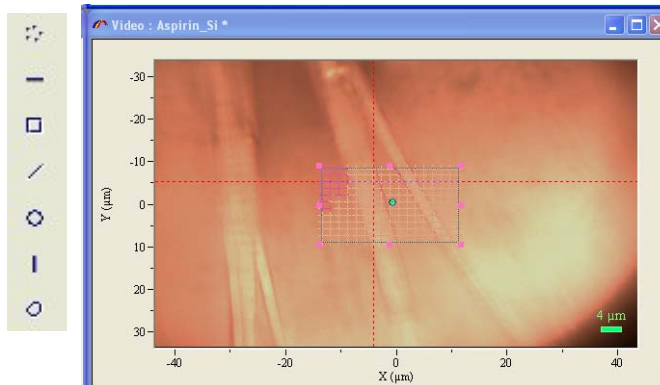


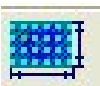
# Horiba Jobin-Yvon LabRam Raman Confocal Microscope (341-K)

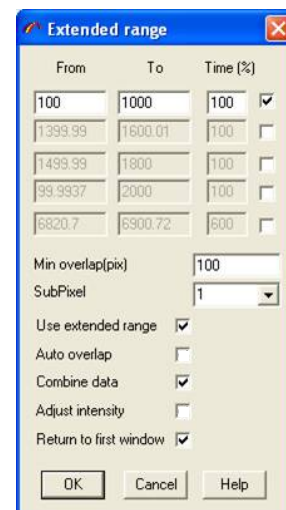
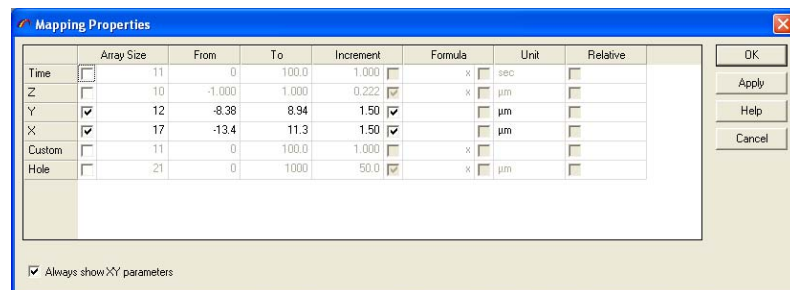
Please contact Dr. Amanda Young for training requests and assistance: 979-862-6845, amandayoung@tamu.edu

## Raman Mapping

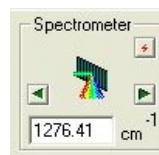
1. Mount sample
2. Choose correct objective on bottom toolbar (sets scale bar)
3. Flip off laser shutter switch
4. Turn on white light
5. Move stem 1 down for video focus
6. Focus on video with white light
7. Set map region shape (circle, rectangle, line, polygon, points, etc.) on the left side toolbar





8. Click  and set the number of points in your mapping region and/or the spacing between points. The increment should be set to approximately the sample spot diameter/3 (see table for spot diameter vs. pinhole size)




9. Press stop on the video; leave it open
10. Turn off white light
11. Flip on laser shutter switch (on silver box)
12. Move STEM 1 to up position



