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Calibration of the OpenRAMAN DIY Raman spectrometer

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

> This is one of many approaches to calibration that you can take, and we found it useful for the types of biological and chemical investigations we're interested in.

Abstract

The **OpenRAMAN system**, created by Luc Boussemaere, is an open-source, low-cost, modular Raman spectrometer. In this protocol, we share a calibration procedure for two configurations of the system, which includes optional steps of assessing system performance using common materials used for Raman analysis. We also share an iPython notebook for data processing and sample data collected on the system. This protocol aims to ensure reproducibility of results, elucidate system limitations and capabilities, and allow for effective comparison to published literature or results from other instruments on solids and liquids.

Image Attribution

Arcadia Science

Materials

- Built and functional OpenRAMAN system (solid or liquid cuvette configuration)
- Neon bulb: An **NE-2H lamp** with a 1000 Ω resistor soldered to **an extension cord with a switch**
- Acetonitrile: **HPLC grade, VWR**
- Ethanol: **USP Spec, KOPTEC**
- Disposable Culture Tubes: **VWR PN: 47729-568** Borosilicate glass — size 10 × 75mm
- Quartz cuvette: **1-Q-10-GL14-S Starna Cells cuvette** filled with 4 mL of acetonitrile
- Calcite crystal: **Iceland Spar Optical Calcite from Ward's** (product number 470014-650)
- Black foil: **Rosco Cinefoil** (Amazon)

Safety warnings

Use appropriate laser safety glasses.

Before start

To follow this protocol, you should already have an OpenRAMAN system built and functional. If you don't, check out the guide here: **https://www.open-raman.org/build/starter-edition/**.

Hardware setup

1 To follow this protocol, you should already have a functional OpenRAMAN system built. Below is a labeled diagram of the system used for this protocol, which is a "Starter Edition."

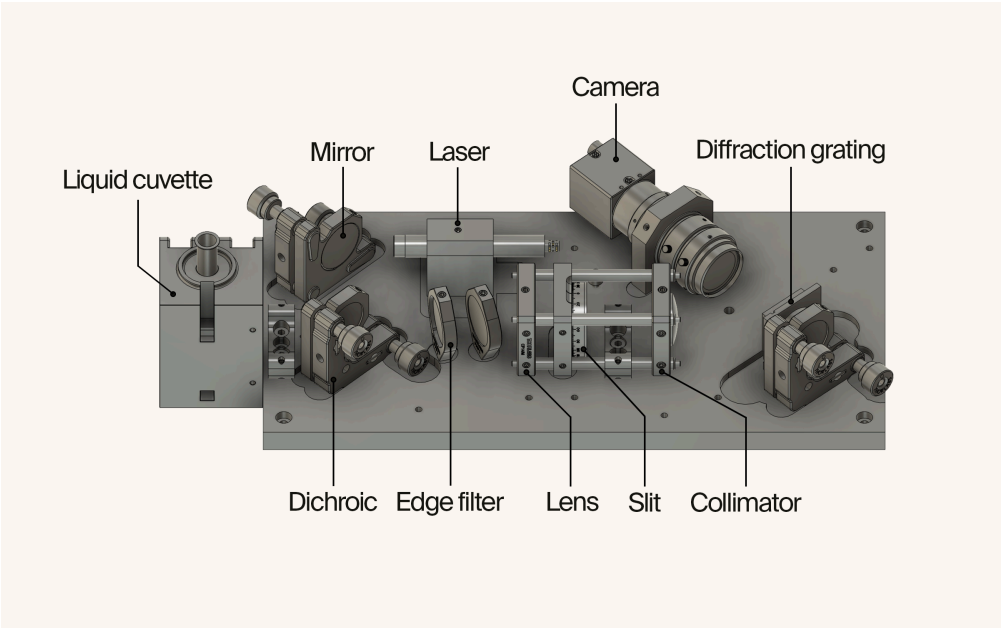
We put our system in a black corrugated box with two layers of black fabric to prevent background room lighting from hitting the detector. When possible, we typically turn off overhead lights and reduce vibration to the table.

2 Select the appropriate tab for your cuvette configuration, liquid (standard) or solid. The liquid cuvette is primarily used for liquid samples (e.g., isopropanol) and high-concentration solutions (e.g., salts). The solid cuvette is primarily used for solid samples, including crystals and powders. The path is slightly different for each configuration, so we calibrate each separately.

