

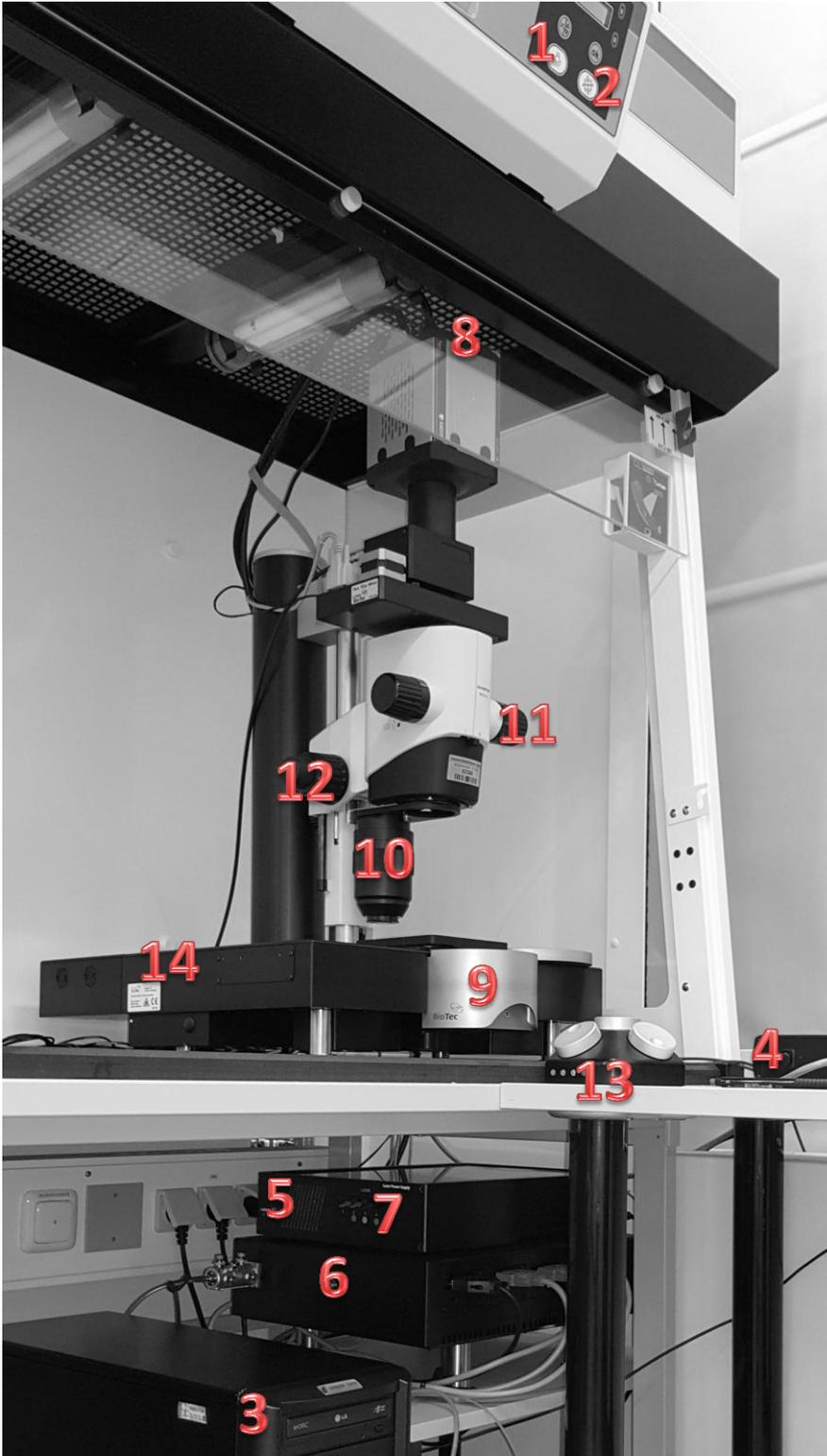
Standard Operating Procedure
LaVision Ultramicroscope

Page 1 von 7

Last change: 08.12.2015

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Start up procedure



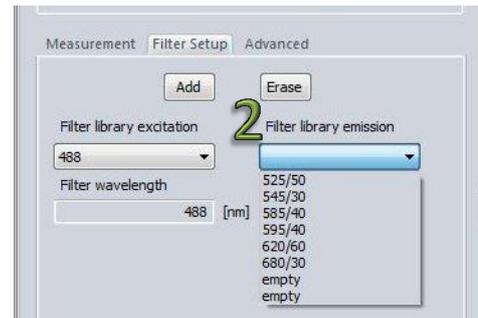
- Turn ON the Light <1>
- And the fan <2>
- Switch ON the computer <3>
- And the instrument power supply <4>
- Now switch ON the laser unit <5>
- And the OPSL <6>
- Then use the laser-line toggle switches <7>
- Activate the camera <8>
- Place your sample in one of the sample holders
- Fill the glass cube with your imaging solution
- Place the gray lid at one side of the instrument <9>
- and use the stamp below the stage to help bringing the cube into the correct position.
- Close the cube-chamber with the gray lid <9>
- Use the smallest magnification <11>

