Acquiring Raman Spectra with Renishaw inVia Reflex Microspectrometer



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|------------------------------|--|--|
| 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

1. Turn on the power outlet to turn on fan (only for system 01).



2. **Remove** the zip-locks on the mirrors used for the laser. Extra care to avoid touching or moving/rotating the mirrors.



3. Choose the desired laser.





4. Determine which mirrors must be active for the laser to pass through the microspectrometer.



The optical setups utilize a fused silica (SiO_2) broadband dielectric 400-750 nm mirror placed on a <u>kinematic mirror mount</u> with 3 adjusters. All mirrors are fixed in position with an <u>optical post</u>, <u>post holder</u>, and a <u>swivel base adapter</u>. However, some mirrors contain, in addition, a <u>flip mount</u> <u>adapter</u>. The flip mount adapter allows the user to either place the mirror upwards and reflect the incoming light source or downwards so that the source continues towards the next mirror.

5. Activate the necessary mirrors by flipping their kinematic mount vertically upwards (perpendicular to the table).





6. **De-activate** the necessary mirrors by flipping their kinematic mount horizontally (parallel to the table).





7. Open inVia spectrometer door with key.







8. Verify that the correct edge filter is placed (varies per laser excitation line).

Edge filter =





9. Close inVia spectrometer door.



10. Connect the following cable to the Cobolt controller of the desired laser.





11. Turn on the desired laser. System 1 and 2 contain Cobolt controllers that need the following cable to be plugged when in use.

Laser has a key parallel to the table with green light indicates that the laser is off. Rotate key vertically to turn on laser.







WAIT 30 MIN BEFORE USING THE LASER THAT WAS TURNED ON

12. Turn on spectrometer AT THE SAME TIME AS THE LASER.





WAIT 20 MIN BEFORE USING THE CCD DETECTOR THAT WAS TURNED ON

BOTH THE LASER AND INSTRUMENT CAN BE TURNED ON SIMULTANEOUSLY

(i.e. you do not have to wait the initial 30 min to start the spectrometer and wait other 20 min)

13. Turn on the tower of the computer.



14. Open Cobolt program.



15. Select desired laser.





16. Select second option and click "Connect".

| Welcome to Cobolt Monitor™ | Cobol: |
|--|---|
| Connected Lasers Cobolt Laser S/N 532008065 found on COM3 | |
| Laser model Unidentified | Laser Head 05-01 on Gen5 Driver V Connect |

17. Place 30-50 mW power and readjust. Laser power depends on the analyte being utilized.



- **18. Press** "ENTER" key after placing the laser power. There is no button, you press ENTER.
- **19. Open** the WiRE 4.4 program.



20. Select "Reference un-referenced motors only" on the "WiRE Motor Reference Options" window.

| iRE Motor Reference Op | tions | | |
|------------------------|--------|---------------|--|
| Motor | Туре | State | Reference un-referenced motors only |
| Beam Expander | serial | un-referenced | O Paferance All Motors |
| CCD lensfocus | serial | un-referenced | O Reference All Hotors |
| Grating Motor | serial | un-referenced | Reference Selected Motors |
| Post slit lensfocus | serial | un-referenced | Celect one or more maters from the list |
| Pre slit lensfocus | serial | un-referenced | Unreferenced serial motors will still be |
| Slit Master | serial | un-referenced | referenced. |
| and and | | | |

21. Click "OK".

| Motor | Type | State | Reference un-referenced motors only |
|--|--------------------------------------|--|---|
| Beam Expander CCD lensfocus | serial | un-referenced un-referenced | O Reference All Motors |
| Grating Motor | serial | un-referenced | Reference Selected Motors |
| Post slit lensfocus Pre slit lensfocus Slit Master Slit Slave | serial serial serial serial | un-referenced un-referenced un-referenced un-referenced | Select one or more motors from the list. Unreferenced serial motors will still be referenced. |
| | | | O Ignore Referencing Parallel Motors |
| | | | If referencing of Parallel motors is skipped they will be assigned their last known positions. Serial motors will always be referenced. Always reference - to always reference the motors, please select the option from the |
| | | | Reference Motors drop-down list on the System's property page accessible through WIRESystemSetup.exe. |



22. Open Raman microscope enclosure door by pushing the "door release" button.





23. Turn on microscope light.



24. Set microscope objective of 100X for calibrating samples. WEAR GLOVES.



25. Set optical magnification on the WiRE software.



CALIBRATING FOR SOLID SAMPLES

1. Place standard sample to calibrate the microspectrometer.



2. Find de optimal distance for the focal point.



3. Move sample until the light is placed between the corner of the plate and sample. **Move** montage vertically to observe a gradient between corner of the plate and sample.