STEP CASE

Liquid cuvette 5 steps

This is a calibration procedure for the **liquid cuvette (also known as the standard cuvette)**.



Materials

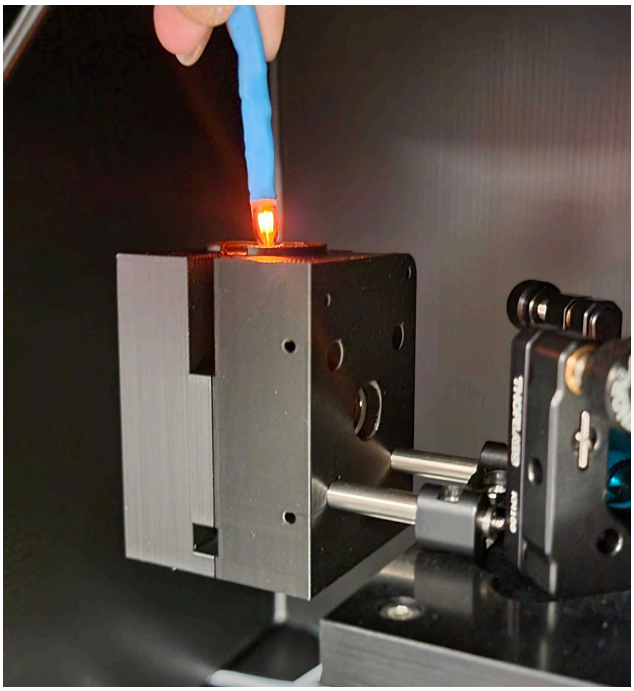
3 Assemble your materials: a neon bulb connected to power and acetonitrile in a disposable culture tube.

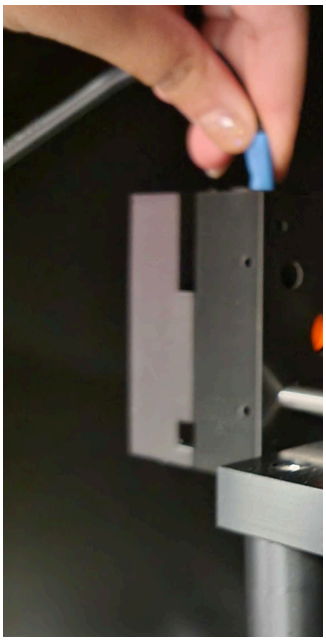
The exact materials we're using are listed below:

- Neon bulb: An **NE-2H lamp** with a 1000 Ω resistor soldered to **an extension cord with a switch**. Ensure all wires are safely insulated.
- Acetonitrile: **HPLC grade, VWR**
- Ethanol: **USP spec, KOPTEC**
- Disposable Culture Tubes: **VWR PN: 47729-568** Borosilicate glass — size 10 × 75mm
- Parafilm or other cover for tube

Neon acquisition

4 Place the neon bulb into the liquid cuvette directly in front of the lens. You should be able to see neon light coming through.

