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

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**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 SpIm windows and dragging them to particular peaks
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.
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)

Green = silicon only
Most intense red area of map (aspirin + silicon)

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