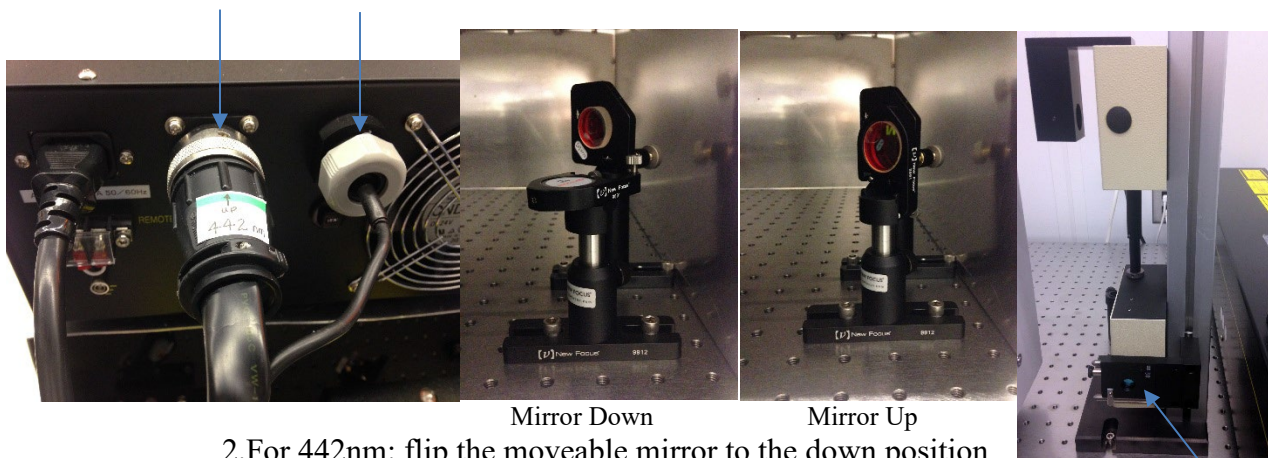


OPT3: Operating Procedure for Horiba Jobin Yvon LabRam Aramis Raman/PL System

See *LabSpec_6_2 General User Quick Start Guide* on the computer desktop

1. Ensure you have an active OPT3 reservation
2. Turn power on to laser(s) that you will be using
 - a. 785nm (Diode Laser): Press the “Laser on” button on the blue Horiba box located on top of the Kimmon power supply (to the right of the Raman). *Click [HERE](#) for 785nm laser start-up video*
 - b. 633nm (HeNe Laser): Turn the key to “On” on the Melles Griot black box. *Click [HERE](#) for 633nm laser start-up video*
 - c. 442nm or 325nm (HeCd Laser):
 1. Plug the desired power cables (325nm or 442nm) into the back of the Kimmon HeCd power supply. *Be gentle when removing and installing the cables – do not pull by the cable itself*



2. For 442nm: flip the moveable mirror to the down position
3. For 325nm: flip the moveable mirror to the up position
4. Insert the appropriate filter (442nm or 325nm) into the optics path as shown above

Do not touch the mirror surface with your fingers

5. Turn the key to “On” on the Kimmon HeCd laser power supply
 1. Allow time for warm-up after powering on (30min or more)
 2. After powering on, leave on for at least 30 minutes before powering off. After powering off, leave off for at least 30 minutes before powering on again.

Click [HERE](#) for 442nm laser start-up video

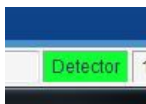
Click [HERE](#) for 325nm laser start-up video