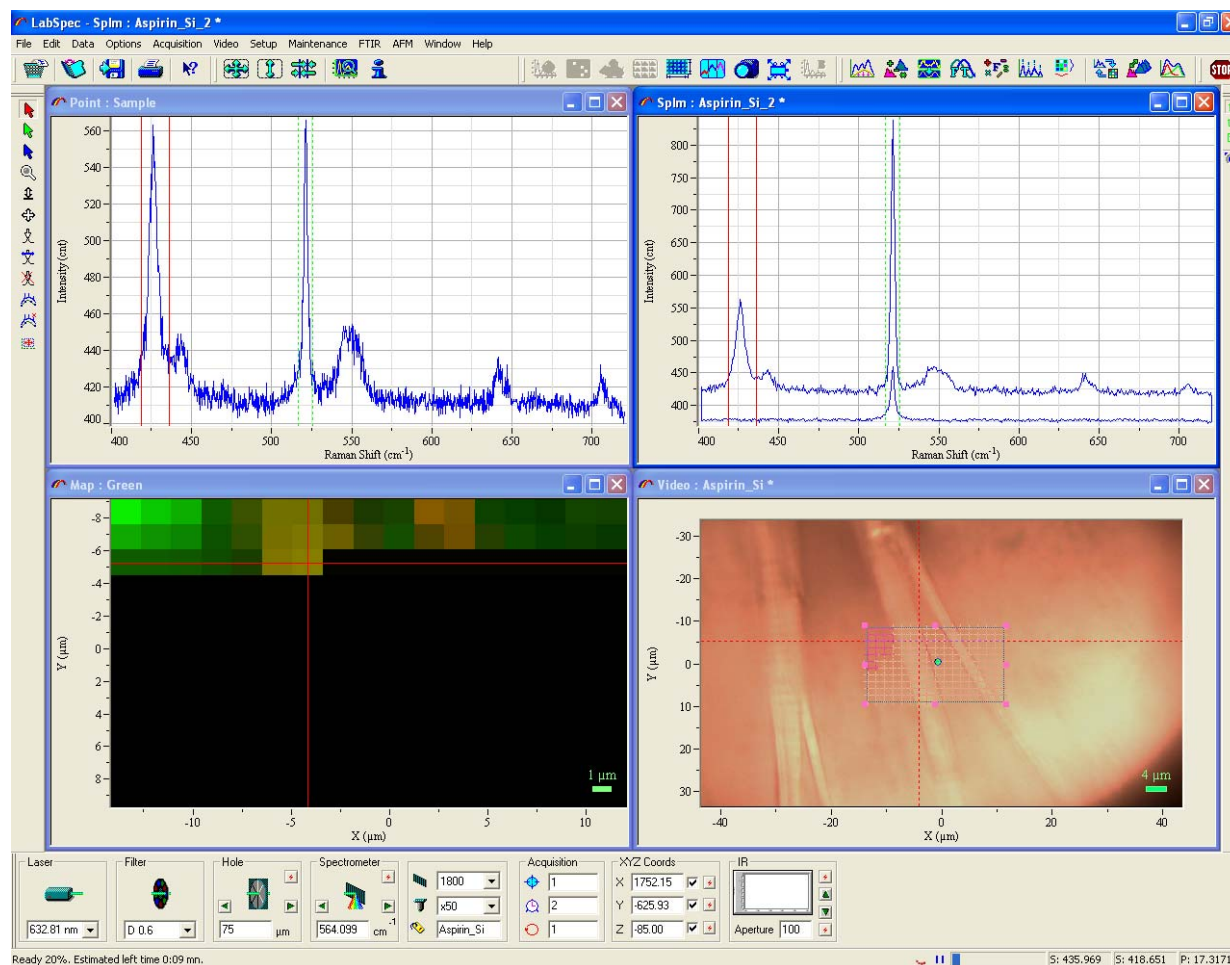
13. Center spectrometer around peak of interest (type in wavenumber into spectrometer and press enter)

14. Perform RTD scan  to optimize exposure time  (shorter is better) and Z-position. You may need to adjust Z-position to see peaks of interest. (If peaks of interest are very weak, then you may not see anything on RTD and changing the Z-position may not improve this.)

