialog. Check the proper sample illumination in the imaging chamber and use the joystick to find the right position for you sample.



11. Lower the objective lens into the clearing solution (yellow arrow) using the focus drive (green arrow). Focus your sample. For example, to make your image lighter, use the contrast "Wizard" (red arrow) symbol. You can also move in the Z plane (up and down) using the joystick to find a better view of your sample.

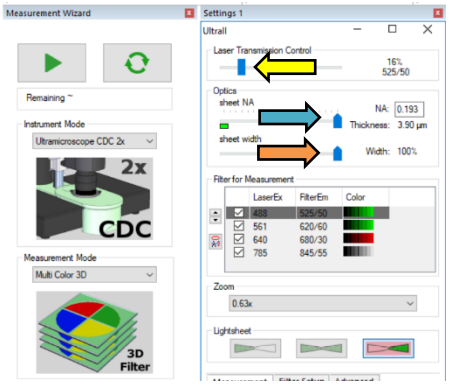


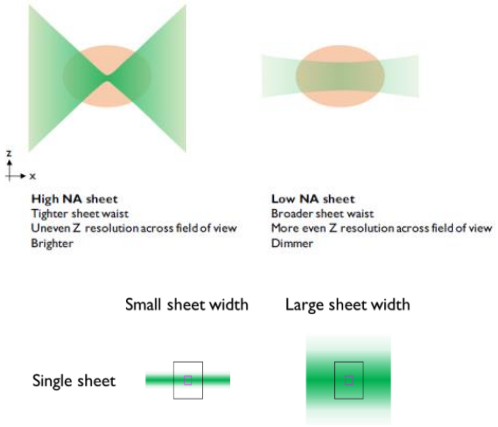


Multi Color 3D Imaging

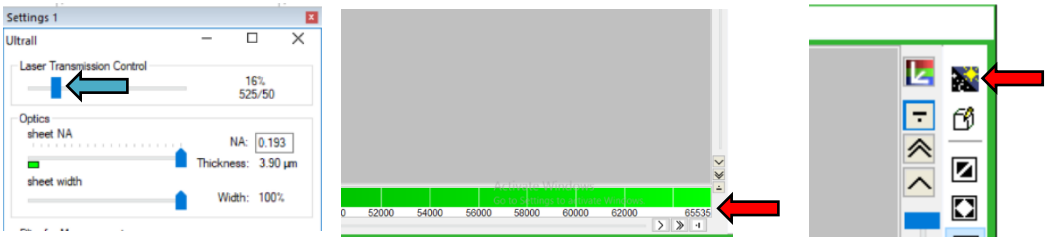
1. Activate the live imaging mode (yellow arrow)

2. Activate the 488 nm laser (yellow arrow)
3. Adjust your sample illumination via the sheet NA adjustment (blue arrow) and the sheet width (orange arrow)

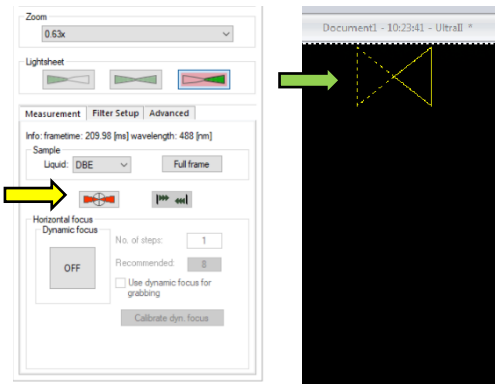




4. Adjust sample illumination by regulating the laser transmission (blue arrow). Check the grey values of several z-positions within your sample via the auto-contrast button to ensure optimal laser transmission settings (red arrows).



5. Position the sheet waist to your region of interest by activating the horizontal focus button (yellow arrow) and moving the focus point in X dimension (green arrow).