3. Turn microscope lamp power on to 5 (intensity can be adjusted as needed). *Click [HERE](#) for microscope lamp video*

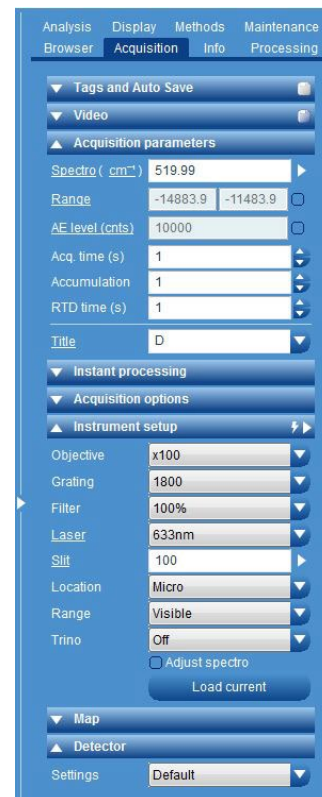


4. Open the LabSpec6 software on the computer by double clicking on the desk top icon.

Click [HERE](#) for Software Start-up and Instrument Set-Up video



5. **Verify that the CCD detector is functioning properly** by checking that the detector tab at the bottom of the software window is green.
6. On the right panel, select the Acquisition tab, and open the “Acquisition Parameters” menu and the “Instrument Set-Up” menu.
7. **To load and use previously saved measurement settings (Template):**
 - a. Click on the Acquisition Parameters menu heading
 - b. Select “Load from File”
 - c. Find your folder in the User Data folder and select the template file you wish to use
 - d. The Acquisition and Instrument Set-Up parameters should change to your saved values
8. **Instrument Set-Up**
 - a. **Objective:** Enter the microscope objective that you will be using. 100x is the typical value as this gives the smallest spot size and best signal. Lower power objectives can be used for volume sampling. The 40x UV objective must be used for the 325nm wavelength (*note that the working distance of the 40x UV objective is similar to the working distance of the 50x standard objective and the samples needs to be raised from the microscope stage in order to focus*)
 - b. **Grating:** Select the grating according to the chosen laser line and desired measurement range. (See the table below) The optimal grating settings for each incident wavelength for Raman measurements are shown in bold.



Laser Line	Grating Setting	Measurement Range (wave numbers)	Measurement Range (nm)
785nm	1800	0-1700	785-900
	1200	0-5400	785-1360
	300	0-10900	785-5440
633nm	2400	0-1140	633-680
	1800	0-4800	633-900
	1200	0-8460	633-1360
	300	0-13900	633-5440
442nm	2400	0-7980	442-680
	1800	0-13645	442-900
	1200	0-15300	442-1360
	300	0-20800	442-5440
325nm	2400	0-16100	325-680
	1800	0-19780	325-900
	1200	0-23440	325-1360
	300	0-28940	325-5440

- c. **Filter:** A filter can be chosen to reduce the laser power that strikes the sample if needed. 100% allows 100% of the intensity thru, while 1% allows 1% of the intensity thru
- d. **Laser:** Choose the laser wavelength you will be using. The optics will automatically change accordingly
- e. **Slit:** Enter a number between 50 and 400um. 100um is the typical value and gives the optimum resolution and counts, while 400um gives the highest number of counts (more light hitting the detector) but less resolution.
- f. **Location:** Choose Micro for measuring samples under the microscope. If using the Macro chamber, choose “Retro” for collection of the reflected signal or “Macro 90” for collection of a signal at 90 degrees.
- g. **Range:** Choose Visible for the 785nm, 633nm, or 442nm lasers. Choose UV for the 325nm laser.
- h. **Trino:** Set to Off

9. Perform Calibration

- a. **Set Acquisition Parameters** *Click [HERE](#) for Calibration Parameters video*

1.Spectro

- 1. **Select cm⁻¹ units** (click on units indicator if need to change)
- 2. **For 785nm, 633nm, or 442nm: type in 520** (for silicon) **and press Enter**
- 3. **For 325nm: type in 1295** (for teflon) **and press Enter**

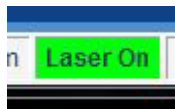
2.RTD time (s)

- 1. **For 785nm, 633nm, or 442nm: type in 1** (for silicon)
- 2. **For 325nm: type in 5** (for teflon)

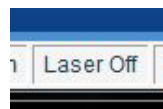
- b. **Load Calibration sample:** Load silicon standard sample if using 785nm, 633nm, or 442nm laser lines. Load Teflon standard sample if using 325nm laser line. *Click [HERE](#) for sample loading video*

- 1. Turn laser off using the Laser On/Off panel at the bottom of the window (if not off already)

