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ACTIVITY/PROJECT SPECIFIC PROCEDURE

SP 12-37
USE OF THE HORIBA SCIENTIFIC XPLORA CONFOCAL RAMAN MICROSCOPE
Revision 0

Effective Date: September 19, 2017

Author: Charlotte Sisk-Scott *Original signed by Charlotte Sisk-Scott* *09/19/2017*
(printed name) (signature) (date)

1.0 Purpose and Scope

This document describes the Sandia National Laboratories (SNL) Waste Isolation Pilot Plant (WIPP) procedure for use of the Horiba XploRA PLUS confocal Raman Microscope. Calibration and operation guidelines are included in the procedure. This SP is in support of activities described in several WIPP Test Plans (TPs) with the majority of the activities for the experimental work in support of WIPP-related studies. Both SNL and contractor personnel will use this SP. This document is not meant to substitute for the manufacturer instruction manuals for the instrument (see references).

Acronyms and definitions for terms used in this procedure may be found in the Glossary located at the Sandia National Laboratories (SNL) WIPP Online Documents web site.

1.1 Instrument Description

The Horiba XploRA PLUS is an integrated confocal microRaman system. The confocal microscope is coupled to a full Raman module mounted above which includes a 200 mm focal length spectrograph equipped with a 4-gratings turret, laser source(s) and detector.

The different excitation wavelengths are supplied by up to three internal lasers. The optical path is split into two parts; the illumination (laser) path and the collection (Raman signal) path. On the incoming laser path, the laser beam is reflected towards the microscope by the use of a special filter (dielectric edge rejection filter). It is used in injection/rejection mode which firstly directs the laser into the microscope and then filters out the Rayleigh scattered light as it returns to the spectrograph allowing only the Raman scattered light alone to be transmitted through to the confocal hole and the entrance of the spectrograph. The spectrograph itself is used to disperse the various multi-chromatic Raman spectral lines onto the CCD detector for detection and analysis.

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2.0 Implementation Actions

The user is responsible for reading and understanding the appropriate manuals. Guidelines for implementing and documenting quality assurance (QA) procedures are presented in this document, and follow the general guidelines given in Nuclear Waste Management Procedure (NP) 12-1 "Control of Measuring and Test Equipment."

2.1 Responsibility, Safety and Training

The technical staff, or designee, whose activities warrant the use of this procedure, are responsible for implementing the requirements of this procedure and shall conform with SNL Environmental Safety and Health programs (ES&H). Laboratory ES&H procedures and specific instrument hazards are described in the ES&H Standard Operating Procedure, *Activities in the Sandia National Laboratories/Carlsbad Program Group Laboratory* and laboratory-specific TWDs and JSAs, which can be found at the WIPP Online Documents website.

In addition, the technical staff or designee is responsible for performing the measurements, following the requirements of this procedure, documenting the results, and assuring that the use of the latest revision of the document is followed.

2.2 Switching On/Off the System

2.2.1 Switching On the System

Normal operation requires only that the lasers and white lamps be turned back on. The two XploRA power supply boxes and the CCD detector remain on permanently and have no switch. In the ***abnormal*** event that the entire system will need to be completely turned back on, please complete the following protocol:

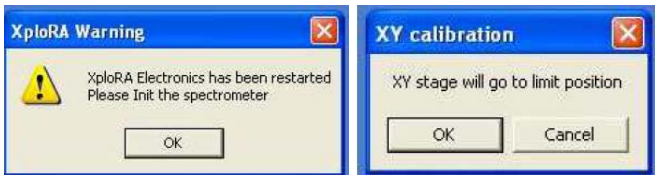
a) **Plug in** the XploRA and CCD power supply.

b) **Switch on** the computer.

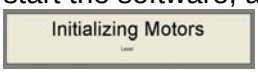
c) **Switch on** the reflection and transmission white lamp power supplies.

d) **Switch on** the XY stage power supply (Tango).

