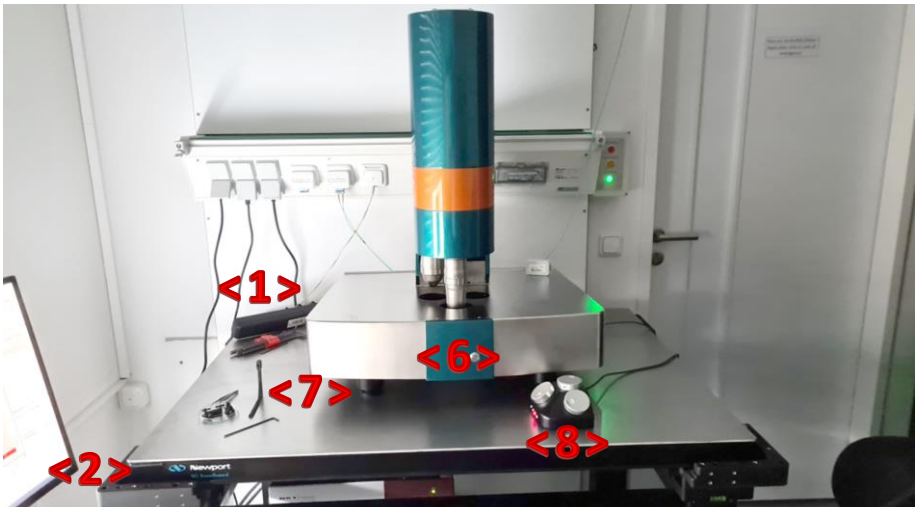
Start up procedure

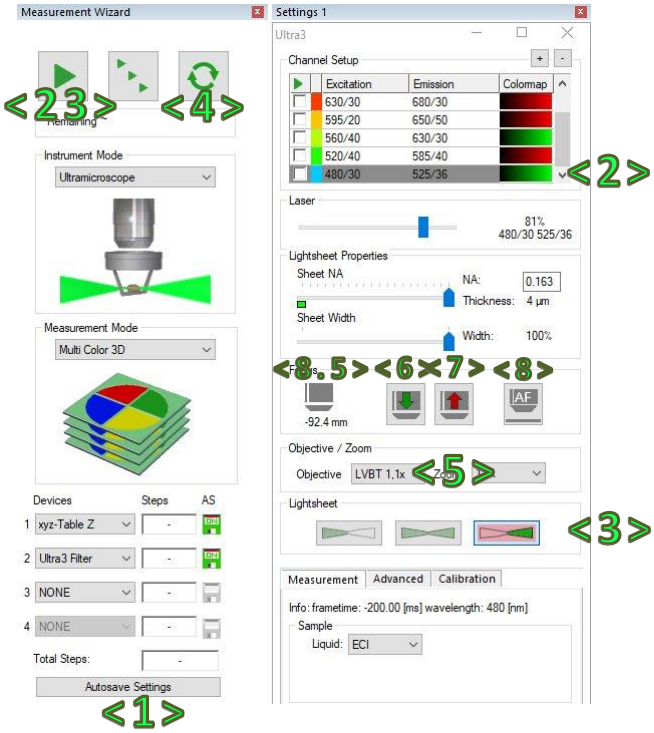
Miltenyi Ultra Blaze



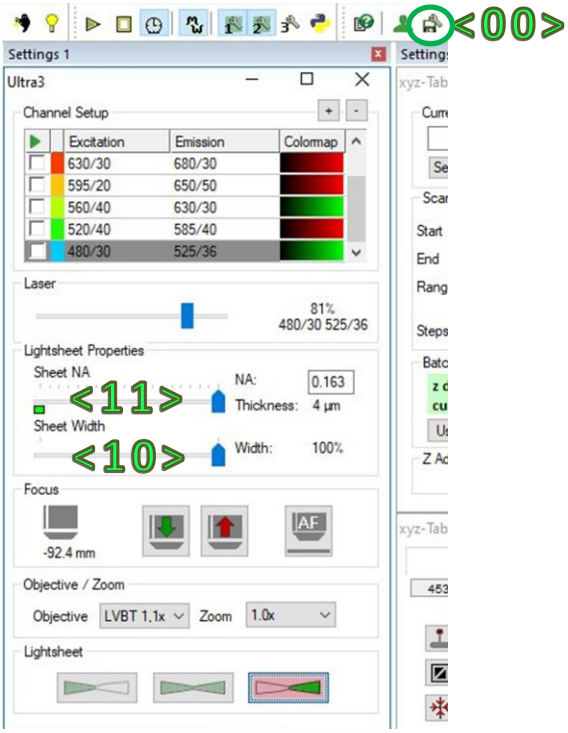
- Turn ON the instrument power supply left behind the instrument <1>
- Switch ON the computer and the monitor <2>
- Turn the keyswitch on the WLL (under the table) to the ON position <3>
- Press first the “return” <4> and then the “emission” <5> button
- Open the instrument stage by dragging the metal drawer <6> (be careful: after you fully took out the drawer the stage will move into a load position mechanically)
- Place the imaging chamber in the stage (must sit correctly on the 3 pins)
- Place the sample holder arm in the imaging position and use the screw to fix it <7>
- Now fill the imaging chamber with your imaging solution (requires ~500ml)
- Take one of the provided sample holders and fix your sample on it
- Place one or more of your samples on the sample holder arm
- Press the button in front of the drawer <6> and the stage will lift mechanically
- Close the drawer