4. Focus until the line between the plate and sample is seen.



5. Place microscope on the place where there are stains.



6. Close microscope door.



7. Change visible light option (1) to a laser (4).





8. Change de light to laser rotating clockwise (CW). There are two clicks.



9. Go to Measurement \rightarrow New \rightarrow Spectral Acquisition in the WiRE program.



10. Change the Grating scan type to "Static" mode from the "Range" tab in the "Spectral acquisition setup" window.

| Range | Acquisition | File | Timing | Temperature | FocusTrack | Advanced | | |
|-------|------------------|------|--------|----------------|------------|--------------------|---|--|
| Grati | ng scan type | | Sp | ectrum Range | | | | |
| 0 | Static | | Low | -4 | 82.49 | | | |
| 0 | extended | | Cen | tre 52 | 0.00 | Raman shift/cm-1 | ~ | |
| | | | High | 1 14 | 09.63 | | 7 | |
| • | Standard High | | Gra | ting name | 1800 l/mm | (vis) | ~ | |
| | | | Det | ector name | Master: R | enishaw CCD Camera | ~ | |
| | | | Cali | bration status | 🖌 Ca | librated OK | | |

11. Choose the Edge filter placing previously from Configuration \rightarrow Laser name.

| Range Acquisition | File Tim | ing Tem | perature | FocusTrack | Advanced | | | |
|-------------------|----------|------------|----------|------------|------------------|----|---|--|
| Grating scan type | | Spectrur | n Range | | | | | |
| Static | | Low | -482 | . 49 | | | | |
| CExtended | | Centre | 520. | 00 | Raman shift/cm-1 | | ~ | |
| | | High | 1409 | 9.63 | | | | |
| Confocality | | Configur | ation | | | | | |
| | | Laser nan | ne | 532 nm ed | ae | | ~ | |
| Standard | | | | 405 nm ed | ge | | | |
| ⊖ High | | Grating n | ame | 532 nm ed | | | | |
| | | 2015 10 | | | | | | |
| | | Detector | name | Master: Re | nishaw CCD Came | ra | ~ | |
| | | Calibratio | n status | 🖌 Cal | brated OK | | | |
| | | | | | | | | |

12. Click "Apply" and go to "Acquisition" tab.

| Acquisition File | Timing Temp | erature | FocusTrack | Advanced | | | |
|----------------------------|-------------|---------|------------|----------------|------|--------|--|
| Grating scan type | Spectrum | Range | | | | | |
| Static | Low | -482 | .49 | | | | |
| ○ Extended | Centre | 520. | 00 | Raman shift/c | m-1 | \sim | |
| | High | 1409 | 1.63 | | | | |
| High | Grating nar | ne | 1800 l/mm | (vis) | | ~ | |
| High | Grating nar | ne | 1800 l/mm | (vis) | | ~ | |
| | Detector n | ame | Master: Re | enishaw CCD Ca | mera | ~ | |
| | Calibration | status | 🖌 Cal | ibrated OK | | | |

13. Adjust laser power, exposure time, and acquisitions.

| Laser power (LP) [mW] | |
|----------------------------------|--|
| Exposure time (ET) [s] | |
| Accumulation number (ACC) | |
| Objective of Magnification (OBJ) | |
| Signal-to-noise Ratio (SNR) | |

| Statement of the second s | File Timing | Temperature Focus1 | rack Advanced | | | |
|--|--|---|------------------|-----------------------|-----------------------|------|
| Exposure time /s | 1.00 | Laser power / % | 100 | ✓ Accumulations | 1 | |
| | | | | Objective | 5 | |
| | | | | | | |
| Live imaging | | | | | | |
| | | | | | New | |
| Not using live in | naging. | | | | Edit 1107 | 1000 |
| | | | | | LuicLui | |
| | | | | | | |
| ītle | Single scan measu | rement | | | | |
| ītle)escription | Single scan measu | irement surement generated by | the WiRE spectra | acquisition wizard. | | |
| itle)escription | Single scan measu A single scan mea | irement surement generated by | the WiRE spectra | acquisition wizard. | | |
| itle Description | Single scan measu A single scan mea | irement surement generated by | the WiRE spectra | l acquisition wizard. | | |
| itle Description | Single scan measu A single scan mea ration | urement surement generated by estore instrument state | the WiRE spectra | l acquisition wizard. | er exposure on sample | |

