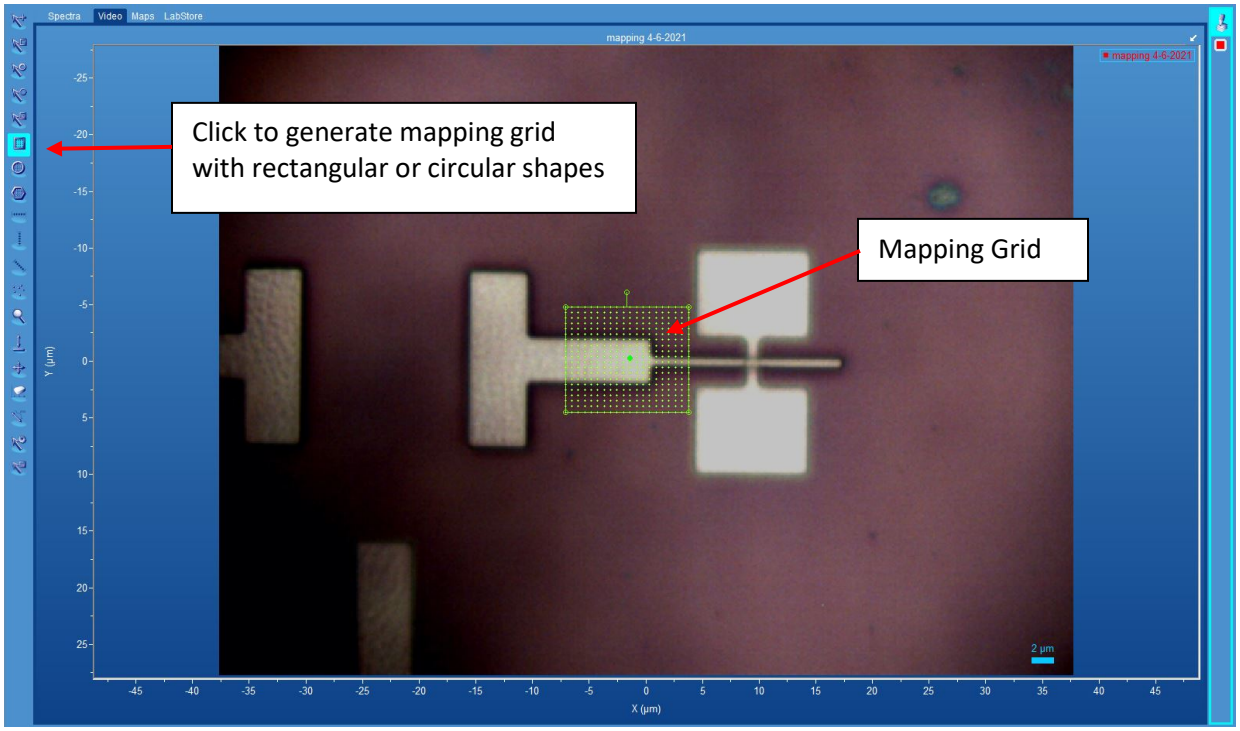
OPT3: Mapping Operating Procedure

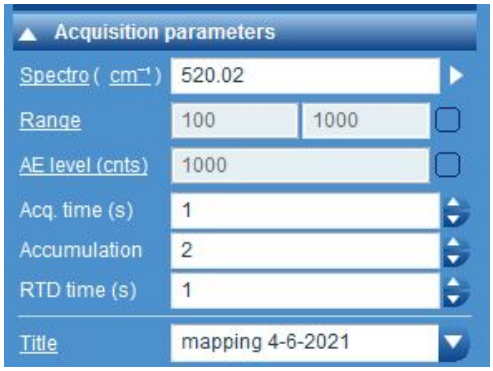
1. Turn on laser and microscope lamp, perform instrument set-up and calibration per the OPT3 Operating Procedure, steps 1-11.
2. **Load your sample and position and focus as follows**:
   a. Rotate the microscope turret to the 10x objective and rotate the focus knob to raise the objectives away from the sample stage

   b. Load sample onto the microscope stage.
   c. Click on the video icon in the software to bring up the video screen
   d. Adjust the microscope focus knobs to bring the camera image of the sample into focus
   e. Move the stage joystick to position the sample in x and y to the desired area
   f. Change the objective to 100x (or to the desired objective), refocus and adjust sample position if needed.