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.

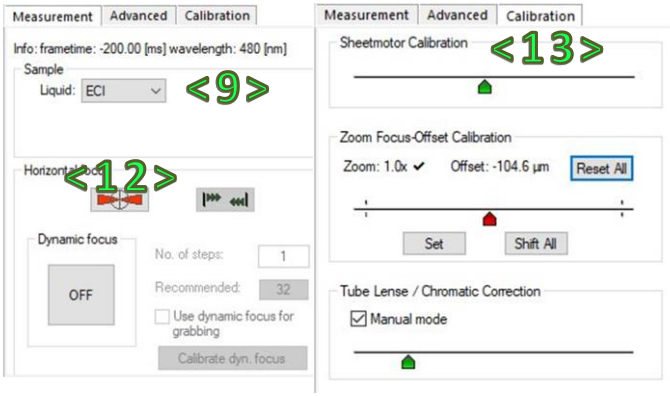
Instrument Setup

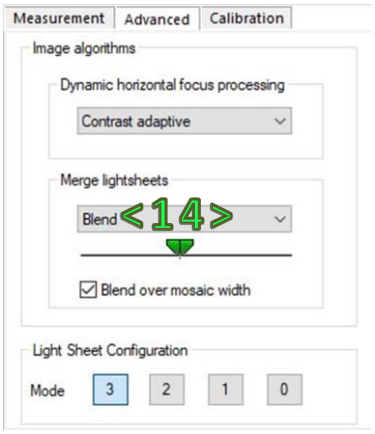


- Start the Imspector Software
- Open the ├Autosave Settings┤ <1>
  and use the correct folder location for saving your data
- Choose one of the preset laser-setups by clicking and highlighting the line <2> adjust the "% Laser power" with the slider below
- To set up the lighsheet start with the ├Right┤ light sheet <3>
- Start imaging with the |- Live-| button <4>
- Position you specimen in the light sheet with the x-y-z adjustment wheels <8> (to do this check with your eyes if your specimen glows bright in the illumination chamber)
- Choose an appropriate magnification for your specimen by selecting from the drop down menu <5> (Make sure the objectives are in the rotate position and can freely move)
- Within this menu you could also apply an additional zoom by selecting different lenses from the drop down menu
- Now move the Objective into the imaging solution by pressing the "green objective button" <6> until the objective stops moving
- 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 autofocus button to focus on y sample <8>, or focus manually with the appearing slider after hitting the focus button <8.5>

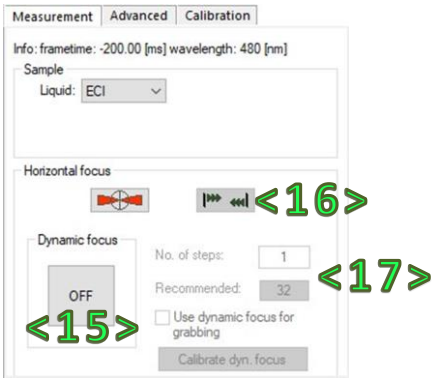


- Make sure you have selected the correct imaging solution <9>
- Adjust sheet width <10> to the size of your sample
  The best z-position to adjust the sheet width is in the middle of your sample. The whole sample should be evenly illuminated
- Move to the top of your specimen (in z) and bring it to the center of the screen (x & y)
- Set “sheet N.A.” to the highest value for the thinnest sheet width <11>
- Activate the horizontal focus <12>
- Move the Horizontal focus triangle to the edge of your specimen
- Switch from the “Measurement” to the “Calibration” tab. <13>
  Now adjust the focus position of the lightsheet by moving the green arrow of the “Sheetmotor calibration” <13> so that the best contrasted point overlaps with the horizontal focus symbol on the screen.
- Now “save as default” (in the task bar <00>)
  After you’ve done this with the right light sheet repeat the procedure with the left sheet
- Now bring the left & the right horizontal focus symbol to the middle of the image (use the crosshair for orientation)
- Deactivate the laser <4>
- Activate both lightsheets at once <3>
- Start the laser again <4>
- Switch from the “Calibration” to the “Advanced” tab
- bring the two green “Merge lightsheet” triangles so close together that they touch each other <14>
- now you observe a line separating the left & right part of the image
- Adjust the left lightsheet to the correct focal plane with the last screw left below the instrument (at a certain point you can see structures matching at the border of the right and left part of the image)
  If you are done seperate the triangles apart to get a smoother transition
- Stop the live measurement and activate the measurement again but with one Lightsheet only