14. Search or Create folder where spectra will be recorded by going to the "File" tab and clicking "Browse".

| ectral 📕 | Open | | | | | × | > |
|----------|-------------|--------------------|----------------------|-----|------------------------------------|--------------|------|
| Range | Look in: | FOR SOP | | ~ | G 🖸 🖻 🗔 - | | |
| | * | Name 🔀 Si-5X-01 | ^ | | Date modified 6/11/2022 3:19 PM | Type WiRE | |
| File na | uick access | | | | | | |
| | Desktop | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | This PC | | | | | | |
| | Network | ۲ | | | | > | |
| _ | | File name: | Si-5X-01 | | ~ | Open | |
| | | Files of type: | WiRE Data Files (*.w | df) | ~ | Cancel | Help |

15. Record record spectra in *.spc and *.txt formats and click "Apply".

| | | 1.1.0 | | | | | |
|-----------|--------------------------------------|-------------|---------------|---------------|----------|------------|---|
| ange Acqu | uisition File | Timing | Temperature | FocusTrack | Advanced | | |
| Cile anna | C:\Users\Ber | aich ann Ou | 027A\Decktor | | V.01 udf | Provine | 1 |
| | te increment | lishaw Q- | 027A (Desktop | NFOR SOF DI-3 | X-01.W01 | browse | |
| | | | | | | | |
| AU | to export to spo to export to typ | | | | | | |
| Au Au | to export to spo to export to txt |) | | | | | |
| Au Au | to export to spo to export to txt | > | | | | | |
| Au | to export to spo to export to txt |) | | | | | |
| Au | to export to spi |) | | | | | |
| Au | to export to spi |) | | | | | |

16. Place title on "Title" and click "Apply". E.g., Si-LP10-ET2-ACC-1-OBJ20X.

| Range Acquisition | File | Timing 1 | [emperature | FocusTr | ack | Advance | d | | | | |
|-------------------|---------------------|-----------------------------|-----------------------------|--------------------|---------|----------|---|---------------|------------|--------------|---|
| Exposure time /s | 5.00 | • | Laser pow | ver / % | 10 | | ~ | Accumulations | 5 | • | |
| | | | | | | | | Objective | 5 | | |
| | | | | | | | | | | | |
| Live imaging | | | | | | | | | | New | |
| Not using live in | naging. | | | | | | | | | Edit LUT | • |
| Title 🛛 | Si-5X-01 | 5 | | | | | | | | | |
| Description | Measure ET=5s, I | ement of silio LS=10%(17 | con standard 7.8mW), ACC | for calibra =5. | ation. | | | | | | |
| Response calibr | ration | Res | store instrum | ent state | on cor | npletion | | Minimize las | er exposur | re on sample | |
| | oval | Clo | se laser shut | ter on con | npletio | n | | | | | |
| Cosmic ray rem | | | | | | | | | 112 | | |

17. Click "Apply" and then "OK".

| Range Acquis | ition File | Timing T | emperature Focus | Track | Advanced | | | | |
|---------------|------------------------------------|---------------|-----------------------|----------|----------|---------------|-------------|-----------|--|
| Exposure time | /s 5.00 | | Laser power / % | 10 | ~ | Accumulations | 5 | | |
| | | | | | | Objective | 5 | | |
| Not using liv | e imaging. Si-5X-01 | | | | | | | Edit LUT | |
| | | ment of silic | con standard for cali | oration. | | | | | |
| Description | Measurer ET=5s, L | S=10%(17 | .8mvv), ACC=5. | | | | | | |
| Description | Measurer ET=5s, L alibration | S=10%(17 | store instrument stat | e on co | mpletion | Minimize las | er exposure | on sample | |
| Description | Measurer ET=5s, L | S=10%(17 | store instrument stat | e on co | mpletion | Minimize las | er exposure | on sample | |