15. Set the extended range exposure time  and accumulations  (based on best RTD exposure time from previous step)

16. Choose scan range under “Extended Acquisition” 

17. Acquire map by clicking  on the top toolbar



18. While acquiring map or after acquisition is finished, set bounds of green/red/blue peaks using Map Analysis




or by clicking green/red/blue arrows in Point or Splm windows and dragging them to particular peaks

The 'Map Analysis' dialog box contains the following options:

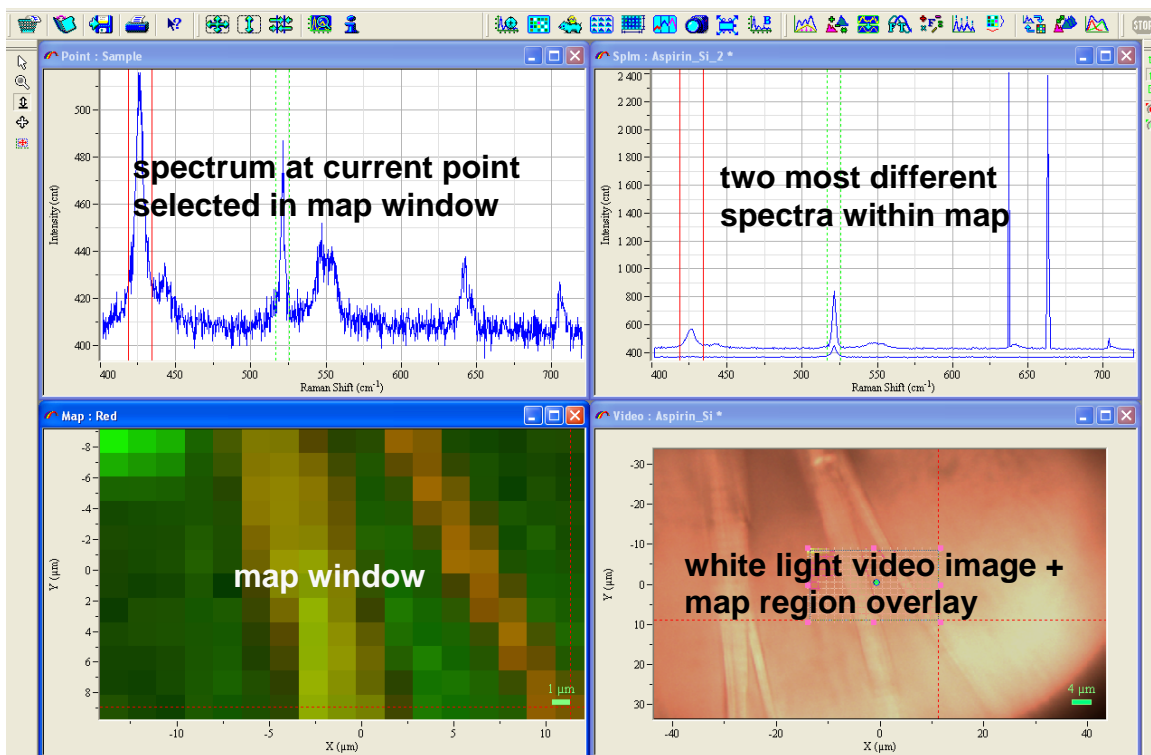
Options	Use	Baseline	From	To
Red	<input checked="" type="checkbox"/>	<input type="checkbox"/>	434.028	418.54
Green	<input checked="" type="checkbox"/>	<input type="checkbox"/>	516.601	525.681
Blue	<input type="checkbox"/>	<input type="checkbox"/>	0	0
Green/Blue	<input type="checkbox"/>			
Spectrum	<input checked="" type="checkbox"/>		Correct	

Buttons: OK, Convert, Help, Cancel

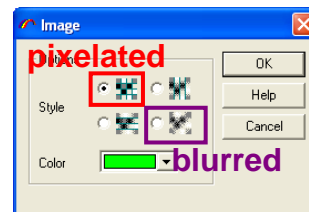


19. During acquisition, press the  while each window is active including the map (but not the video window) to auto-adjust intensity and improve contrast on map