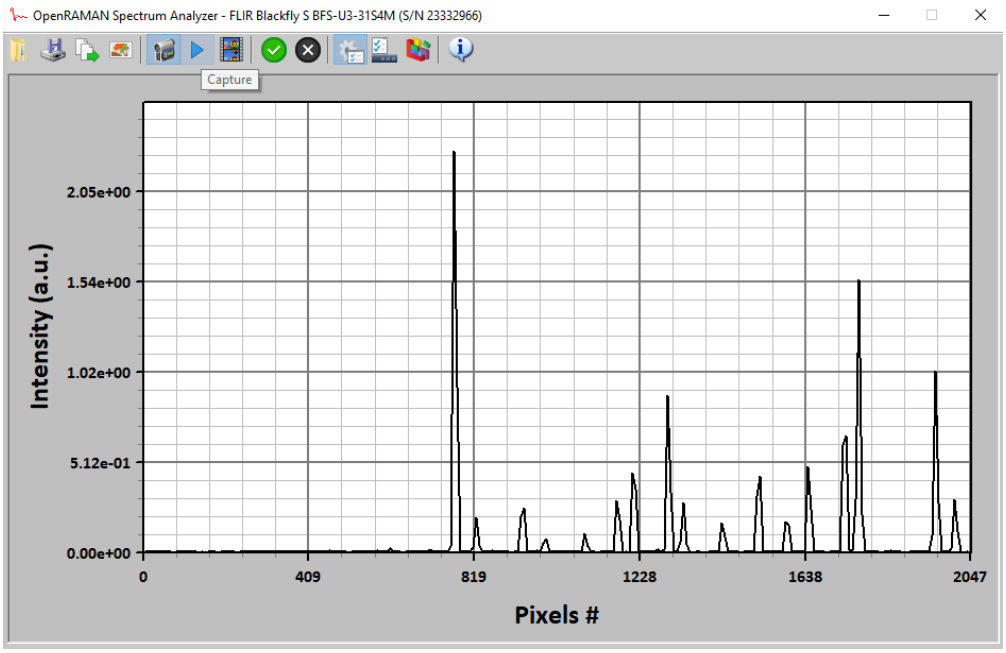




Take a live measurement and move the bulb around until you get a spectrum like the one below. Make sure 'Enable Baseline Removal (Schulze et al. Algorithm)' and 'Enable Median Filtering' are both **OFF** and use the following settings:

	A	B
	Exposure	1000 ms
	Gain	0.0
	ROI	100 px
	Num avg	5

You can manually save your file as a ".csv" or ".spc" by selecting the save icon in the upper left corner of the toolbar. We recommend saving files as ".csv" because ".spc" files can only be viewed on the Spectrum Analyzer software.

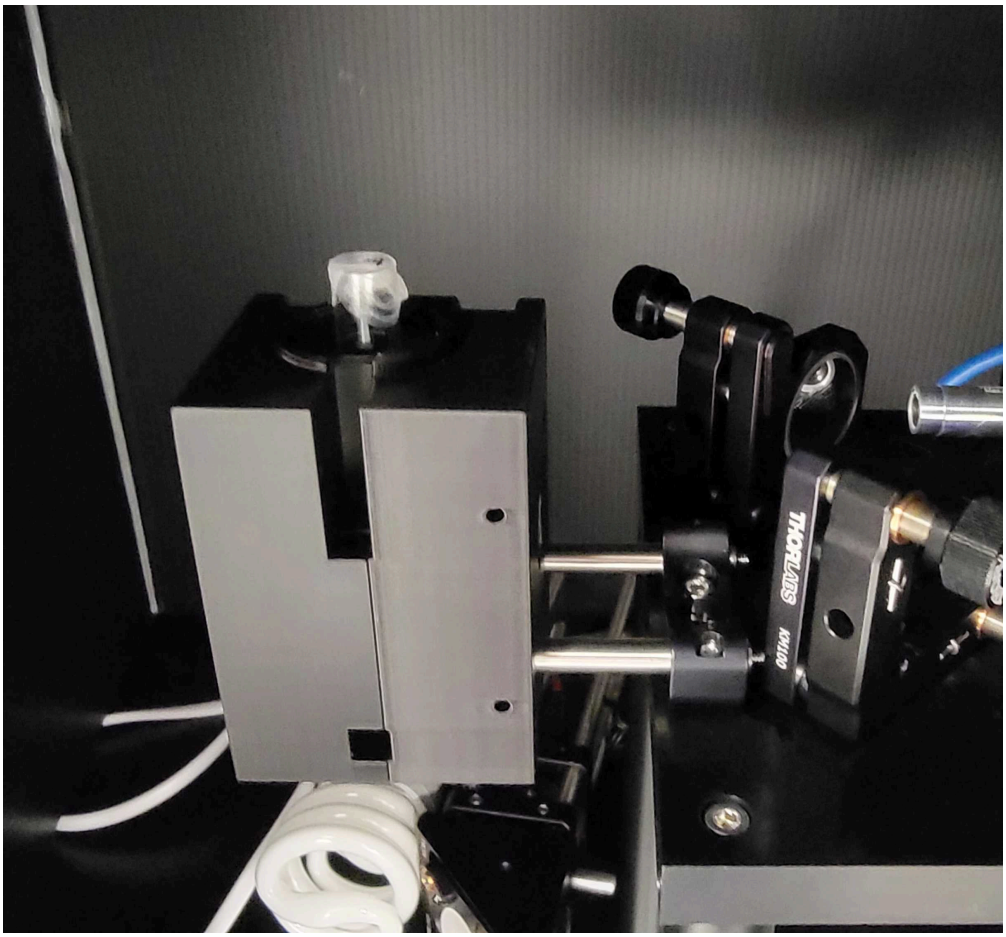


A screenshot of the Spectrum Analyzer software displaying the spectrum of neon from a liquid cuvette.