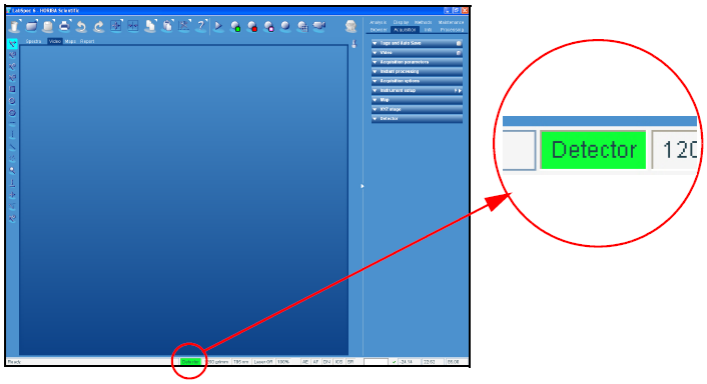
e) **Open** LabSpec6 software. When you start Labspec6 just after the XploRA power supply connection, a message appears:



The XY stage gets in its limits. Make sure that objectives are not too close to the stage. When you start the software, all motors are initialized. Wait for few seconds until this initialization ends.



The CCD gets cold automatically when you start LabSpec6. Wait for the temperature of the detector to indicate green on the status toolbar. The status bar runs along the very bottom of the LabSpec main window and is green indicating the detector is at working temperature and red indicates the detector is above the working temperature and should not be used.



The system is now ready to work.

2.2.2 Switching Off the System

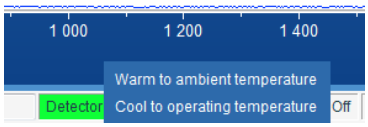
Normal operation requires only that the lasers and white lamps be turned off. In the abnormal event that the entire system will need to be completely turned off, please complete the following protocol:

a) **Switch off** lasers from the safety box:

b) **Switch off** Reflection and transmission white lamp internal and/or external power supply:

c) **Switch off** XY stage power supply (Tango):

d) **Heating the CCD**. It is important to heat the Detector before unplugging it to avoid any damage. For this, right click on the Detector icon in the Status Bar and click “Warm to ambient temperature. The cooling temperature is -70°C for IDUS CCD:



e) Wait for the temperature to be close to room temperature in the Linkham drop down menu located under the Acquisition tab.

f) **Close** LabSpec6 software.

g) **Switch off** the computer.

h) **Unplug and Turn off** the toggle to the XploRA power supply.

2.3 Alignment/Calibration Standard(s)

The software allows the operator to do automatic calibration and alignment directly for the system.

2.3.1 **Alignment** - ONLY a service technician can perform alignment.

[**Note**: Alignment requires the Class 1 enclosure to be removed. When the Class 1 enclosure is not in place, the raman spectrometer is classified as a Class 3B laser.]

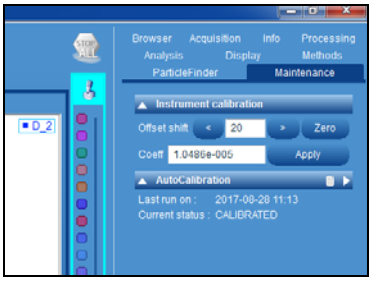
2.3.2 Calibration of the system

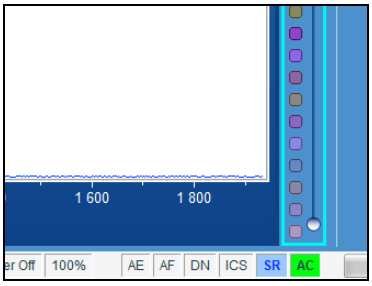
The PI or designee is responsible for performing the calibrations and measurements following the requirements of this procedure, documenting calibrations, and assuring that the latest revision of this document is followed. The instrument will be calibrated on the day of use, or prior to use. It will also be calibrated immediately before use if instrument irregularities are observed.