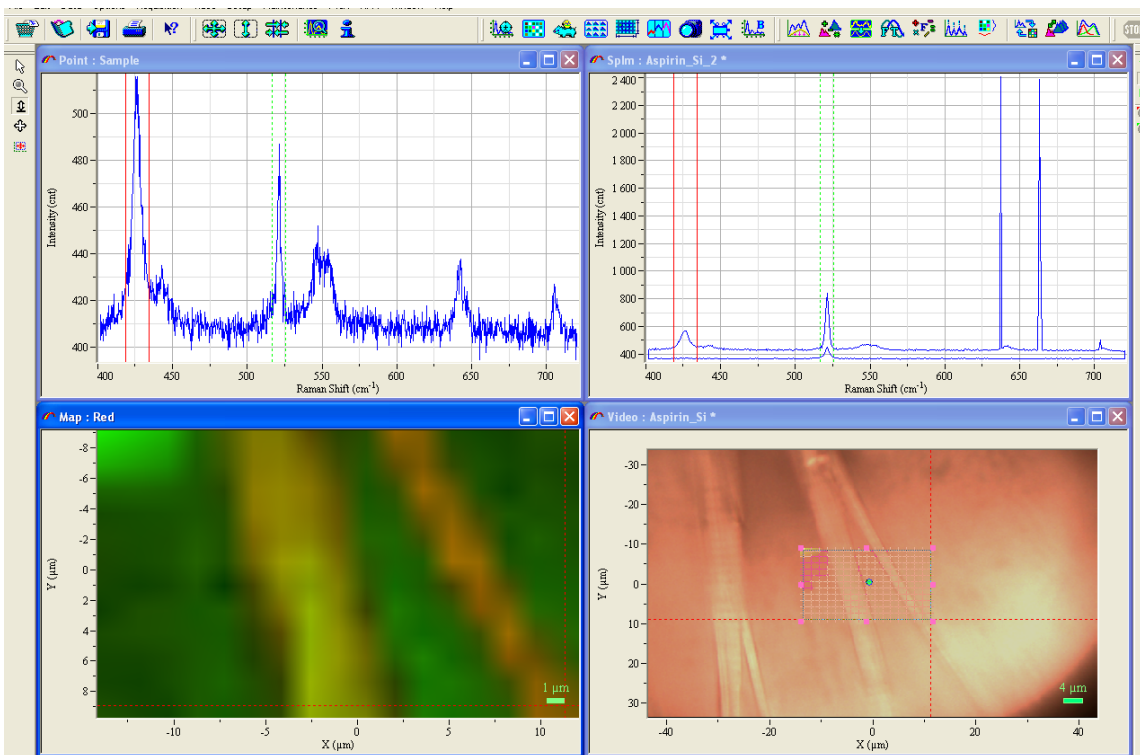
### FINISHED MAP (pixilated image mode)