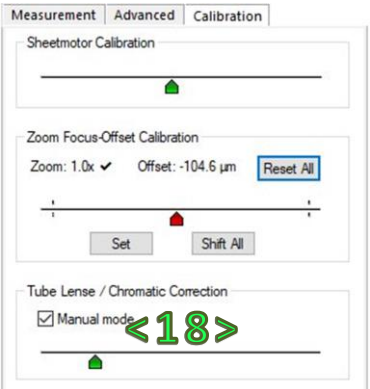




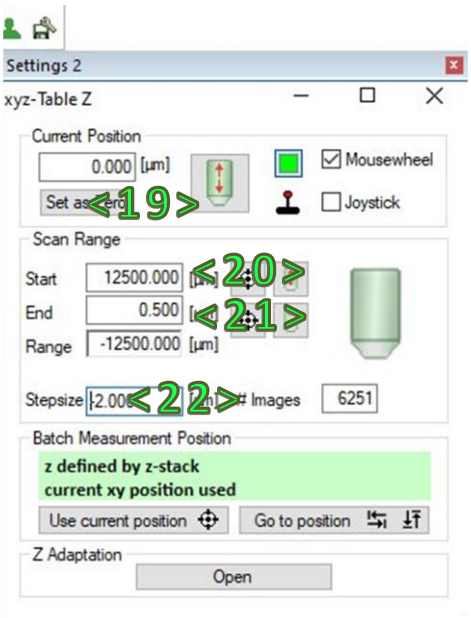
- Activate the crosshair and make sure your sample is in the middle of the field of view. Try to adjust a z plane with the largest extension of your sample to both sides (or at least you should know how far the largest extension of your sample goes)
- **For global overview images :**
- Readjust the horizontal focus triangles with the “sheet N.A” <11>. Reduce the “sheet N.A.” until the size of the focus triangles are aproximately half the size of your sample and place it in the middle of the left/right specimen half.
- Deactivate the “horizontal focus” button
- **For best z resolution over the sample :**
- Be absolutely sure the focus position is perfectly adjusted with the sheetmotor calibration <11>. Set the Sheet N.A. to the largest value
- Turn “ON” the “Dynamic Focus” <15>
- Activate the “green arrows” <16> drag lines appearing in the sample image as big as is needed for your region of interest in your sample
- The program will suggest a recommended number of focus steps. Depending on you region size. Take the suggestion by clicking on the number, or write your own No. of steps in the white box above <17>



- If you want to acquire several channels make sure they are all in the same focal plane, or correct by using the “Tube Lens / Chromatic Correction” <18>
- “save as default” (in the task bar <00>)