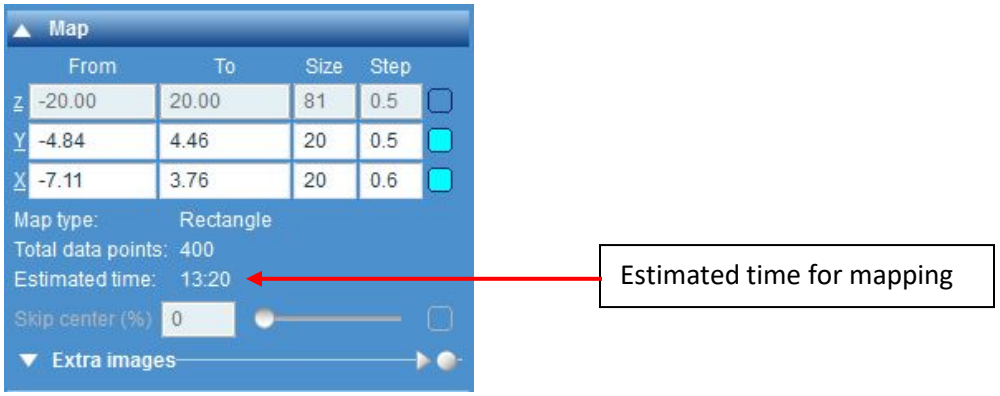
   g. Turn the laser On using the Laser On/Off panel at the bottom of the window, and fine focus to minimize the laser spot size. Then turn the laser off.
   h. Click the STOP button to stop and freeze the video image
   i. Turn off the microscope light and close the sample chamber doors
3. With the image frozen in the Video tab
   a. You can zoom into the image if desired
   b. Click on the mapping grid options on the left hand side of LabSpec6 interface.
   c. You can either select the grid to move it or drag at its corners to change its size. Resolution setting can be done manually by inputting number of points per row or column. (*See Step 4 Acquisition Parameters*)



4. **Set Scan Parameters**
    a. Select the desired **units** by clicking on the units indicator after the word "Spectro"
    cm^{-1} (used for Raman) is the amount of wavenumber shift from the laser line
    nm (used for PL) is the actual wavelength value measured
    b. Deselect the box beside the **Range** values to deactivate the range setting. It is best to enter the desired wavenumber in the Spectro field, and then choose the appropriate grating value to give the desired spectral range of measurement.
    c. Enter desired **Acq. Time (s)** – this is the count time; higher values result in more counts and longer acquisitions
    d. Enter desired **Accumulation** – this is the number of measurement averages; high values result in smoother data and longer acquisitions
    e. **Title**: enter a sample ID