NIST reference for neon

Acetonitrile Acquisition

5 Pipette 2 mL of acetonitrile into a disposable glass culture tube and cover it with a piece of Parafilm or similar.



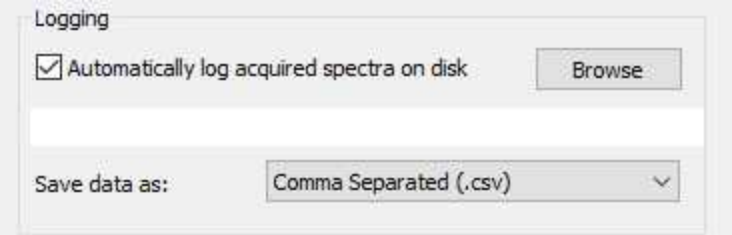
Acetonitrile in tube in the liquid cuvette attachment.

Make sure "Enable Baseline Removal (Schulze et al. Algorithm)" and "Enable Median Filtering" are both **OFF** and use the following settings:

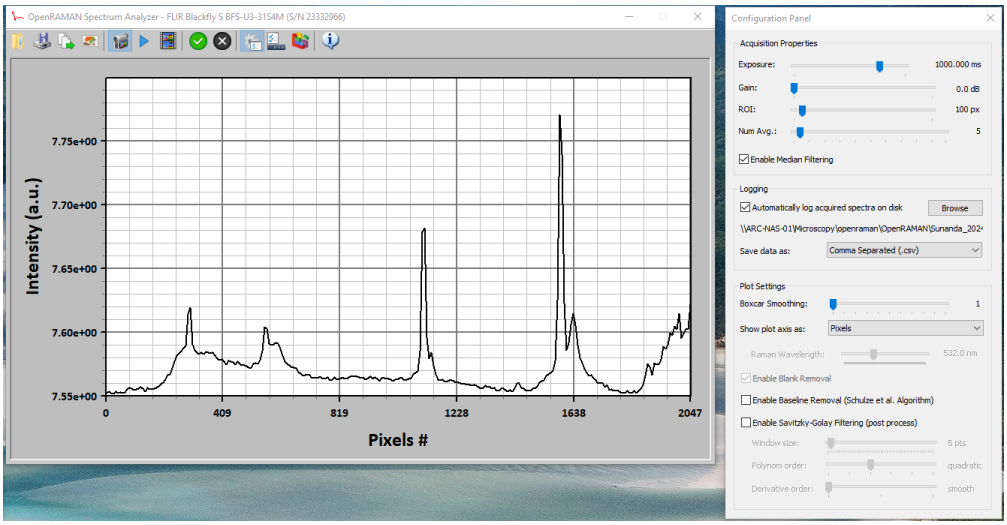
	A	B
	Exposure	1000ms
	Gain	0.0
	ROI	100px
	Num Avg	5

Capture a spectrum using the play button.

If you select "Automatically log acquired spectra on disk," your data will be saved automatically. You can save data as either a ".csv" or ".spc." We recommended saving files as ".csv" because ".spc" files can only be viewed on the Spectrum Analyzer software.



File path has been removed in this image.



A screenshot of the Spectrum Analyzer software displaying the spectrum of acetonitrile from a liquid cuvette. Configuration settings are shown on the right.

Sym. Species	No	Approximate type of mode	Selected Freq. Value	Rating	Infrared Value	Phase	Raman Value	Phase	Comments
a_1	1	CH3 s-str	2954	A	2954.1 M	gas	2942 VS	liq.	
a_1	2	CN str	2267	A	2266.5 M	gas	2249 S	liq.	
a_1	3	CH3 s-deform	1385	C			1376 M	liq.	OC(ν_3+ν_4)
a_1	4	CC str	920	A	920.2 S	gas	918 S	liq.	
e	5	CH3 d-str	3009	A	3009.2 S	gas	2999 S	liq.	
e	6	CH3 d-deform	1448	D	1447.9 S	gas	1440 M b	liq.	FR(ν_7+ν_8)
e	7	CH3 rock	1041	A	1040.8 M	gas			
e	8	CCN bend	362	B	362 S	gas	380 S	liq.	

Source: Shimanouchi, 1972

NIST reference for acetonitrile

Dark spectrum acquisition

6 A "dark spectrum" is just a measurement with the laser off. This spectrum gives you information about background noise in your environment. You can do this without a sample in the cuvette.