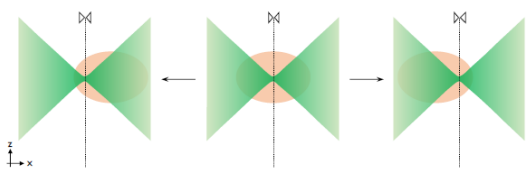
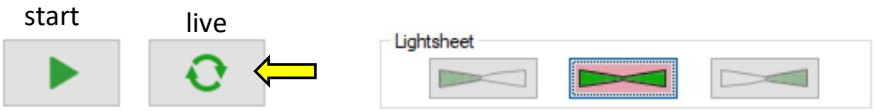


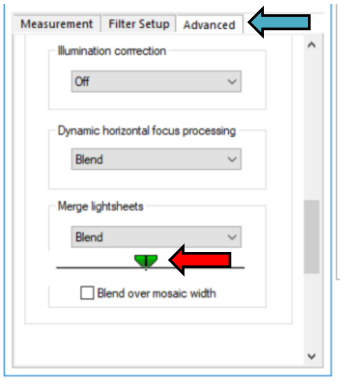
Figure 8.8. The horizontal focus can be moved to shift the location of the light sheet's waist (where it is narrowest in Z) in the X dimension.

6. Switch from the right-side illumination to left-side illumination and position the sheet waist according to step 5.

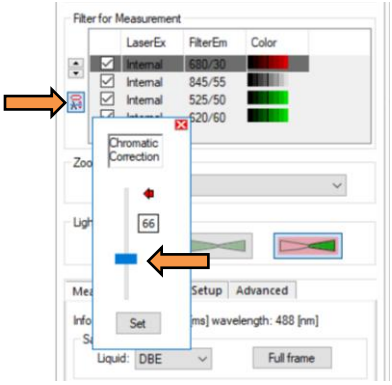
7. Stop the live imaging mode (yellow arrow), activate the two-sided illumination mode, and activate the live mode again



8. Switch from “measurement” to “advanced” mode (blue arrow)
9. Scroll down to the “Merge lightsheets” field and define the blending range by the green sliders (red arrow).

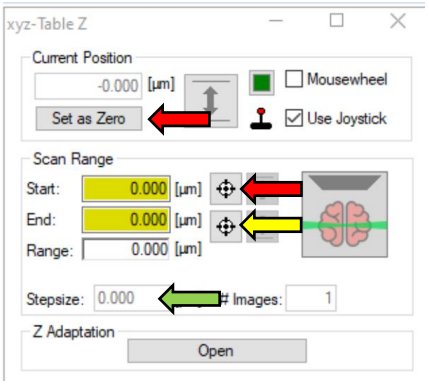


10. Activate next excitation laser according to step 2 and adjust the chromatic shift via the “chromatic correction” tool (orange arrows)
11. Adjust the sample excitation for this laser line according to step 4

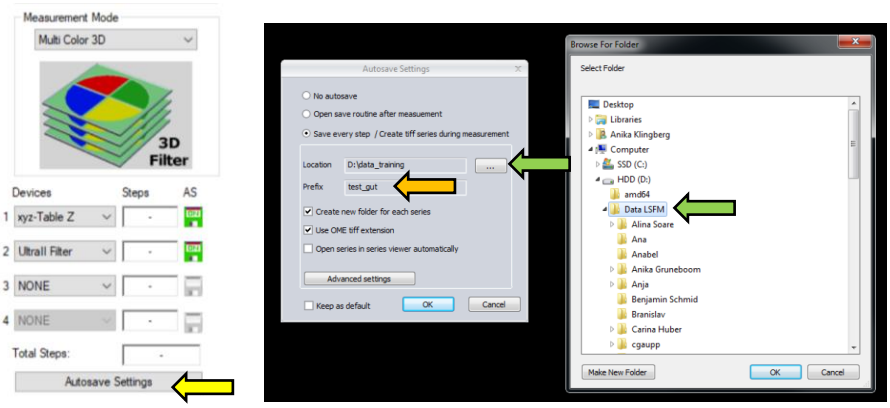


12. Repeat step 10-11 for all required excitation lasers.

13. Define start position of your z-stack by setting the current position as zero and confirm zero-position as start-position (red arrows)
14. Define the endpoint of your z-stack (yellow arrow)
15. Adjust the z-step size according to the defined sheet thickness (eg. 5µm, green arrow)



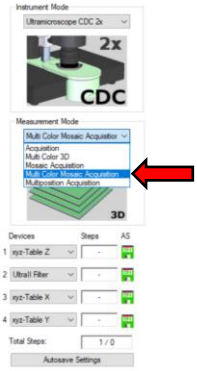
16. Stop the live-mode and define the autosave settings (yellow arrow) including the path to the target folder (green arrows) and the file name (orange arrow)