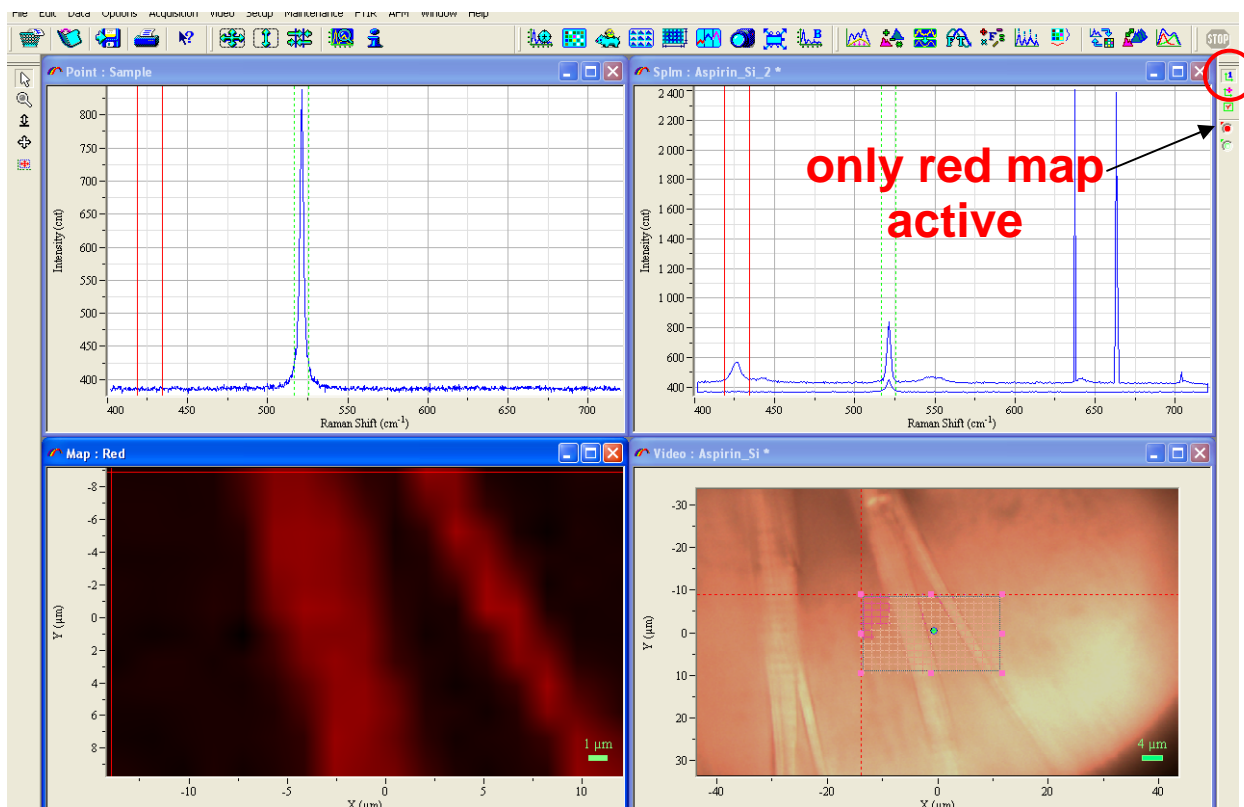
20. Right click on map image and choose "Image" to change from pixilated to blurred image mode

