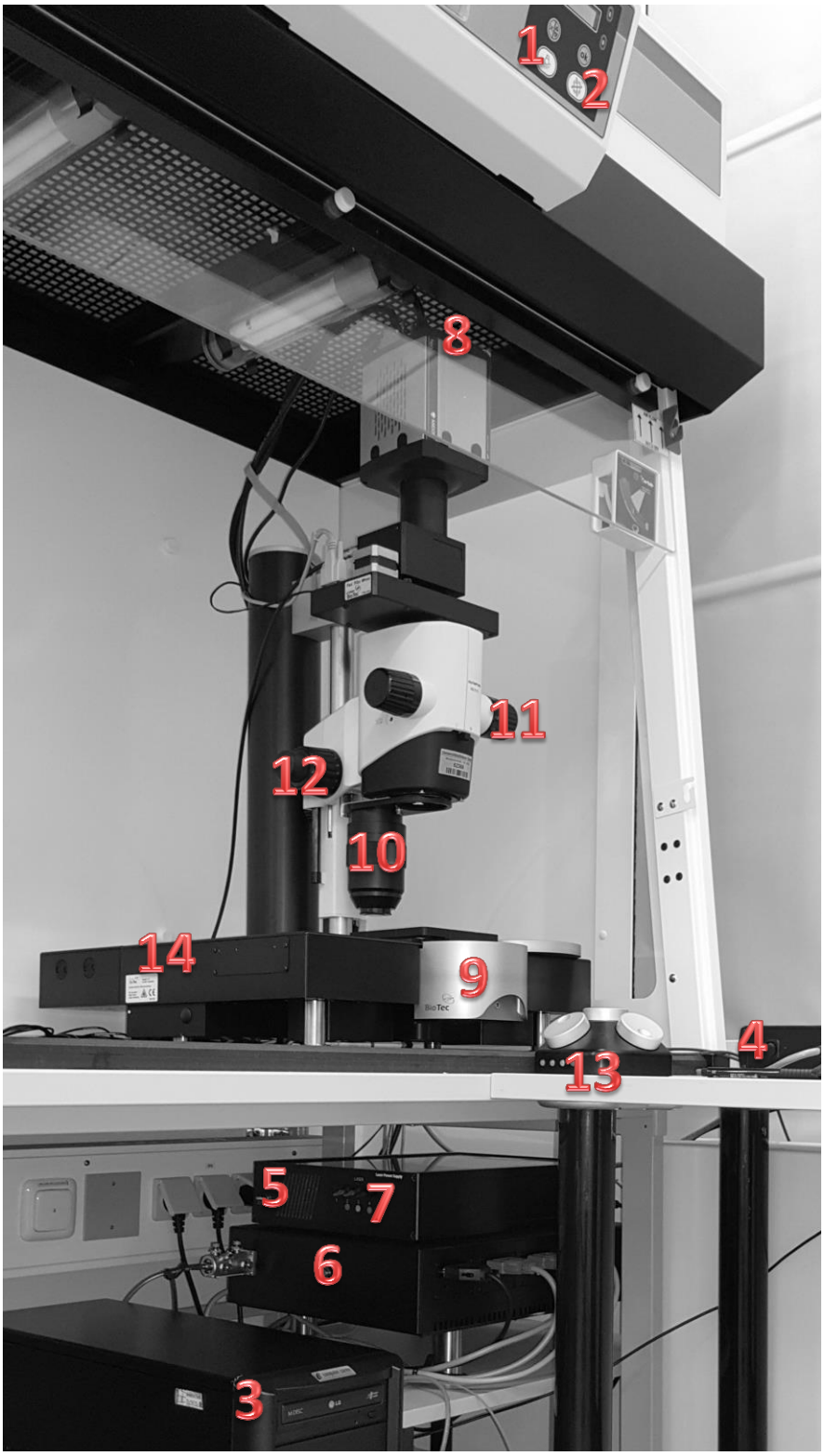
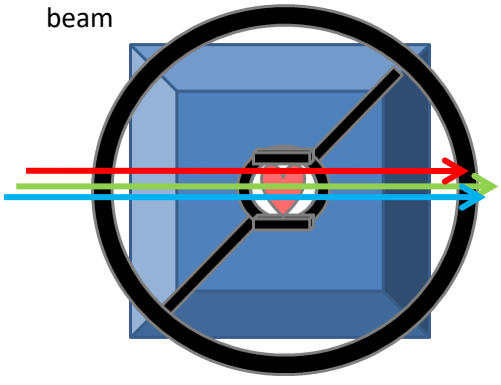
Start up procedure