18. If it runs in "Static" mode, **turn off** laser from the "Sample Review" window.

| Sample Review White light ar | nd view control | | 1 | Laser state and view control | | Spec | trometer state | |
|---------------------------------|-----------------|---|---------|------------------------------|-----|------|-----------------------------|---|
| - | ų. | | x5 ~ | | 10% | | 1800 l/mm (vis) | ~ |
| | | | <u></u> | Laser Shutter Closed e | ~ | | Master: Renishaw CCD Camera | ~ |
| | 0 | 0 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

19. Click "Run" to acquire spectra of the sample.



20. Identify peaks from the "Peak Pick" option.



21. Fix peak shift from Tools \rightarrow Calibration \rightarrow Offset.

Solid Samples -> Silicon (520.744 cm⁻¹)





22. Place value to fix offset (positive values are subtracted and negative values are added) and click "OK".



23. Click "Run" to acquire a new spectrum.

| UVIRE - Si-5X-01 | | | | | | |
|------------------|---------------------|-------------------|--------------|-----------------|------------|-----|
| File Edit View | Surface Measurement | Live video Analys | s Processing | Procedures Tool | s Window H | elp |
| 🦓 🏶- 🛴 | 🗅 🚔 - 日 | \times | - 😁 😝]: | 1 🖢 🕇 🗲 | • 🔿 🦊 🛛 | |
| * | * X 2 7 1 | 7 🖗 🕺 🛛 |) - 🔏 🔛 | B & (1) | 🏵 🔳 🚀 | |
| Renishaw XYZ | X -32602.1 Y 6585.9 | Z 107.0 Go | a | Ru | n | |
| Video | | | | | | |
| inVia 60r | ns | | | | | |

24. [OPTIONAL] Run again in "Extended" mode from 100 to 1000 cm⁻¹ to validate offset.



| nange | Acquisition | File Timing | Temperature | FocusTrack | Advanced | | | |
|-------|--------------|-------------|----------------|------------|--------------------|--------|--|--|
| Grati | ng scan type | Sp | ectrum Range | | | | | |
| 0 | Static | Low | 10 | 0.00 |] | | | |
| | Extended | | ntre | | Raman shift/cm-1 ~ | 1 | | |
| | | Hig | h 10 | 00.00 | | | | |
| 0 | High | Gra | ting name | 1800 l/mm | (vis) | ~ | | |
| ١ | High | Gra | ting name | 1800 l/mm | 1800 l/mm (vis) | | | |
| | | Det | ector name | Master: Re | enishaw CCD Camera | \sim | | |
| | | Cali | bration status | 🖌 Ca | ibrated OK | | | |

CALIBRATING FOR LIQUID SAMPLES

1. Place standard sample to calibrate the microspectrometer.

Liquid Samples -> Cyclohexane (801.484 cm-1)



ACQUIRING SAMPLE SPECTRUM

1. Open microscope door.



2. Place sample to analyze inside.



3. Focus the sample.



4. Close door.



5. Go to Measurement \rightarrow New \rightarrow Spectral Acquisition.



6. Place "Extended" mode from the "Range" tab and the "Spectral acquisition Setup" window with the desired spectrum range.

| Range | Acquisition | File | Timing | Temperat | ture Focu | Track | Advanced | | |
|-------|----------------|------|--------|-------------|-----------|------------|------------------|--------|--|
| Grati | ng scan type - | | Sp | ectrum Rai | nge | | | | |
| 0 | Static | | Low | [| 100.00 | | | | |
| | Extended | | Cen | tre | | | Raman shift/cm-1 | \sim | |
| | | | High | n [| 1000.00 | | | | |
| 0 | Standard | | Lase | er name | 53 | 2 nm edge | 2 | ~ | |
| 0 | Standard | | cut | | 53 | 2 nm eage | | ~ | |
| | | | or cr | ang name | 18 | JU I/mm (\ | 'IS) | ~ | |
| | | | Det | ector name | e Ma | ster: Ren | shaw CCD Camera | ~ | |
| | | | Calil | oration sta | itus 🖌 | Calib | ated OK | | |

