# Acquiring Raman Spectra with Renishaw inVia Reflex Microspectrometer


Created by: Francheska Colón, Annette Colón, Maria Villarreal, Edwin Caballero

Manager: Samuel Hernandez-Rivera

| | | | |
|---|---|---|---|
| SOP-01 | Francheska Colón, Annette Colón, Maria Villarreal, Edwin Caballero | | University of Puerto Rico at Mayagüez |
| Effectivity: June/11/2022 | Acquiring Raman Spectra with Renishaw inVia Reflex Microspectrometer | | Revised by: |
| Revised: | | | Approved by: |

This SOP uses the following:

- Instrument: Renishaw inVia Reflex Microspectrometer
- Laser

| Company | Product | Wavelength | Laser Power |
|---|---|---|---|
| Hubner Photonics | Cobolt Flamenco™ | 660 nm | $\leq 500\ mW$ |
| Hubner Photonics | Cobolt Jive™ | 561 nm | $\leq 500\ mW$ |
| Hubner Photonics | Cobolt Samba™ | 532 nm | $\leq 1500\ mW$ |
| Hubner Photonics | Cobolt Twist™ | 457 nm | $\leq 300\ mW$ |
| | | 405 nm | |

- Filter: Vary
- Program: Cobolt, WiRE

**TURNING ON MICROSPECTROMETER**

| | |
|---|---|
| | Turn on the power outlet for system 01 to activate the fan. |
| | Remove the zip-locks on the mirrors used for the laser, being careful not to touch or move/rotate them. |
| | Choose the desired laser wavelength for system 01 or 02 (660 nm, 561 nm, 405 nm, 457 nm, or 532 nm). |
| | Determine which mirrors must be active for the laser to pass through the microspectrometer, depending on the system (system 01 or system 02). |
| | Activate the necessary mirrors by flipping their kinematic mount vertically upwards (perpendicular to the table). |
| | De-activate the necessary mirrors by flipping their kinematic mount horizontally (parallel to the table). |
| | Open the inVia spectrometer door with the key. |
| | Verify that the correct edge filter is placed for the laser excitation line being used. |
| | Close the inVia spectrometer door. |
| | Connect the cable to the Cobolt controller of the desired laser. |
| | Turn on the desired laser by rotating the key parallel to the table until the green light turns on, and wait for 30 minutes before using it. |
| | Turn on the spectrometer at the same time as the laser and wait for 20 minutes before using the CCD detector. |
| | Turn on the tower of the computer. |
| | Open the Cobolt program. |
| | Select the desired laser. |
| | Select the second option and click "Connect." |
| | Set the laser power to 30-50 mW, depending on the analyte being utilized, and readjust as necessary. |
| | Press the "ENTER" key after setting the laser power. |
| | Open the WiRE 4.4 program. |
| | Select "Reference un-referenced motors only" on the "WiRE Motor Reference Options" window and click "OK." |
| | Open the Raman microscope enclosure door by pushing the "door release" button. |
| | Turn on the microscope light. |
| | Set the microscope objective to 100X for calibrating samples, and wear gloves. |
| | Set the optical magnification on the WiRE software |

## CALIBRATING FOR SOLID SAMPLES

| | |
|---|---|
| | Place the standard sample to calibrate the microspectrometer. |
| | Find the optimal distance for the focal point. |
| | Move the sample until the light is placed between the corner of the plate and sample. Move montage vertically to observe a gradient between the corner of the plate and sample. |
| | Focus until the line between the plate and sample is seen. |
| | Place the microscope on the place where there are stains. |
| | Close the microscope door. |
| | Change the visible light option (1) to a laser (4). |
| | Change the light to a laser rotating clockwise (CW). There are two clicks. |
| | Go to Measurement → New → Spectral Acquisition in the WiRE program. |
| | Change the Grating scan type to “Static” mode from the “Range” tab in the “Spectral acquisition setup” window. |
| | Choose the Edge filter placing previously from Configuration → Laser name. |
| | Click “Apply” and go to the “Acquisition” tab. |
| | Adjust laser power, exposure time, and acquisitions according to the following values: |
| | Laser power (LP) [mW] |
| | Exposure time (ET) [s] |
| | Accumulation number (ACC) |
| | Objective of Magnification (OBJ) |
| | Signal-to-noise Ratio (SNR) |
| | Search or create a folder where spectra will be recorded by going to the “File” tab and clicking “Browse”. |
| | Record spectra in *.spc and *.txt formats and click “Apply”. |
| | Place a title on “Title” and click “Apply”. E.g., Si-LP10-ET2-ACC-1-OBJ20X. |
| | Click “Apply” and then “OK”. |
| | If it runs in “Static” mode, turn off the laser from the “Sample Review” window. |
| | Click “Run” to acquire spectra of the sample. |
| | Identify peaks from the “Peak Pick” option. |
| | Fix peak shift from Tools → Calibration → Offset. Solid Samples -> Silicon (520.744 cm-1). |
| | Place a value to fix offset (positive values are subtracted and negative values are added) and click “OK”. |
| | Click “Run” to acquire a new spectrum. |

| | |
|---|---|
| | [OPTIONAL] Run again in “Extended” mode from 100 to 1000 cm-1 to validate offset. |

**CALIBRATING FOR LIQUID SAMPLES**

| | |
|---|---|
| | Prepare the standard sample, Cyclohexane (801.484 cm-1), to calibrate the microspectrometer. |
| | Open the microscope door and place the sample to analyze inside. |
| | Focus the sample. |
| | Close the microscope door. |
| | Go to Measurement → New → Spectral Acquisition. |
| | Set the desired spectrum range by selecting "Extended" mode from the "Range" tab in the "Spectral acquisition Setup" window. |
| | Click "Apply" and go to the "Acquisition" tab, then click "OK". |
| | Adjust the laser power, exposure time, and accumulations as per the requirement. (LP [mW], ET [s], ACC, OBJ, SNR) |
| | Search or create a folder where spectra will be recorded by going to the "File" tab and clicking "Browse". |
| | Record spectra in *.spc and *.txt formats. |
| | Place a title on "Title", click "Apply" and then "OK". |
| | Click "Run" to acquire spectra of the sample. |
| | If "Static" mode ran, turn |

**TROUBLESHOOT**

1. Optical setup is misaligned.

***DO NOT TOUCH*** *contact authorized personnel for help.*

If authorized personnel cannot be contacted, move the mirror closest to the laser.

Advisor Signature _____

Co-Advisor Signature _____