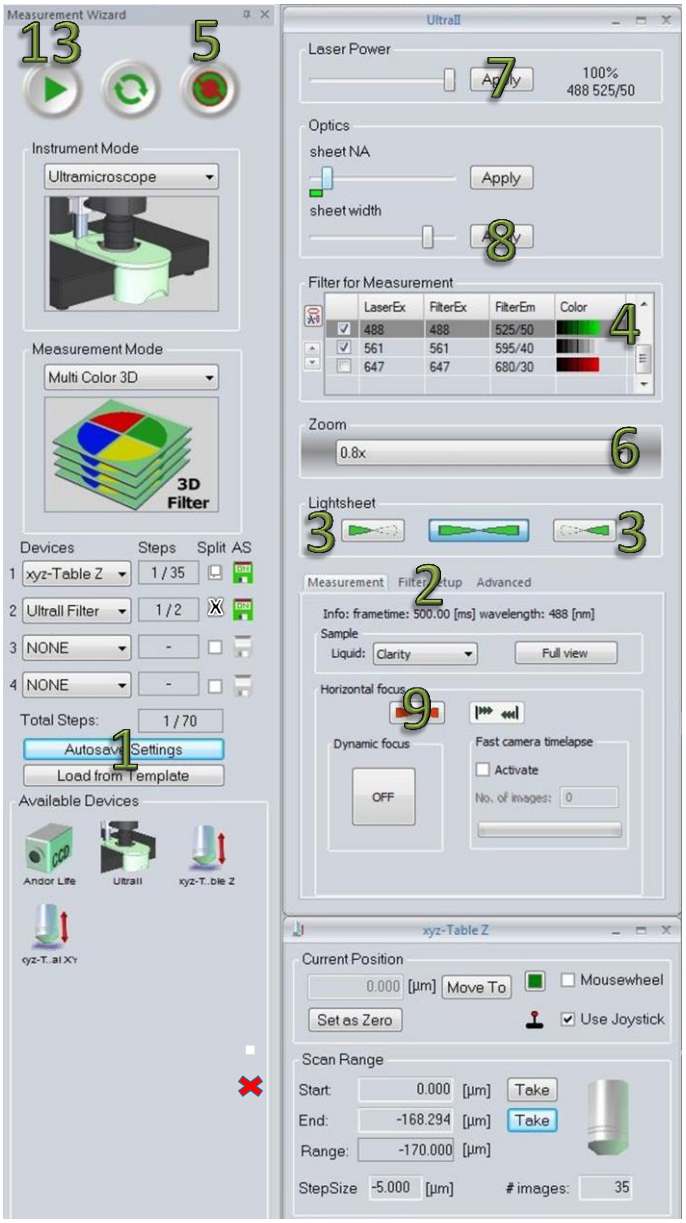


- Activate the timer to use the system
- Turn ON the Light <1>
- And the fan <2> (always active)
- Switch ON the computer <3>
- And the instrument power supply <4>
- Turn ON the laser unit <5>
- And the OPSL <6>
- Then use the laser-line toggle switches <7>
- Activate the camera <8>
- Fill the glass cube with your imaging solution
- Place the cube into the microscope. Use the stamp below the stage as a support.
- Close the cube-chamber with the gray lid <9>
- Place your sample in one of the sample holders
- Take care for the right orientation of the sample according to the laser beam

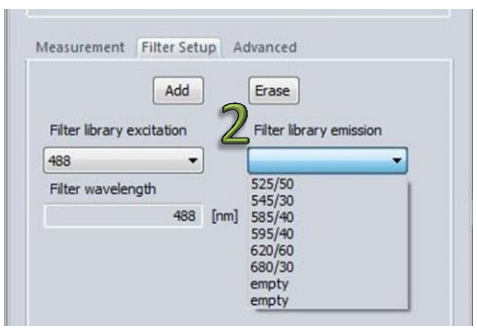


- Place the sample holder with your specimen into the imaging chamber
- Use the smallest magnification <11>

