

Standard Operating Procedure Raman Microscope - Renishaw inVia

The Renishaw inVia Raman Microscope can efficiently provide Raman spectra and photoluminescence measurements for chemical composition and structure analysis. It is supplied with the powerful WiRE[™] software for intuitive operation and easy data manipulation, as well as the high sensitivity ultra-low noise RenCam CCD detector.

1.0 Operation Procedure

Laser Safety

- The user is reminded that when used in accordance with the operating instructions the user should not be exposed to a laser hazard greater than Class 1. However, the system uses Class 3B lasers and as such, ALL necessary safety conditions for such instruments must be adhered to.
- Class 3B lasers are potentially hazardous if a direct beam or specular reflection is viewed by the unprotected eye. Precautions should be taken to avoid direct beam viewing, and to control specular reflections.
- It is strongly recommended to use two sets of safety glasses and/or goggles suitable for each laser wavelength used with the system.
- The system is a fully interlocked system. It ensures laser safety by automatically shuttering lasers during excitation wavelength changes. The control software tests the integrity of the interlocks during routine operation.

1.1 Startup the System

- a. Turn on the main power switch
- b. Turn on the laser
 - ✓ If you use 514 nm or 488 nm laser, the laser switch is separate from the generator. Please see the left picture below. Turn the key to "Closed" position, then turn the laser power to the marked level.
 - ✓ If you use 785 nm laser, the laser switch key is on the generator. Please see the right picture below. Just turn the key to "Closed" position.
- c. Preheat the laser for 30 minutes





514 nm and 488 nm laser



785 nm laser

- d. Launch the control software WiRE4.2 from the desktop
- e. When there is an error message about the XYZ stage X Motor, click OK to acknowledge it



When the "WiRE Motor Reference Options" window pops up, select "Reference All f. Motors", then click OK.

Page





Motor	Туре	State	Reference un-referenced motors only
Beam Expander CCD lensfocus Grating Motor Holographic Notch Filter Podule Lower Selector Wheel Podule Upper Selector Wheel Post slit lensfocus Pre slit lensfocus	serial serial serial serial serial serial serial	un-referenced un-referenced un-referenced un-referenced un-referenced un-referenced un-referenced un-referenced	Reference All Motors Reference Selected Motors Select one or more motors from the list. Unreferenced serial motors will still be referenced. Innore Referencing Parallel Motors
Slit Master Slit Slave	serial serial	un-referenced un-referenced	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.

g. Wait 2-3 minutes for the system to finish initialization and display the main screen

& WRE				
File Edit View Surface Measur	ement Livevideo Analysis Processing Procedures Tools Window Help			
🗅 🖨 · 🖬 × 🛛 🕼	· 8 X 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		物金田	
國際常常改圖	🖓 🚰 🔀 🙀 - Remainlance MSC 33 X 3033.4 Y 22096.4 2 -120	11.9 Com 🔗 🙊 🏖 🖉 🖗 🗞		- 2 3
Indeo				
25050 - m//iz 12mi		*		
16767				
25070 -				
25000 -				
25050				
25100				
25110				
25120				
25130				
28740				
25150 -		1253		
🛃 Data 🔲 View				
	Secole Renews White Tipht and view, control Laser (date and new control Scienciferentia		
		🔶 100% 🔢 (200 (iver (200/700)	-	
	· · · · · · · · · · · · · · · · · · ·	¥ 403 mm edge - 🔒 🔐 Master: Renshaw 50/500	-	
	20 17 20 2			
Basely		1 B		III Y TETTA Y PENEL 7 JUNIT D. OPE-DE 🚔 🚇 🍙

1.2 Align the Laser

- a. In "Sample Review" bar, click "Sample Illumination On" and "View the Sample under Video And Eyepieces" buttons
- b. Adjust the white light intensity by dragging the "Lamp" slider
- c. Adjust the "AStop" and "FStop" sliders
- d. Select the correct objective lens (5x, 20x, 50x, L 50x, 100x)



Sample Review			×
Mintelight and view control	Laser state and view control	Spectrometer state	
	50X	u 100% III (1200 l/mm (633/780) ▼ Je ▼ III (Master: Renishaw 537/10 ▼	

- e. Use the joystick to focus on your interested point
- f. In "Sample Review" bar, click "Laser Shutter Open" and "View the Sample under Video And Laser" buttons
- g. Adjust the ND% to display a tiny small laser spot (0.0001% for 514 nm and 488 nm laser, 5 x 10⁻⁸% for 785 nm laser)
- h. Select the desired laser wavelength (514 nm, 488 nm, 785 nm)
- i. Select the desired grating (1200 l/mm, 2400 l/mm, 1800 l/mm, 600 l/mm)
- j. Select the desired detector (Master detector, Andor detector)

Sample Review		×
White light and view control	Spectrometer state	
	0.0001% III 1200 l/mm (633/780)	
() ※ ※ () ※)	🖉 🔰 💥 514 nm edge 🔹 🖬 Master: Renishaw 537/10 👻	
100 100		

 k. In the "Video" window, right-click the mouse → Crosshairs → Circle. This will display the alignment marker.