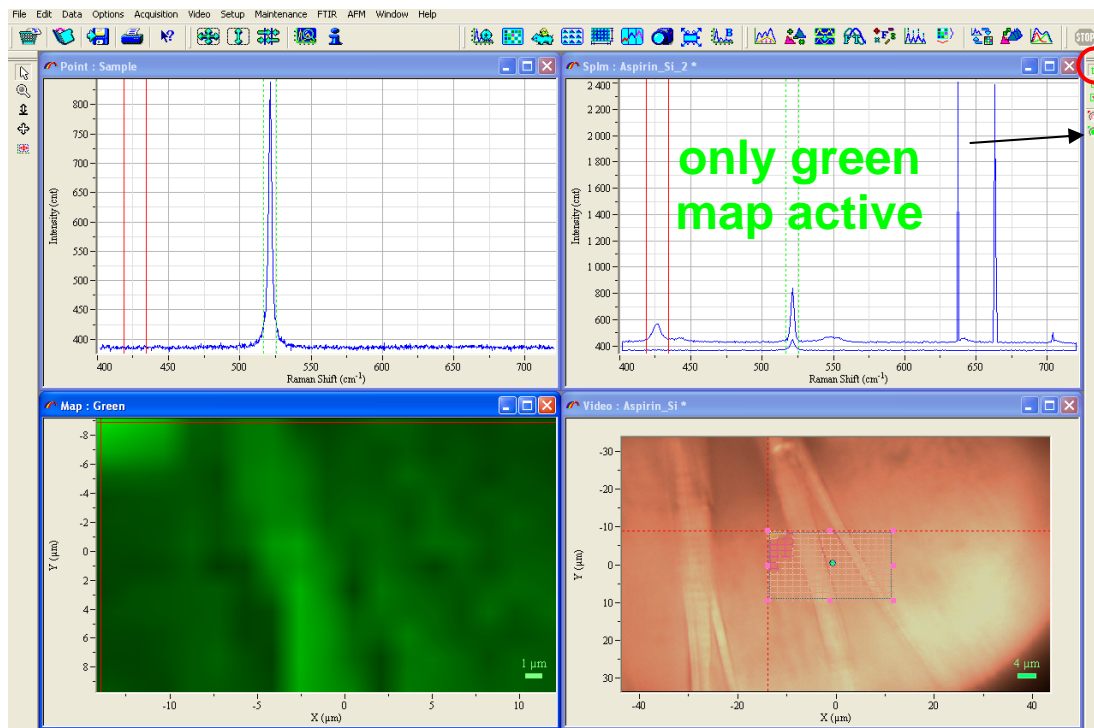


### BLURRED MAP



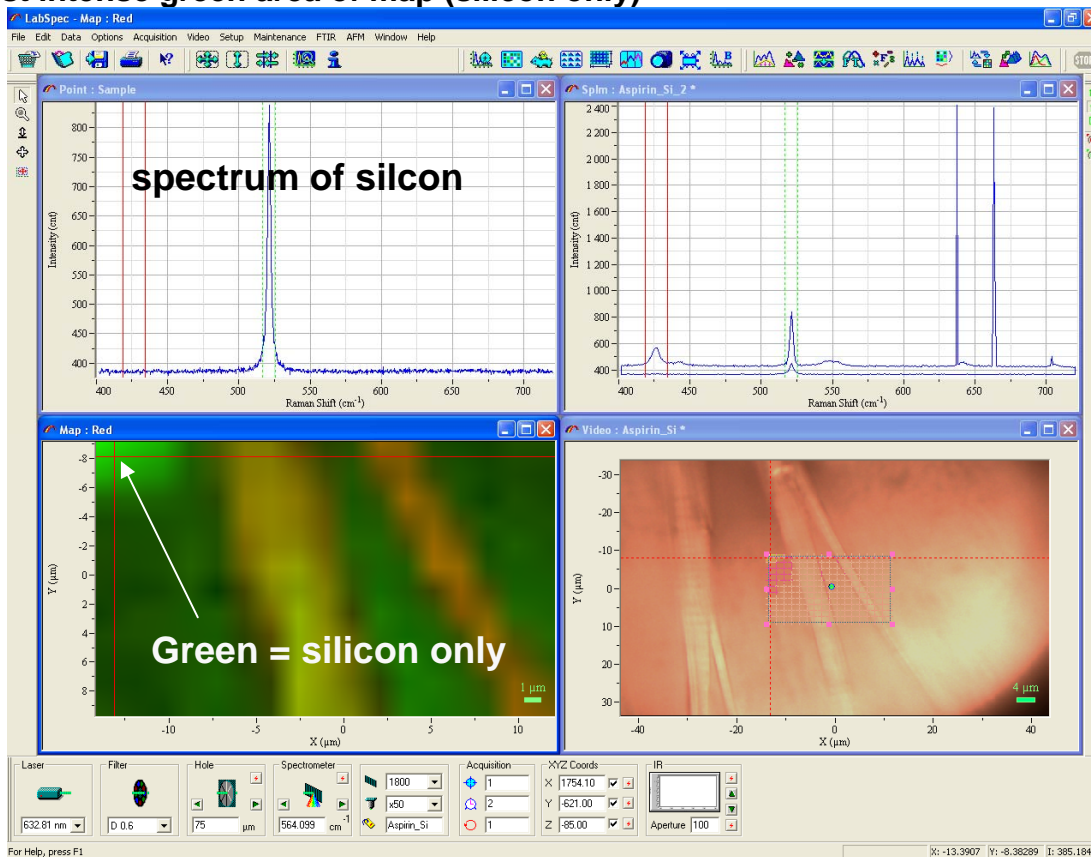
21. While Map window is active, choose “1” on the upper right toolbar to display only one color of map; click colored circle for each map to view it separately