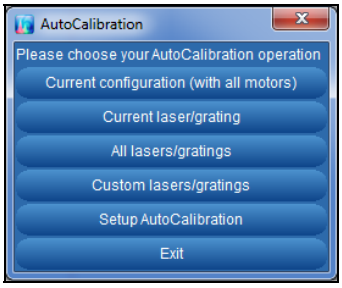
Calibration will be performed using commercially obtained standards that are certified or other nationally recognized standards. The identity, manufacturer, lot number and expiration date of the standards used shall be recorded.

- To do the auto-calibration, a Silicon sample is needed:
  - For each grating, a silicon spectrum will be saved with the reference laser (YAG 532nm). The software will adjust the zero order for each grating.
  - For laser diodes (785nm and 638nm), just one spectrum is collected with the reference grating. If the Silicon line is not perfectly at the right position (520.7 cm-1), the software will adjust the laser wavelengths.

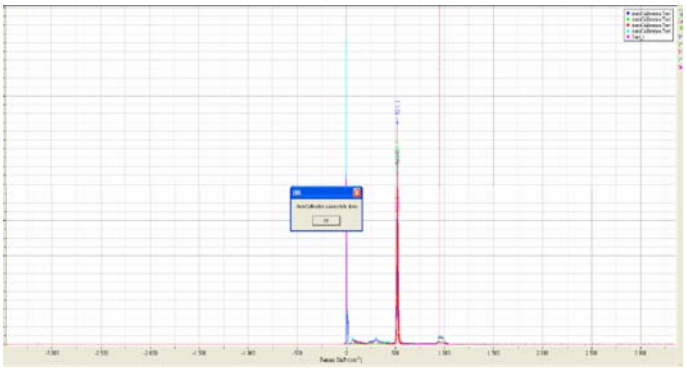
    a) First click on the “calibration” icon in the Maintenance tab of the LabSpec6 software or click the AC icon located in the Status Bar at the bottom of the screen: Note there are four modes of operation for autocalibration, depending on the specific system configuration. Calibration will need to be ran with the 100x and 50x objective lenses.







  - At the end of the calibration, a message appears to indicate that the system is now calibrated:



2.3.3 Standards

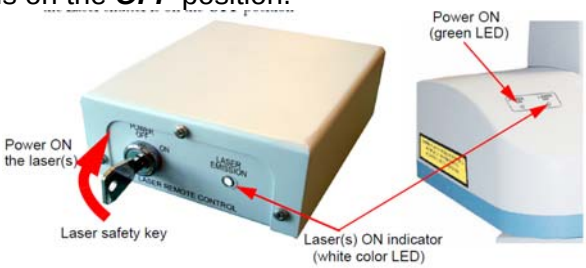
Only use QA certified standards that are specified by the manufacturer (refer to specific operating instructions). A certified reference standard will not be used past its expiration date listed on the container by the manufacturer. Primarily, silicon is used as the certified standard, but other standards such as aspirin, polymer styrene, etc. can be used.

The standards are chosen so that their values provide a validity of instrument alignment. If the calibration fails, the instrument will read a FAIL and must be recalibrated with the appropriate standard, grating, and laser setting until calibration passes.

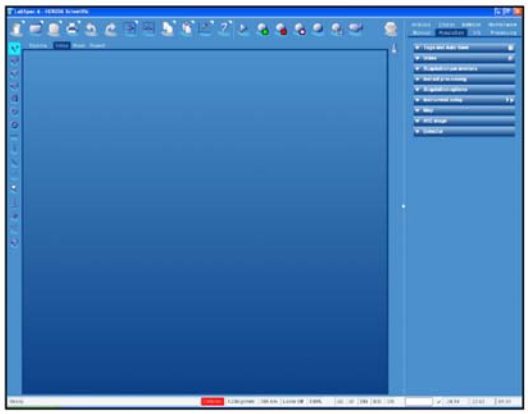
2.4 Procedure

a) Verify that the caps are mounted on the unused microscope exits and that the 10X objective is in the imaging turret position.