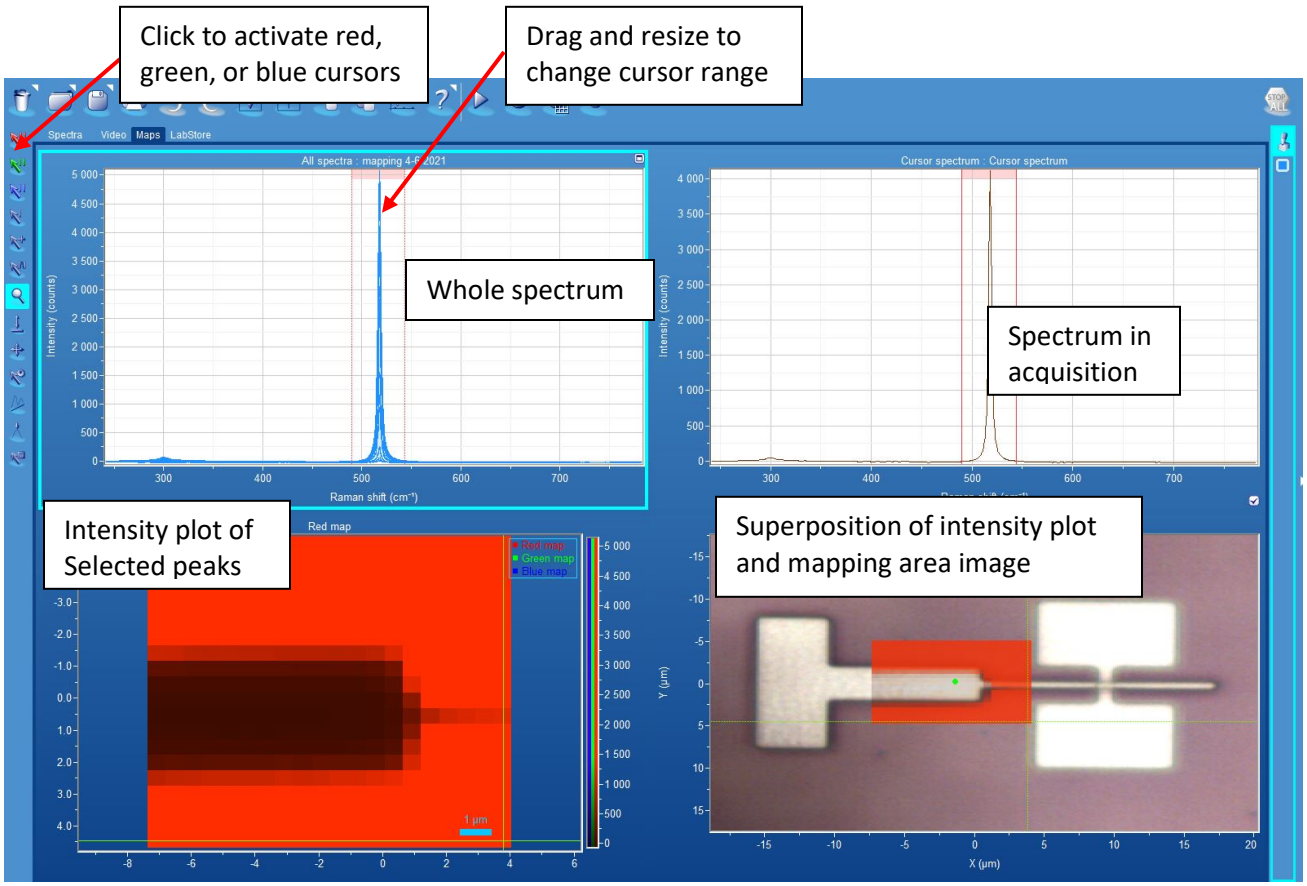


5. **Set Map Parameters**
    a. **Note -** The real size of mapping grid is related to the microscope objective used. A wrong objective selection in the instrument set-up will lead to incorrect movement of XY stage
    b. Do not select Z settings as our system currently doesn't have automated Z capability.
    c. For X and Y:
        i. The "From and "To" numbers are determined by the size of the mapping grid that was drawn. The size can be further adjusted using these numbers.
        ii. The "Size" number is the number of points (pixels) per row or column.
        iii. The "Step" number is the size of each pixel in micrometers. This is automatically calculated from the size settings. A larger size number will produce smaller pixels, but take longer to acquire.



6. **Acquire Map Data**
   a. Select the Maps tab to display the mapping window.
   b. Click on the Mapping Icon to start the mapping process.

7. During or after mapping you can choose up to three peaks of interest to be displayed in the intensity plot and superposition plot



8. **Save Data**

To save all of the information in the mapping data, both the whole spectrum (top left plot) and the superposition (bottom right plot) must be saved.
   a. Click on the Whole spectrum plot so that a blue box appears around it. Then click the "Save as Data" icon. This will save the first three plots.
   b. Click on the Superposition plot so that a blue box appears around it. Then click the "Save as Data" icon. This will save the superposition plot.

If the whole spectrum is saved into txt format, it will have the following data arrangement:

#Grating=	1800									
#Filter=	100%									
#Laser (nm)=	633									
#Slit=	100									
#Location=	Micro									
#Range=	Visible									
#StageXY=	Marzhauser									
#X (µm)=	-45.6									
#Y (µm)=	198.8									
#Full time(mm:ss)=	18:01									
#Project=	A									
#Sample=	B									
#Site=	C									
#Title=	test map									
#Remark=										
#Date=	12.03.2021 14:27									
#Acquired=	12.03.2021 14:27:51									
#Base:Fit Sub=	12.03.2021 14:45:49									
		351.082	351.634	352.187	352.739	353.291	353.842	354.394	354.946	355.498
-5.89323	-6.8408	5.03083	-4.19568	-4.74269	-4.08808	-2.78051	-0.483213	0.925149	2.04939	3.02584
-5.89323	-5.70462	-0.79991	-1.8453	-2.26767	-1.57813	-0.548413	0.414918	1.78152	2.95068	3.54843
-5.89323	-4.56843	8.40356	7.16041	5.32325	5.34584	2.96087	-0.17613	-3.3094	-2.29615	0.550715
-5.89323	-3.43225	6.5756	8.01729	6.44837	5.25568	4.05806	1.79248	0.624992	0.322071	0.367917
-5.89323	-2.29606	0.250883	1.10502	1.91856	2.74633	0.874449	-0.219863	-1.50117	-2.29296	-2.12712
-5.89323	-1.15988	-4.11972	-0.361105	2.52439	3.47821	3.09611	2.92935	2.87325	2.59655	2.6565
-5.89323	-0.0236961	-8.5554	-5.75245	-2.95737	-0.693559	0.472981	1.2619	1.60886	2.14834	2.08638
-5.89323	1.11249	-3.66845	0.327719	-0.915668	-1.95864	-2.31548	-1.92842	-1.17947	-0.71886	-0.202254
-5.89323	2.24867	-2.41234	-2.7153	-2.93054	-3.17064	-1.80415	-0.437246	1.03735	1.99372	2.03136
-5.89323	3.38486	-2.06734	-1.21562	-0.645875	-0.0078709	-0.376398	-0.719961	-1.07667	-1.40032	-1.69742
-5.89323	4.52104	-3.10875	-3.09163	-2.11981	-1.06672	-0.084511	0.851075	1.67056	1.6081	1.13256
-5.89323	5.65722	-3.69429	-1.85436	-0.0691111	1.10011	1.78936	1.94084	1.88173	1.87448	1.80517
-5.89323	6.79341	1.24238	6.77195	7.17659	5.67678	3.30411	0.0382817	-2.95894	-2.19371	-0.885683

Acquisition Information

Counts, intensity

Wavenumber (cm-1)

(y,x) position in um