**Standard Operating Procedure
LaVision Ultramicroscope**

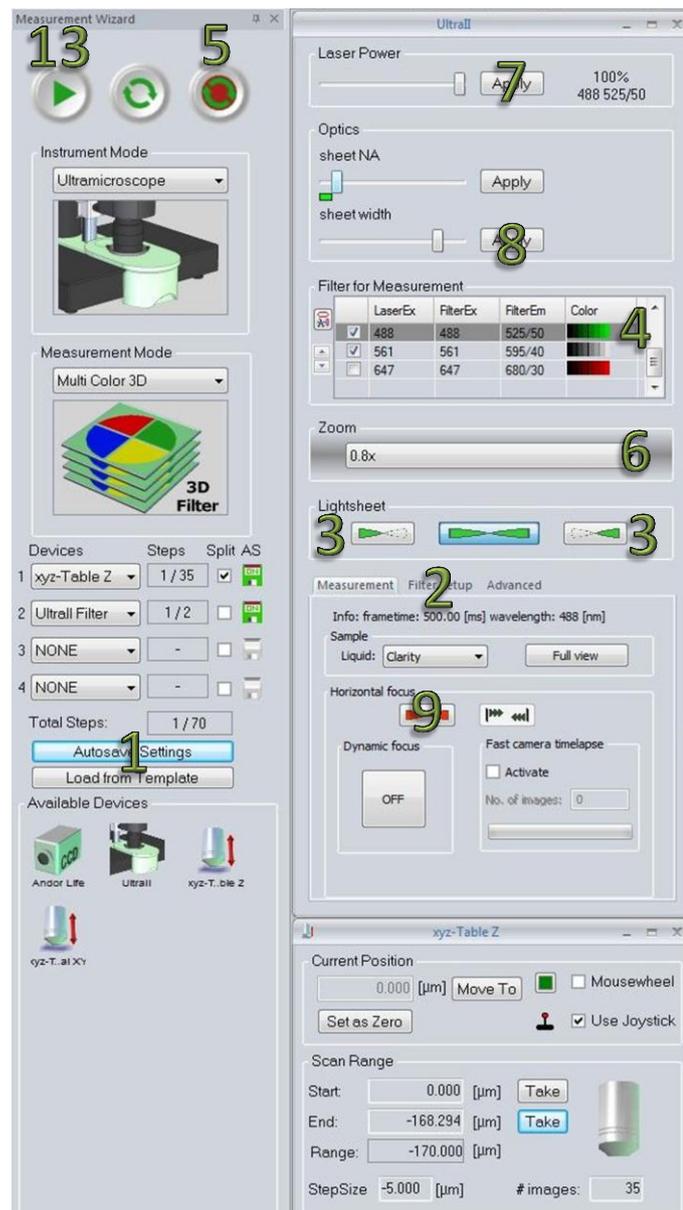
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The Software

- Start the Inspector Software
- Open the **[-Autosave Settings-]** <1> and use the correct folder location for saving your data
- Choose one of the preset laser-setups or define yourself <2>
 - Choose from the **"Filter Library Ex"** drop down menu
 - GFP/FITC = blue (488nm)
 - TRITC = green (561nm)
 - Cy 5 = red (647nm)
 - Choose Emission filter from the **"Filter Library Em"**
 - GFP/FITC = 525/50
 - AF594 or Cy3 = 620/60 or 585/40
 - Cy 5 = 680/30
 - Click the **[-Add-]** button



- To set up your images use either the **[-Left-]** or the **[-Right-]** light sheet button <3>
- 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!!!
- Activate one laser line <4> (highlight) and press **[- 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)
- Rotate the objective arm 180° counterclockwise <10>
- Now move the Objective into the imaging solution with the coarse focus wheel <12>
- 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
- For changing the laser intensity choose the **Laser Power** slider and press **[-Apply-]** <7>
- Adjust sheet width <8> to the size of your sample
- Activate the horizontal focus <9>
-