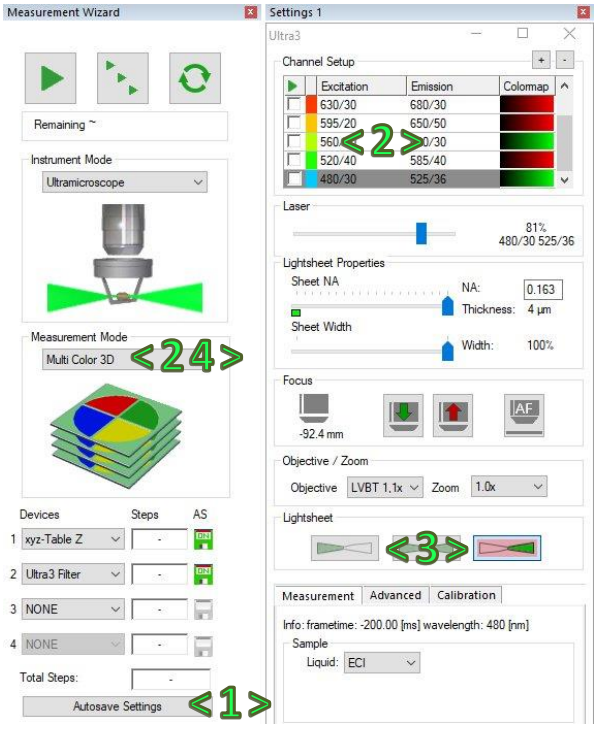


Acquiring single images



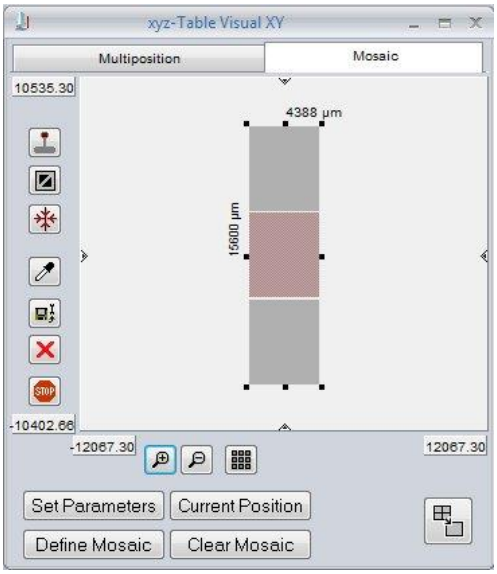
- Activate the laser line of interest <2>
- Tick as many laser lines you need <2>
- Bring your sample to the position you want to image and
- Choose the “xyz Table Z” menu
- press “set as zero” <19> ;
- Start-position “crosshair”-button <20> and
- End-position “crosshair”-button <21>
- Press the “Start measurement” button <23>

Acquiring a Z-stack



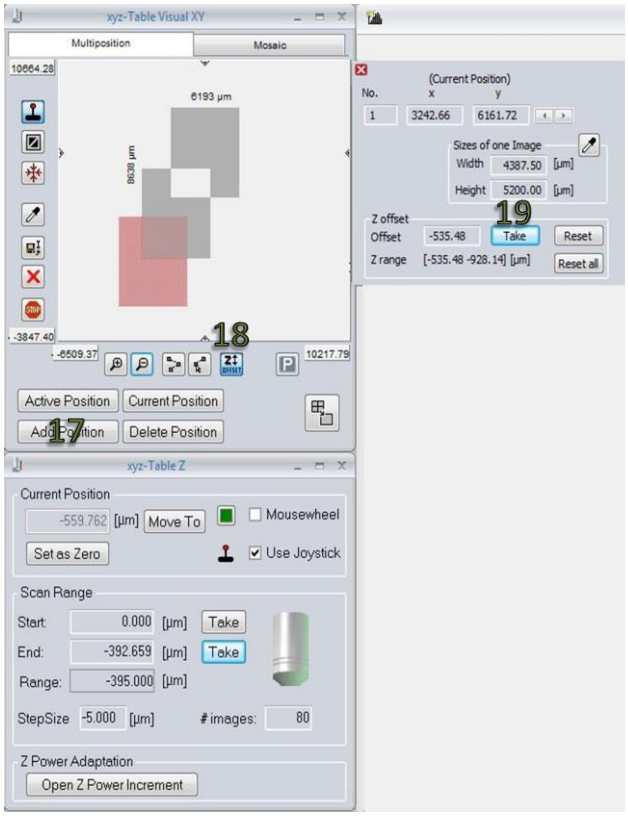
- Go to the top of your specimen, press “Set as Zero” <19> and the Start-position-crosshair <20>
- Then move to the bottom of your sample and press End-position “crosshair”-button <21>
- Now choose a “StepSize” value for e.g. 5µm <22>
- Now you can see the predicted number of images (“# images”) right beside the “StepSize”
- If you want to image several channels don´t forget to tick the desired laserlines <2>
- Check your “Autosave settings” <1>
- And don´t forget to apply the appropriate lightsheet selection <3>
- Tick as many laser lines as needed <2>
- Make sure you have selected “Multi color 3D” as Measurement Mode <24>
- Then press the Start button <23>

Mosaic Measurement



- Activate the „Mosaic“ 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:

- Move the Objective Arm up with the red arrow button <7>
- Disassemble the sample holder arm
- Shut down the ImSpector Software
- Turn OFF the laser key switch <3>
- The main power supply <1> and the Computer <2>.
- Pour the imaging solution back to the storage container (help yourself with the pipette)
- Clean the sample chamber and the sample holder with tissue and cleaning solution (alcohol or Bacillol)
- Also pour some in the cube and wipe clean with tissue
- Clean all the surfaces
- Clean the objective with alcohol and lens cleaning tissue
- Put every kind of waste in the little plastic bags and seal properly
- Place your sealed little plastic bags in the provided red bins