Laser On



Laser Off



- 2. Rotate the microscope turret to the 10x objective and rotate the focus knob to raise the objectives away from the sample stage
- 3. Load sample onto the microscope stage.
- 4. Click on the video icon in the top menu of the software to see a video image
- 5. Adjust the microscope focus knobs to bring the video image of the sample into focus
- 6. Change the objective to 100x (or to the desired objective)
- 7. Turn the laser On, and fine adjust the focus to minimize the laser spot size
- 8. Click on the STOP button to stop the video image





c. **Run a Real Time Scan** by clicking on the Real Time Display Icon
 Click [HERE](#) for calibration acquisition and peak location measurement video

d. **Measure the peak location**

1. Zoom into the sample peak using the zoom tool

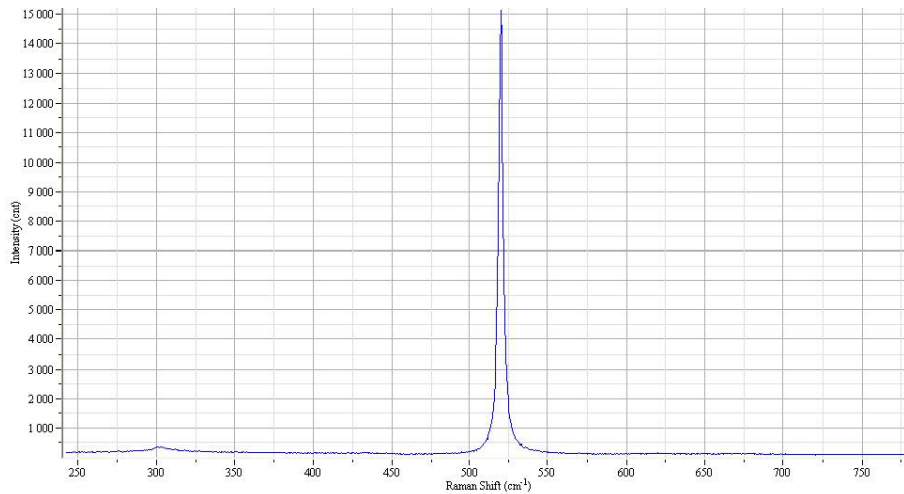


and measure the location of the peak using the line cursor tool.

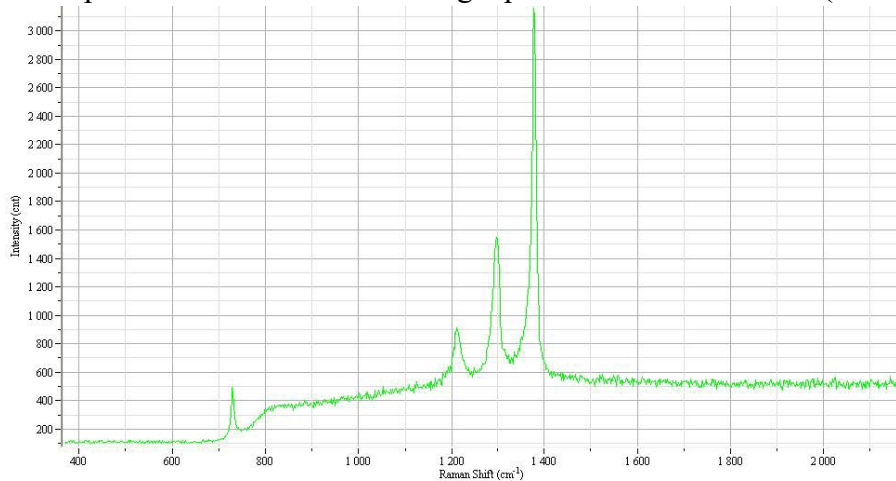
These tool icons are located on the left panel.