7. Click "Apply" and go to the "Acquisition" tab and then click "OK".

| Range Acquisiti | on File | Timing Temperatu | re FocusTrack | Advanced | | | | |
|------------------|-----------------------|---|-------------------------------|-----------|---------------|-------------|-----------|--|
| Exposure time /s | 5.00 | 🖨 Laser (| oower / % 10 | ~ | Accumulations | 5 | - | |
| | | | | | Objective | 5 | | |
| Not using live | imaging. | | | | | | Edit LUT | |
| Description | Measurem ET=5s, LS | ent of silicon stand =10%(17.8mW), A | ard for calibration ACC=5. | ı. | | | | |
| | bration | Restore inst | rument state on c | ompletion | Minimize las | er exposure | on sample | |
| Response cali | | | | | | | | |

8. Adjust laser power, exposure time, and accumulations.

| Laser power (LP) [mW] | |
|----------------------------------|--|
| Exposure time (ET) [s] | |
| Accumulation number (ACC) | |
| Objective of Magnification (OBJ) | |
| Signal-to-noise Ratio (SNR) | |

9. Search or Create folder where spectra will be recorded by going to the "File" tab and clicking "Browse".

| Range | Look in | FOR SOP | | ~ | G 🜶 🖻 🗔 - | | |
|---------|--------------|----------------|-------------------------|---|-------------------|--------|------|
| | 3 | Name | ^ | | Date modified | Туре | |
| File na | Quick access | 10-5X-01 | | | 6/11/2022 3:19 PM | WIKE | |
| | Desktop | | | | | | |
| 1 | Libraries | | | | | | |
| | | | | | | | |
| | N | < | | | _ | > | |
| | Network | File name: | Si-5X-01 | | ~ | Open | |
| | | Files of type: | WiRE Data Files (* wdf) | | ~ | Cancel | Help |

10. Record spectra in *.spc and *.txt formats.

| ange Acqu | uisition File | Timing | Temperature | FocusTrack | Advanced | | |
|-----------|------------------|------------|--------------|---------------|----------|--------|--|
| | | | | | | | |
| File name | C:\Users\Ren | nishaw Q-0 | 27A\Desktop\ | FOR SOP\Si-5) | (-01.wdf | Browse | |
| Aut | te increment | | | | | | |
| | to export to spc | | | | | | |
| - Har | | | | | | | |
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| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

11. Place title on "Title", click "Apply" and then "OK".

| nange | File T | liming Te | mperature | FocusTra | k Advan | ced | | | | |
|-------------------|-------------------------|-----------------------------|-------------------------|---------------------|-------------|-----|---------------|-------------|-----------------|--|
| Exposure time /s | 5.00 | \$ | Laser pow | er / % | 10 | ~ | Accumulations | 5 | - | |
| | | | | | | | Objective | 5 | | |
| Not using live in | naging. | | | | | | | | New Edit LUT | |
| Title | Si-5X-01 | | | | | | | | | |
| Description | Measureme ET=5s, LS= | ent of silico = 10%(17.8 | n standard 3mW), ACC | for calibrat =5. | ion. | | | | | |
| | 12 | | ore instrume | ent state o | n completio | n | Minimize las | er exposure | e on sample | |
| Response calibr | ation | LIResu | | | | | | | | |

12. Click "Run" to acquire spectra of the sample.

| 🐌 WiRE - Si-5X-01 | | | | | | |
|-------------------|---------------------|----------------|------------------|-----------------|-------------|----|
| File Edit View | Surface Measurement | Live video Ana | lysis Processing | Procedures Tool | s Window He | lp |
| 🦓 🏶- 🛴 | 🗅 🚔 - 🔒 | X | 🎄 🔤 😝 | 11 🖢 🕇 🗲 | • 🔿 🦊 💯 | |
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| Renishaw XYZ | X -32602.1 Y 6585.9 | Z 107.0 | Go to | Ru | n | |
| Video | | | | | | |
| inVia 60n | (f)5 | | | | | |

13. If "Static" mode ran, **turn off** laser from the "Sample Review" window.

| Sample Review White light and view control | | | Laser state and view control | | | Spectrometer state | | |
|---|--------------|--------------|------------------------------|--------------|--------------|-----------------------------|---|--|
| | | x5 ~ | | 10% | | 1800 l/mm (vis) | ~ | |
| Toma and | | e | Laser Shutter Closed e | ~ | | Master: Renishaw CCD Camera | ~ | |
| 0 | 0 | <u> </u> | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | view control | view control | view control | view control | view control | view control | view control x5 Laser state and view control 10% Isoo l/mm (vis) Master: Renishaw CCD Camera | |

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