I. Use the joystick to align the small laser spot on your interested point



1.3 Acquire the Spectrum

a. Click Measurement \rightarrow New \rightarrow Spectral acquisition. This will open a "Spectral acquisition setup" dialog box. Usually, you only need to edit the first three tabs.

ነ 🖻	- 🖬	×	New	•	Spectral acquisition		지 남
		8 ****) Open measurement templat Save measurement template Setun measurement	e	Filter image acquisition Depth series acquisition Map image acquisition	Go to	
ii- - 000	nVia 12ms		ImportXY, NavigateXY Run batch measurements		StreamLine Image acquisit StreamLineHR image acquisit Volume (StreamLineHR) ac	tion cquisition	ŧ
1000 -			Run remote measurement			and the second	
1100			Run Cycle Abart	F5 Shift + F5		a. 	
1200 -		R	Measurement queue	Ctrl + 0	-		
1300 -							
1400			1.	Y	and the		
					14		
1500 -							
1500							
1500 1600 1700							

- b. In "Range" tab:
 - ✓ Select Static or Extended for the "Grating scan type"
 - ✓ Select "Raman shift/cm-1" for Raman tests; select "Wavelength/nm" for PL tests
 - ✓ Input the Center value of the Spectrum Range if Static is selected; input the Low and High edge values of the Spectrum Range if Extended is selected
 - ✓ Select Standard for the "Confocality"
 - ✓ Double-check the "Configuration"
- c. In "Acquisition" tab:
 - ✓ Input the "Exposure time" in second
 - ✓ Select the appropriate "Laser power %"
 - ✓ Input the number of "Accumulations"
 - ✓ Check "Close laser shutter on completion"

Shared Instrumentation Laboratories

Range Acquisition File	Timing Advar	nced					
Grating scan type	Spectrum	Range					
Static	Low	-1204	.09				
Extended	Centre	520.00	D	Raman shift/cm-1 👻			
] High	1968.3	30				
● Standard ◎ High	Laser name Grating name	e me	514 r	nm edge ▼ I/mm (633/780) ▼			
	Detector n	Detector name Master: Renishaw 537/10					
	Calibration	status	1	Calibrated and healthchecked OK.			
				OK Cancel Apply Help			

Range Acquisit	ion File 7	Firming Ad	dvanced					
Exposure time /s	; 10.00	×.	Laser power / %	10	•	Accumulations	βο	-
						Objective	5	
Live imaging								
								New
Not using live	: imaging.							Edit LUT
Title	Single scar	n measurer	ment					
Description	A single sca	an measur	ement generated by	v the WiRE spectra	al acq	uisition wizard.		
Description				on completion		Minimize las	er exposure	on sample
Response ca	ibration	Rest	ore instrument state	- on compression				
Response ca	libration emoval	Clos	e laser shutter on co	ompletion				

- d. In "File" tab:
 - ✓ Click the Browse button to specify the directory for saving the results. The results will be saved as .wdf file.

Page **6**



✓ Check "Auto export to txt"

Range Acqu	isition File	Timing	Advanced				
File name	C:\Users\Ra	man\Deskt	op\user data\Be	ei∖demo.wdf	 	Browse	
Aut	o increment	2					
Aut	o export to sp o export to txi	L 1					
					 		S

- e. Click Apply, then click OK.
- f. Click "Run" to start the spectral acquisition; if you wish to abort the acquisition, click "Abort"

WiRE	- Single scan	measurem	ent								
File	Edit View	Surface	Measurement	Live video	Analysis Proc	essing Proced	lures Tools \	Nindow Help			
	🎽 - 日	X	1 - 1	1 🔛 🔛 🛛	K 🔊		1 🗖 🔏	ele la		🚮 🕍 🐱 🖥	
	1997 - 19		1	7 Q. Q	R Run	w MSC 10 X	14040.6 Y 2	1338.4 Z -991	2.7 Go to	🖉 🖉 🖉	
Video										×	Circula and a
	inVia 12m	15		*							Single scan measuren
20900	-										-
21000				·		- States					8 —
21100	-					1.	· ~ ?	1. 1			_
21200	-							and in			°



