

Standard Operating Procedure for the Raman Microscope

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These instructions are for reference with the assumption that you have already been trained on the instrument and are subject to modification.

IF YOU HAVE NOT BEEN TRAINED ON THE INSTRUMENT DO NOT USE IT!

TURNING ON THE INSTRUMENT

1. Reserve time online at <http://www.nanocenter.umd.edu/scheduler/>
2. Load LabSpec software and open the Video Camera window .
3. Check that the detector temperature is $-70^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
4. Turn on the light (top is reflection, bottom is transmission).
5. Turn on the desired laser (532nm or 633nm). For 532 nm, turn the switch to on position and press the “blinking” enable button. For 633 nm, turn the switch to on position (figure 1).

CALIBRATING THE INSTRUMENT

6. Note: Calibration is needed whenever the instrument is started and when the grating is changed.
7. Start with the 10X Objective and load Silicon calibration standard. Set the position with joystick in XY.
8. Coarse focus manually using the knob inside the microscope, (only use this knob with the 10X lens).
9. Fine focus with the Joystick (twist the top of the handle). Twisting counter clockwise bring the objective closer to the sample.
10. Once focused at 10x, switch to 50x and fine focus, then repeat at 100x (ONLY USE THE FINE FOCUS WITH THE 50X AND 100X).
11. Close the microscope doors (laser will not work with doors open)
12. Set the laser filter to D1 and the exposure time to 0.5 seconds.
13. Select grating to calibrate
 - a. Finer gratings (higher number) give higher spectral resolution but shorter wavelength range.
 - b. Each grating must be re-calibrated before each use, even in the same session.
14. Select slit width and hole size:
 - a. Default settings: slit size $100\ \mu\text{m}$, hole size $400\ \mu\text{m}$.
 - b. Slit setting 100 provides 96% of max intensity, hole size 400 provides 96% intensity.
 - c. Smaller hole gives better height resolution but lower intensity. With low height resolution, the laser will probe the substrate if the sample is transparent.
 - d. Smaller slit give higher spectral resolution but lower intensity
15. Press the stop button  to deactivate camera.



Figure 1. Laser power switches.

16. Press the realtime display (RTD) icon  to acquire spectra in real time (spectra cannot be saved).
17. Set "Spectrometer," offset to 0 by clicking the  button (figure 2). The spectrometer reading **HAS** to be **0(><) cm⁻¹** as shown in figure. **DO NOT TYPE 0 IN THE BOX.**
18. Click the normalize button .
19. Select "Instrument Calibration," from the setup menu at the top of the screen.
20. Adjust the zero parameter until the peak on the display is at 0.
21. Click the  button again (spectrometer offset will change slightly).
22. Repeat steps 19 and 20 until a sufficient calibration is achieved (zero peak should be within ± 1 pixel).
23. Enter 500 cm⁻¹ into the spectrometer offset.
24. Silicon has a characteristic Raman shift at 520.7 cm⁻¹. Adjust the Koeff parameter until the peak on the display is at 520.7 cm⁻¹.
25. The instrument is now calibrated.

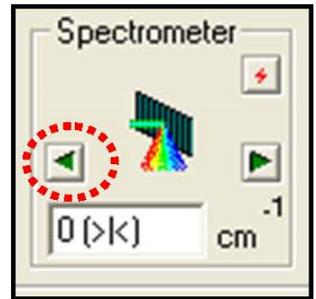


Figure 2. Setting spectrometer to zero

ANALYZING YOUR SAMPLE

1. Click the stop button, open the doors.
2. Rotate the objective turret until an empty slot is over the standard. Remove the standard.
3. Insert your sample, making sure it's flat enough to avoid scratching the lenses.
4. Repeat position and focus procedure used during the calibration until sample is focused at the desired magnification.
5. Check the extended range setting . If multi-window is selected change it to single window.
6. Activate the RTD and adjust the instrument parameters (exposure time, laser filter, spectrometer offset, ect.) until the desired spectrum is shown on the display.
7. Press stop and turn off the lamp.
8. Set acquisition time and accumulation number to desired values (example: 5 second exposure and 3 accumulations).
9. Enter a file name
10. Press the piggy bank icon  to acquire a spectrum.

ENDING THE SESSION

1. Save your data by clicking "Save All" under the file menu. Save all spectra in the labspec format. Select "Save As" under the file menu. Save each spectrum individually as a text file.
2. Rotate the objective turret until an empty slot is over the sample. Remove the sample.
3. Turn off light(s) and lasers. Remove all samples and clean workspace.
4. **IF YOU USED THE EXTENDED RANGE FEATURE**  **TURN IT OFF WHEN FINISHED.** Change multi-window to single window.
5. Exit software

APPENDIX

Default Setting

Menu: Acquisition > Options

Options dialog box showing default settings:

- Exposure time(sec): 6
- Accumulation number: 5
- Data name: (empty)
- Photo-bleaching time: 0 sec
- Binning factor: 1
- Accumulation mode: Average
- Spike filter: On
- Autofocus: Off + 0 μm
- Scanning device: Stage
- Use detector: Active
- Signal mode: Signal
- Auto exposure: Off (Set)
- Shutter mode: Auto
- Intensity correction: Off (Correct)
- Denoise: Off

Menu: Acquisition > Detector

Detector dialog box showing default settings:

- Type: (empty)
- Sensor: Synapse CCD
- Mode: Spectrum
- Sizes: 1024 (width), 256 (height), 100 (width), 142 (height), 1 (width)
- Options: High Gain, Shift 980
- ADC: 20 kHz
- T°C: -73.0 (Get/Set)

Troubleshooting:

1. **Fluorescent samples.** Some samples emit light that can interfere with the raman spectrum. This can be minimized or removed in some cases by switching to a different laser. Another option is to photobleach the sample by subjecting it to high intensity laser light.
2. **Sample decomposes during analysis.** High intensity laser light may cause chemical changes to some samples. Avoid this by using high density filters to decrease the laser intensity. Keep in mind, the 532 nm laser is rated at 100 mW while the 633 nm laser is rated at 9 mW.