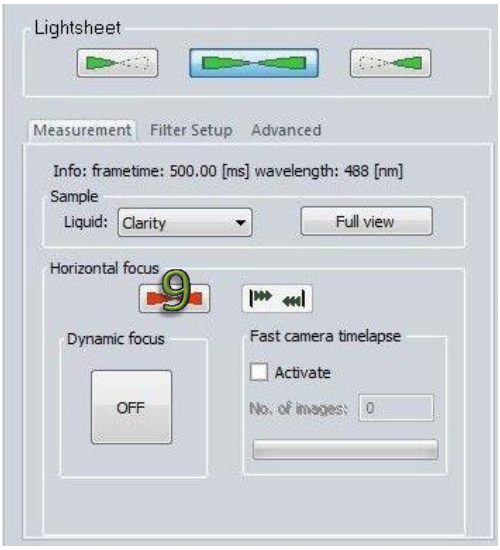
The Software

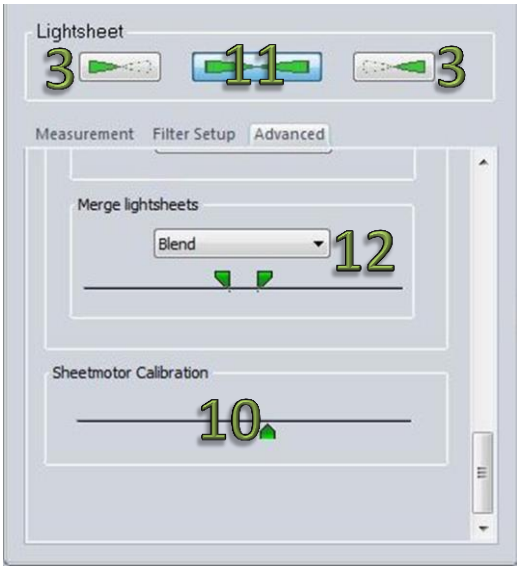


- Start the Imspector Software
- Open the ├Autosave Settings┤ <1>
  and use the correct folder location for saving your data
- Choose one of the preset laser-setups <4>
- or define yourself from the library <2>
- To set up your images use the ├Right┤ light sheet button <3>
- Start your imaging process with pushing the “record button” |- Live-| <5>
- Take care to put you specimen in the range of the light sheet with the x-y-z adjustment wheels <13> (to do this check with your eyes if your specimen glows bright in the illumination chamber)
- If the instrument is set to the smallest magnification <11> update the Zoomfactor <6> in the software and always correct it after changing the magnification!!!
- Rotate the objective arm 180° <10>
- Now move the Objective into the imaging solution with the coarse focus wheel <12>
- Just when you think the objective might dip into the imaging solution only look at the screen for finding your sample
- To set up a better contrast on screen: draw an area around a bright and/or dark area and hit the contrast wizard symbol in the upper right corner of the software 
- Use the coarse focus wheel until you can see your sample in focus



Please note: If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements.





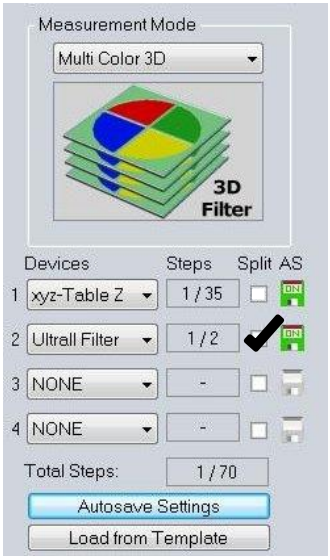
- For changing the laser intensity choose the Laser Power slider and press |-Apply-| <7>
- Adjust sheet width <8> to the size of your sample
  The best z-position to adjust the sheet width is in the middle of your sample
- Move to the top of your specimen (in z) and bring it to the center of the screen (x & y)
- Activate the horizontal focus <9>
- set “sheet N.A.” to the smallest possible
- Move the Horizontal focus triangle to the edge of your specimen
- Switch from the “Measurement” to the “Advanced” tab and scroll down
- Now adjust the focus of the lightsheet by moving the green arrow of the “Sheetmotor calibration” <10> (choose the point when the edge of your specimen appears the smallest and as sharp as possible)
- Now “save as default” (task bar upper left)
  After you’ve done this with the right light sheet repeat the procedure with the other one
- Now bring the left & the right horizontal focus to the middle of the image (use the crosshair for orientation)
- Deactivate the laser <5>
- Activate both lightsheets at once <11>
- Start the laser again <5>
- bring the two green “Merge lightsheet” triangles so close together that they touch each other <12>
- now you observe a line separating your image
- Adjust the left lightsheet to the correct focal plane with the screw driver <14> (at a certain point you can see structures matching at the border of the right and left lightsheet)
  If you are done tear the triangles apart to get a smoother transition
- Stop the live measurement and activate the measurement again with one Lightsheet only
- Readjust the horizontal focus triangles with the “sheet N.A”. Set the “sheet N.A.” half the size of your sample and place it in the middle of the left/right specimen half. Let them overlap in the middle / or use smallest horizontal focus to use the dynamic focus option
- Deactivate the “horizontal focus” <9>