H WIRE	Single scan measurement	
File	Edit View Surface Measurement Live-video Analysis Processing Procedures Tools Window Help	
	≠ • ⊟ ×	🖾 🖾 🖪
	👷 🐄 🐺 📰 🔐 🕼 🥨 Renishaw MSC Abort 🗴 14040.6 y 21338.3 Z -9912.7 Go to	a a a 1
Video	× Db see	
2090		3500 -
2100		3000
2110		2500
2120		2000

g. After the spectral acquisition, you can review the measurement parameters in the "Navigator" window

Navigator	x
提 Data 🔟 View	
⊟- 🔁 demo.wdf	
🗄 🖑 🚯 Summary information	
• path: C:\Users\Raman\Desktop\user data\Bei	
- • title: Single scan measurement	
• description: A single scan measurement generated by the WiRE spectral acquisition wizard.	
🔍 user: Raman	
🤐 🥥 laser: 514 nm edge (mode: Regular)	
- • grating: 1200 l/mm (633/780)	
	H.
- 30 accumulations	
- • laser power: 10%	
🧰 🧉 centre: 520 Raman shift/cm-1	
slit opening 50μm, centre 2738μm	
- video field of view 8902 5836	
- objective x5	
🔠 🕁 Static scan	
a-BB Measurement status: Completed	
a - 1 File modified by	
Advanced file information	-
Ready	

- h. If you wish to capture and save an image of your sample:
 - ✓ Close the measurement result window(s)
 - ✓ Click Live video → Snap → Single
 - ✓ Click File → Save view as..., the captured image will be saved as .emf file





1.4 Shutdown the System

- a. Close the WiRE4.2 control software
- b. Turn the laser power to the minimum level (Min.)
- c. Turn the laser switch key to "Open" position
- d. Turn off the main power switch



2.0 Specifications/Features

- Three laser wavelengths: 488 nm, 514 nm, 785 nm
- Raman shift range: up to 4000 cm⁻¹
- Photoluminescence (PL) range: up to 950 nm
- Objectives: x5, x20, x50, x100, L x50, SLW x20

	x5	x20	x50	x100	L x50	SLW x20
WD (mm)	14.0	1.15	0.5	0.22	8.2	25.0
Cryostat use	Yes	No	No	No	Yes	Yes

- Two sets of gratings: 1200 l/m + 2400 l/m, 600 l/m + 1800 l/m
- Measurement capabilities:
 - Spectral acquisition
 - Depth series acquisition
 - Map image acquisition
 - StreamlineHR image acquisition
 - Automatic image montaging
- Basic Raman spectra analysis capabilities (e.g. peak pick-up, substrate baseline, etc.)
- WiRE[™] 4.2 control and analysis software

3.0 User Requirements

The Renishaw inVia Raman Microscope must be used by authorized personnel only. All authorized users are expected to read and understand this SOP and follow the operation instructions carefully. No unauthorized user may operate this Raman unless accompanied by an authorized user. All visitors must be briefed on proper safety protocol and must wear appropriate personal protective equipment. To become an authorized user, one must:

- 1. Complete Environment, Health & Safety (EH&S) training
- 2. Complete initial orientation and training for the Materials Characterization Laboratory
- 3. Receive training on this piece of equipment from lab personnel
- 4. Schedule equipment time using the calendar
- 5. Read and fully understand this SOP



4.0 General Safety

4.1 Required Personal Protective Equipment

Users must wear lab coats, safety glasses, and gloves. Shorts, open-toed shoes, high heels, and skirts, are forbidden.

4.2 Emergency Procedures and Contacts

<u>For non-life threatening emergencies</u>: notify the MCL facility manager and your PI immediately. Facility manager: Zongmin (Shirley) Bei, Ph.D. Office: 109B Furnas Hall, Tel: (716) 645-5165, Cell: (585) 354-5623 Email: <u>zongminb@buffalo.edu</u> or for police / ambulance, call 645-2222

In case of fire or other life threatening emergency: Exit the laboratory through an emergency exit door. Pull one of the fire alarms located in the main hallway. Dial campus police / ambulance at 645-2222.

4.3 University after hours laboratory use policy

No working alone, use the buddy system!

#	Revised by	Date	Modification
1	Zongmin (Shirley) Bei	05/10/2016	Document initial release
2	Zongmin (Shirley) Bei	07/07/2016	2.0 Specifications
3			
4			
5			