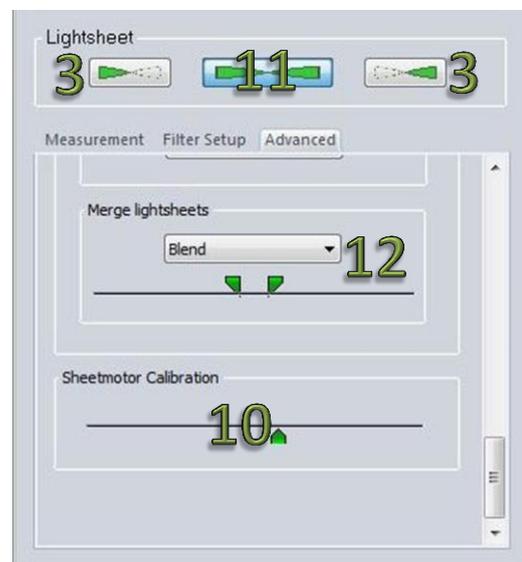
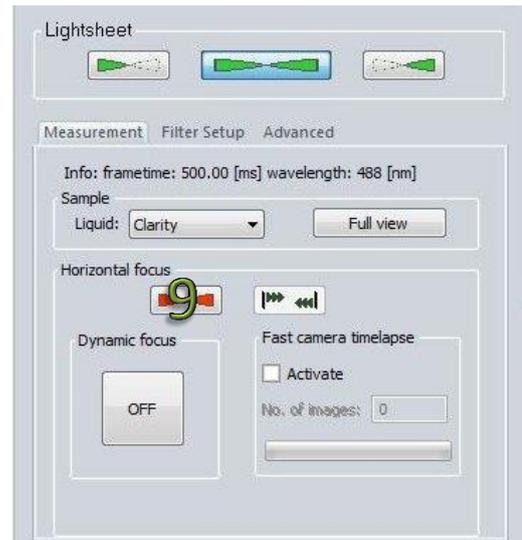
Standard Operating Procedure
LaVision Ultramicroscope

Page 3 von 7

Last change: 08.12.2015

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- Switch from the “Measurement” to the “Advanced” tab and scroll down
- Activate either the left lightsheet <3>
- Move to the top of your specimen and bring it to the center of the screen
- Move the Horizontal focus triangle to the middle of the screen and set it to the smallest possible size with “sheet N.A.”
- Now adjust the focus of the lightsheet by moving the green arrow of the “Sheetmotor calibration” <10> (choose the point when your specimen appears the smallest and as sharp as possible)
- Now “save as default”
- After you’ve done this with one light sheet repeat the procedure with the other one
- Now deactivate the laser <5>
- Activate both lightsheets at once <11>
- Activate 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 with the screw driver in the hole near to the wall <14> the left lightsheet to the same illumination height as the right lightsheet (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
- Now readjust the horizontal focus triangles with the “sheet N.A”. Move to the widest point of your specimen in Z. Set the sheet N.A. half the size of your sample and position it according to the active lightsheet. Let them overlap in the middle
- Deactivate the “horizontal focus” <9>



**Standard Operating Procedure
LaVision Ultramicroscope**

Page 4 von 7

Last change: 08.12.2015

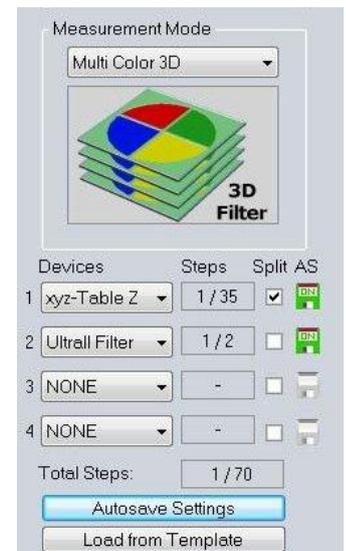
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Objective settings

- It's important for data analysis that you always update the "Zoom" in the Software <6> according to the Hardware Zoom <11>

Filter settings

- If you want to acquire a multicolor image / Z-stack you have to tick the tickboxes left besides the laserlines <4> you adjusted earlier
- Focus in the green channel and use the "chromatic correction". For this option you have to perform a "right-click" on the other laser line you want to measure and move the appearing slide bar till you get the best focus. Press "save" and close the new window.
- Also make sure the correct "Measurement Mode" settings are chosen for a Multicolor image you need for example:
 - Xyz-Table Z
 - Ultra Filter
 - Both "AS" floppy discs NEED to be GREEN
 - And tick the "Split" Tickbox at the xyz-Table Z
- If you only need a single channel you only need the xyz-Table z row



Acquiring 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
- Press the Start-button <13>