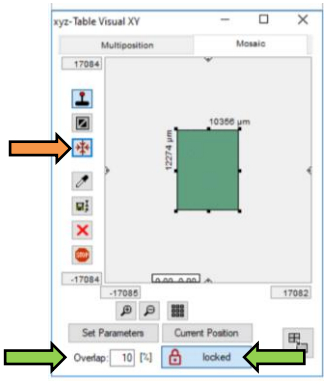
17. Start the measurement

Mosaic Imaging

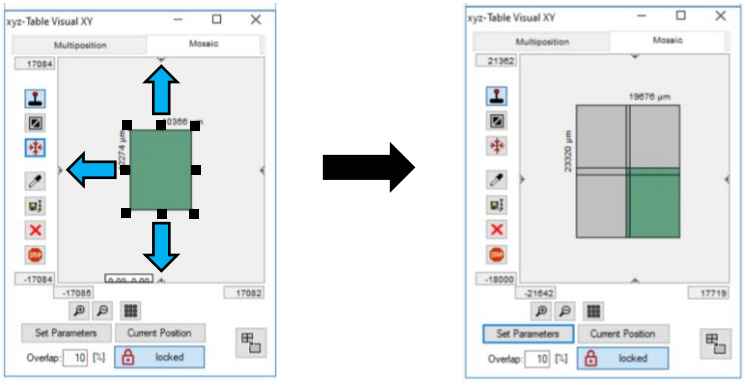
1. Adjust your settings according to “Multi color 3D imaging” step 1 to 12
2. Activate the “Multi Color Mosaic Acquisition” Measurement Mode (red arrow)



3. Define the centre position of the mosaic via the joystick
4. Safe the centre position by the “Define Mosaic” Button (orange arrow)
5. Set the mosaic overlap to 10% and save settings via the “locked” button (green arrows)



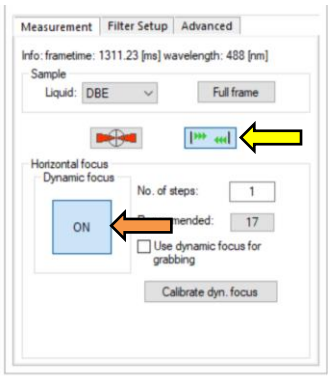
6. For defining further mosaic positions move with the cursor on the centre mosaic position. The black squares appear around the centre position.
7. Move the black squares in x or y position to define the entire mosaic (blue arrows).



7. Proceed with “Multi color 3D imaging” step 13 to 17 for defining the z-step and starting the measurement.

Dynamic horizontal focus

1. Adjust your settings according to “Multi color 3D imaging” step 1 to 12
2. Activate the “dynamic focus” button (orange arrow)
3. Activate the dynamic focus area (yellow arrow)



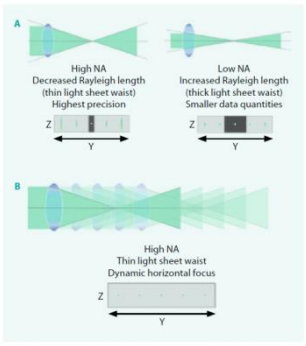
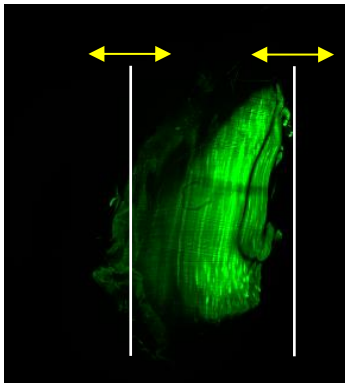
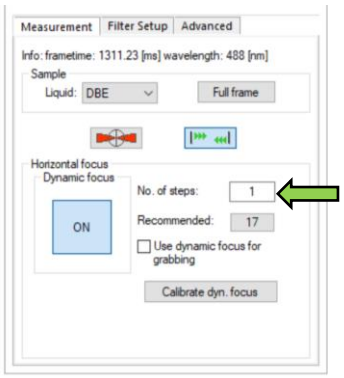


Figure 2: Trade-off between field of view, light sheet thickness and image quality (A). Dynamic horizontal focus (B).

4. Define the dynamic focus range by moving the white lines in X dimension (yellow arrows)



5. Adjust the number of imaging steps according to the recommended number (green arrow)



6. Proceed with “Multi color 3D imaging” step 13 to 17 for defining the z-step and starting the measurement