Objective settings

- It´s important for data analysis that you always update the "Zoom" in the Software <6> according to the Hardware Zoom <11>
- If you changed the zoom after setting up the horizontal focus you have to check the setting again!

Filter settings

- If you want to acquire a **multicolor** image / Z-stack you have to tick the tickboxes of the desired laserlines <4>
- Choose the option "Ultra Filter" in the second "Devices" drop down menu
- Focus in the green channel and use the "**chromatic correction**" for all other channels. For this option you have to perform a "right-click" on the laser line you want and move the appearing slide bar till you get the best focus. Press "set" and close the pop up window.
- Make sure the green (AS) floppy disks are in a dark green color having the label "ON" in the middle.

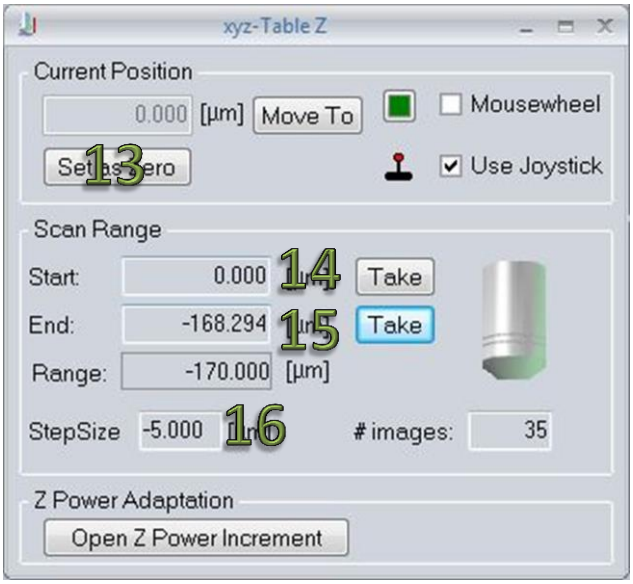


Acquiring single images

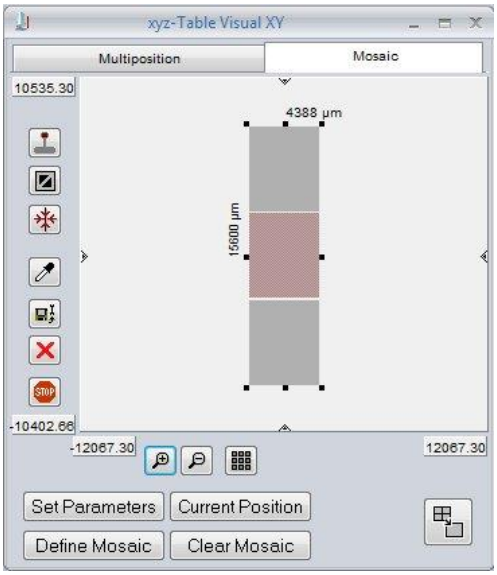
- If you only want to acquire a single image make sure you set the "Autosave Settings" <1> properly
- Activate the laserline <4>
- Tick as many laserlines you need <4>
- Bring your sample to the position you want <13> to image and
- Choose the "xyz Table Z" menu
- press "set as zero" <13> ; Start-position "take" <14>and End-position "Take" <15>
- Press the "Start measurement" button