Standard Operating Procedure
LaVision Ultramicroscope

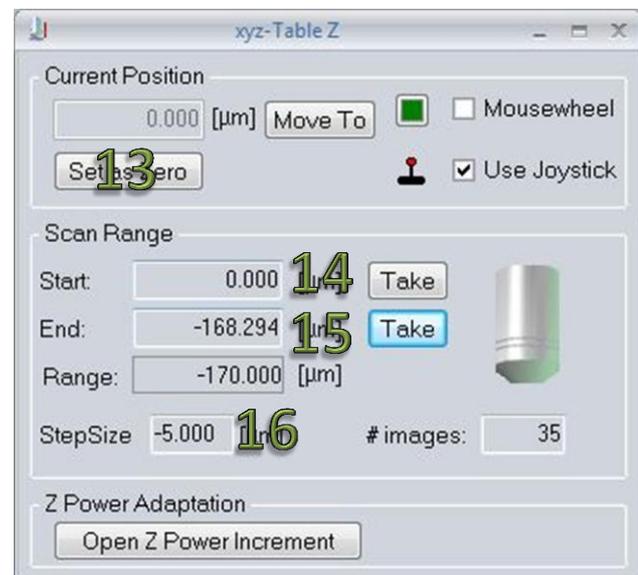
Page 5 von 7

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Acquiring a Z-stack

- After adjusting the Software settings activate a laser line <4>, only one lightsheet and press the Live button <5>
- Go to the top of your specimen, press “Set as Zero” <13> and “Take” <14>
- Then move to the bottom of your sample and press “Take” <15> (if you start with the top, or bottom doesn't matter)
- Now choose a “StepSize” value for e.g. 4µm <16>
- And you can see the predicted number of images right beside the “StepSize”
- If you now want to image several channels don't forget to tick the desired laserlines <4>
- 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



Standard Operating Procedure
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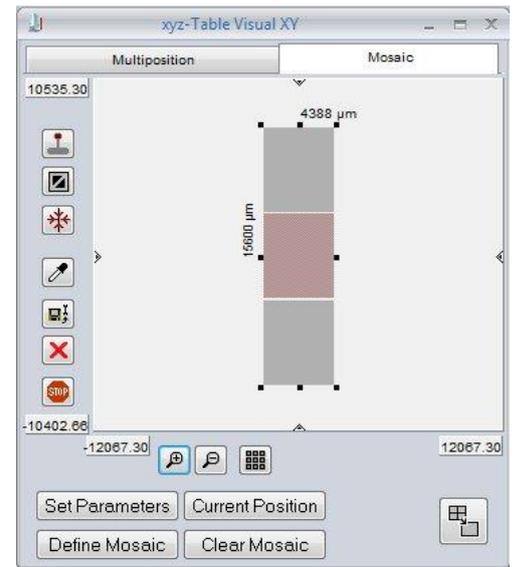
Page 6 von 7

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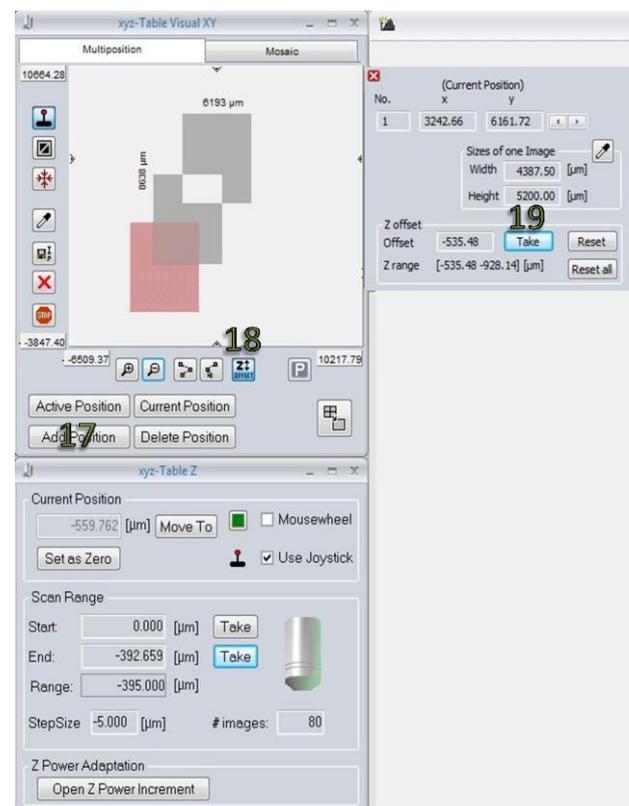
Mosaik Measurement

- 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





**Standard Operating Procedure
LaVision Ultramicroscope**

Page 7 von 7

Last change: 08.12.2015

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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 Plastic Cube 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