Turn the laser off and acquire a dark spectrum to record the background environment.

Make sure "Enable Baseline Removal (Schulze et al. Algorithm)" and "Enable Median Filtering" are both **OFF** and use the following settings:

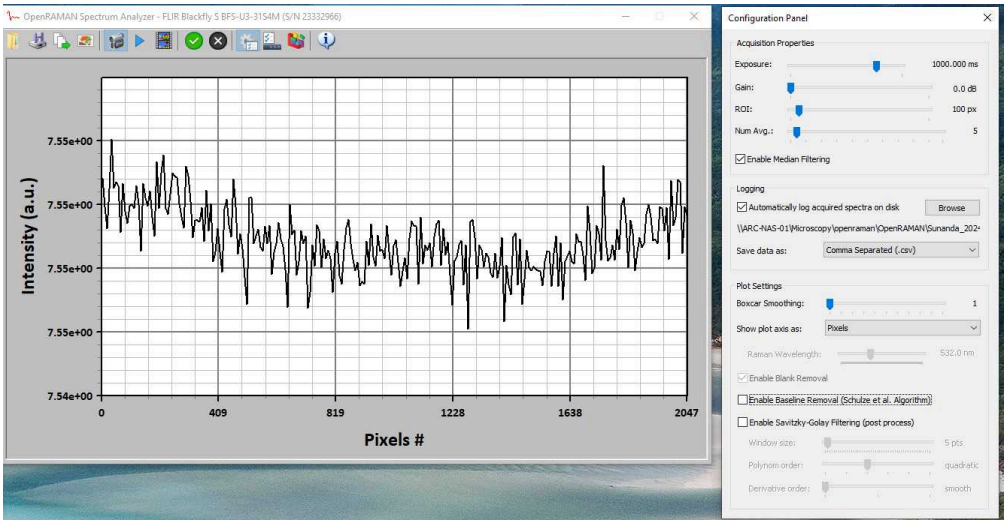
	A	B
	Exposure	1000 ms
	Gain	0.0
	ROI	100 px
	Num avg	5

Acquisition parameters for the dark spectrum.

Capture a spectrum using the play button. Note that you can change the exposure to match the typical acquisition settings you'll use for your samples.

If you select "Automatically log acquired spectra on disk," your data will be saved automatically. You can save data as either a ".csv" or ".spc" file. We recommend saving files as ".csv" because ".spc" files can only be viewed on the Spectrum Analyzer software.

File path has been removed in this image.



A screenshot of the Spectrum Analyzer software displaying a dark spectrum. Configuration settings are shown on the right, but make sure to uncheck "Enable median filtering."

Data processing

7 All the data collected with the liquid cuvette can be processed using a Python calibration notebook available on **GitHub**. The references quoted above are used to create the calibration equations. This notebook has four general steps:

1. Use the neon spectrum to create a linear equation to convert from pixel # to wavelength (nm). Atomic emission sources are great for this since they have known

absolute wavelength positions. They also have well-defined, sharp peaks that aren't affected by environmental factors like temperature and pressure.

2. Convert wavelength to wavenumber (cm-1). The equation is: Raman_shift_in_cm-1 = (10^7)((1/532) - (1/wavelength_in_nm).

3. Test conversion and apply an additional adjustment based on the acetonitrile spectrum. Acetonitrile is a known, commonly referenced material with strong peaks across a wide range. This additional step helps catch systematic errors that might occur in the previous two steps, acting as a check. The acetonitrile spectrum, unlike neon, is from Raman scattering and can help correct for small variations in laser behavior and track laser power over time.

Protocol references

1. Boussemaere, L. **https://www.open-raman.org/build/**. Accessed 2024.

2. *Strong lines of Neon (NE)*. Available at: https://physics.nist.gov/PhysRefData/Handbook/Tables/neontable2.htm (Accessed: 25 September 2024).

3. **Shimanouchi, T.**, Tables of Molecular Vibrational Frequencies Consolidated Volume I, National Bureau of Standards, 1972, 1-160.

4. *Calcite R150020 - RRUFF*. Available at: https://rruff.info/R150020 (Accessed: 25 September 2024).

5. Okuno, M. (2021) 'Hyper-Raman spectroscopy of alcohols excited at 532 nm: Methanol, ethanol, 1-propanol, and 2-propanol', *Journal of Raman Spectroscopy*, 52(4), pp. 849–856. doi:10.1002/jrs.6066.