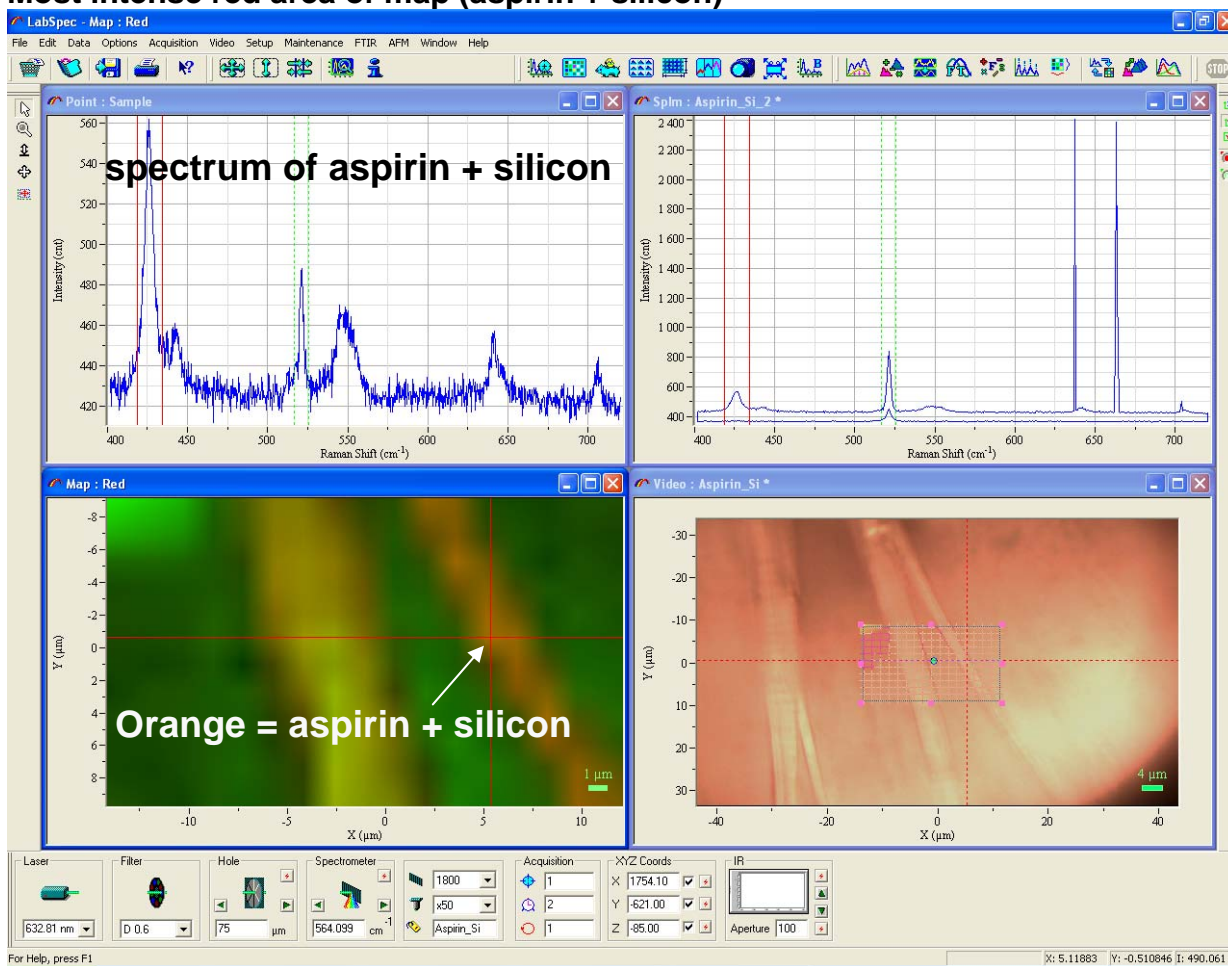


22. In map window, choose the cursor (arrow) and click at various points within the Map window. The spectrum for that point is displayed in the Point window.

### Most intense green area of map (silicon only)



### Most intense red area of map (aspirin + silicon)



23. Save video image and SpIm file (SpIm contains all info about your sample—each point spectrum, two most different spectra, and maps)