Raman spectra of silicon standard: Target peak location: 520cm^{-1}



Raman spectra of teflon standard: Target peak location: 1295cm^{-1} (middle peak)



e. **Calibrate** (if measured value is shifted more than one wavenumber from target value) Click [HERE](#) for calibration video

1. Select the Maintenance tab on the right panel

2. Adjust the offset shift value You can either type in a new number or click on the arrows

1. To move measured peak to the right, decrease the offset value

2. To move measured peak to the left, increase the offset value



3. Keep adjusting the offset shift value until the measured peak value is within one wavenumber of the target value.
4. Click the STOP button to stop the real time acquisition
5. Select the Acquisition tab on the right panel
6. Rotate the microscope turret to the 10x objective and rotate the focus knob to raise the objectives away from the sample stage, and unload the standard sample from the microscope stage

10. **Load your sample and focus as follows:** *Click [HERE](#) for sample loading video*
- a. Rotate the microscope turret to the 10x objective and rotate the focus knob to raise the objectives away from the sample stage
 - b. Load sample onto the microscope stage.
 - c. Click on the video icon in the software to bring up the video screen
 - d. Adjust the microscope focus knobs to bring the camera image of the sample into focus
 - e. Change the objective to 100x (or to the desired objective)
 - f. Turn the laser On using the Laser On/Off panel at the bottom of the window, and fine focus to minimize the laser spot size
 - g. Click the STOP button to stop the video image
 - h. Turn off microscope light and close the sample chamber doors

11. **Set Scan Parameters** *Click [HERE](#) for setting scan parameters video*
- a. Select the desired **units** by clicking on the units indicator after the word “Spectro”
 cm^{-1} (used for Raman) is the amount of wavenumber shift from the laser line
 nm (used for PL) is the actual wavelength value measured
 - b. Select the **Range**
 1. Select the box beside the range numbers to activate the range selection
 2. Enter the desired start and end values
 - c. Enter desired **Acq. Time (s)** – this is the count time; higher values result in more counts and longer acquisitions
 - d. Enter desired **Accumulation** – this is the number of measurement averages; high values result in smoother data and longer acquisitions
 - e. **Title:** enter a sample ID

Acquisition parameters	
Spectro (cm^{-1})	520.01
Range	100 2000 <input checked="" type="checkbox"/>
AE level (cnts)	1000 <input type="checkbox"/>
Acq. time (s)	5
Accumulation	5
RTD time (s)	1
Title	Sample 1

12. Acquire Spectra *Click [HERE](#) for Spectra Acquisition video*



- a. Click on the Spectra Acquisition icon to perform the full range scan
- b. Click on the rescale icon to auto scale
- c. Right click on the graph and select display mode:
 1. Single spectra
 2. Overlay spectra
 3. Stack Spectra



13. Saving data *Click [HERE](#) for saving data video*



- a. Click “Save as Data” icon of type CTRL S
- b. Select or create your own folder in the User Folder
- c. Enter a filename
- d. Select the format for the saved data under the “Save As Type” pull down menu:
 1. Labspec6: saves the data in the Labspec format, and also saves the measurement parameters that were used
 2. Text: saves the data at x,y pairs in a text file. This does not save the measurement parameters.
- e. The spectra can be saved as a picture file by copying (CTRL C) and pasting into the Paint program

14. Saving Your Measurement Settings (Template)

- a. Click on the “Acquisition Parameters” menu heading
- b. Select “apply to all frames”
- c. Enter a template name
- d. Click “Save to file”
 1. Select or create your own folder in the User Folder
 2. Enter a filename and save as a template file

15. Process the data (if desired)

- a. Select Analysis tab at the top of the right panel for peak searching and fitting
- b. Select Processing tab at the top of the right panel for:
 1. Smoothing and filtering
 2. Application of math functions
 3. Baseline correction

16. When finished: *Click [HERE](#) for system shut-down video*

- a. **Unload your sample**
 1. Make sure laser is off using the Laser On/Off panel
 2. Rotate the microscope turret to the 10x objective and rotate the focus knob to raise the objectives away from the sample stage
 3. Unload sample from the microscope stage

b. Turn off the microscope lamp power

c. Turn off the laser(s) power

- d. **Remove all data files from the active screen** by clicking on the “Close Active Data” icon for each file



e. Exit the LabSpec6 software

f. End your active OPT3 reservation