Acquiring a Z-stack

- After adjusting the Software settings activate a laser line <4> of only one lightsheet and press the Live button <5>
- Go to the top of your specimen, press “Set as Zero” <13> and “Take” <15>
- Then move to the bottom of your sample and press “Take” <14> (if you start with the top, or bottom doesn´t matter)
- Now choose a “StepSize” value for e.g. 5µm <16>
- And you can see the predicted number of images (“# images”) right beside the “StepSize”
- If you now want to image several channels don´t forget to tick the desired laserlines <4> and the “Ultra Filter” Option in the second drop down menu of the “Devices” menu
- Check if you chose proper “Autosave settings” <1>
- And don´t forget to activate both lightsheets again <11>
- Then press the Start button <13>
    - Just control now if the steps the software calculates in the “Measurement Mode” fit to your settings
    - And check if you truly get one image per Z-Step in the folder you preset

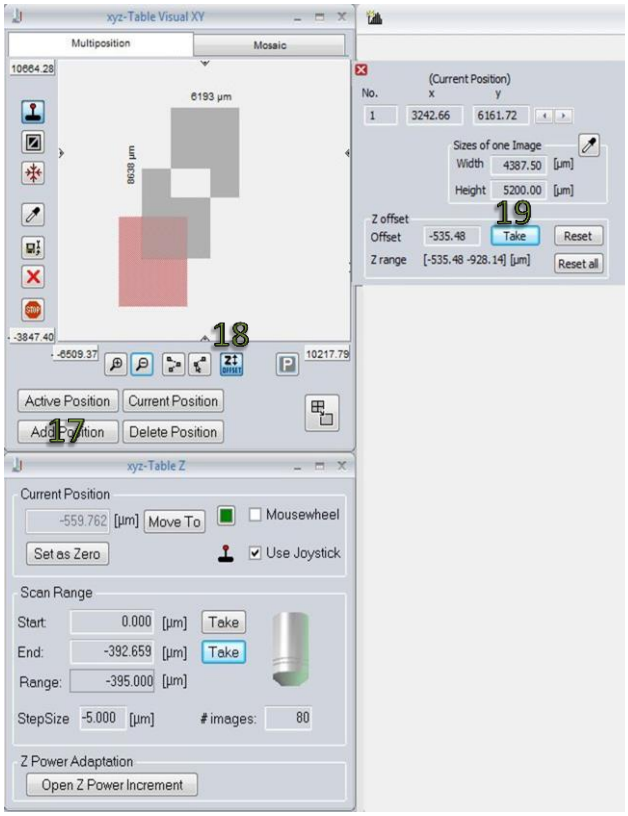


Mosaik Measurement



- Activate the „Mosaik“ tab in the „xyz-Table Visual XY“ window
- The pink square you can see is your actual position
- If you double-click on this square you get the option to enlarge your square by drag and drop on the freshly appeared black spots on the corner of the pink square
- Now define a Z-Stack and the instrument will run this Z-Stack on all the positions you defined.
    - If you now move the stage the pink square always indicates your actual position
    - By double-clicking on one of the other squares the stage will move to the field of view you activated

Multiposition Measurement



- Activate the „Multiposition“ tab in the „xyz-Table Visual X window
- The pink square you can see is your actual position
- Use the “Add Position” button <17>
- If you now move the stage the pink square will move accordingly
- Use the “Add Position” <17>button as often, as you need
- Now define a Z-Stack and the instrument will run this Z-Stack on all the positions you defined. If you use the Start button
- You could also activate the “Offset” button <18> here you can define different offsets for your positions by
    - Double-click on one of your positions
    - Move in the Z height till you want the Z-stack to start and use the “Take” button <19> in the new window

Shut down the Instrument:

- Shut down the ImSpector Software
- Turn OFF the Camera <8>
- Turn OFF the laser toggle switches <7>
- Switch OFF the OPSL <6>, the laser Unit <5>,
- The main power supply <4> and the Computer <3>.
- Pour the substances you want to keep for imaging back to the container it belongs
- Clean the sample chamber and the sample holder with tissue
- Pour some alcohol (or Bacillol) in the cube and wipe clean with tissue
- Only do your entire work under the fume hood
- Put every kind of waste in the little plastic bags and seal properly
- Place your little plastic bags in the provided red bins NOT THE S2 BIOSAFETY BINS
- Keep the fan running for at least 10 more minutes after you are done with the cleaning procedure
- Shut down the hood