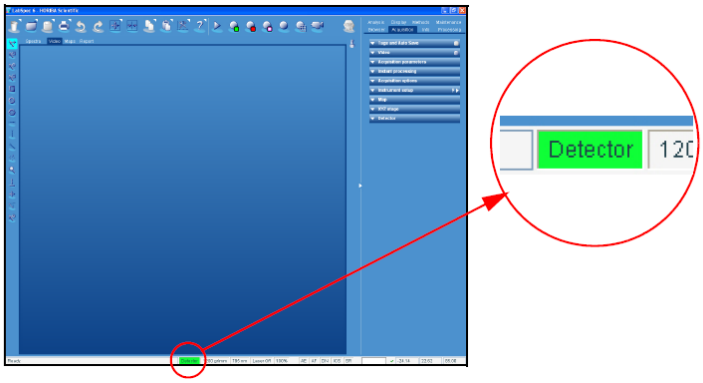
b) **Turn *ON*** the Laser Safety key located on the Remote Control front panel and verify that the Laser Shutter is on the ***OFF*** position.

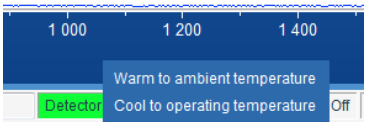


c) **Switch on** the Computer. When the Desktop screen is displayed, click on the LabSpec6 icon to launch the software. The following screen is displayed:



d) When the XploRA is powered ON, the CCD detector Peltier device begins the cool down process. The temperature status is visualized by the background color of the Detector field located on the Satus Bar (bottom of the window). When the working temperature is not reached, the background is red. As soon as the background color is green, the CCD detector reaches -70°C and the System is ready to work.





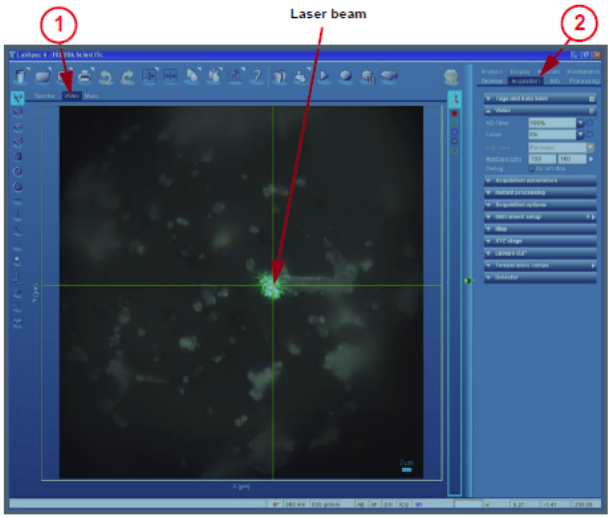
2.4.1 Acquiring Spectrum with LabSpec6 Software

The LabSpec6 is an interface that allows visualization of the sample and measurement set-up, interpretation of the data, and final reporting of the results.

a) Place sample on the microscope stage.

b) Use a visualization device to correctly position microscopic samples. The video camera can be used and will highly increase the positioning precision and ease of use.
   1. **Marker 1:** Switch to **Video** Data Tab; the video camera image is displayed in the main window. The sample and/or the laser beam is visible.
   2. **Marker 2:** The **Acquisition** Control Panel is activated. This is done to control the acquisition parameters, particularly **Acquisition > Video** Section.

c) The sample is visible through the video camera or the microscope binocular lenses. Using the sample stage (manual or automatic), move the sample to select the analysis position.

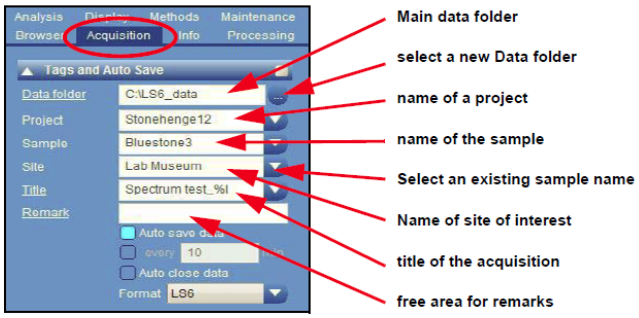


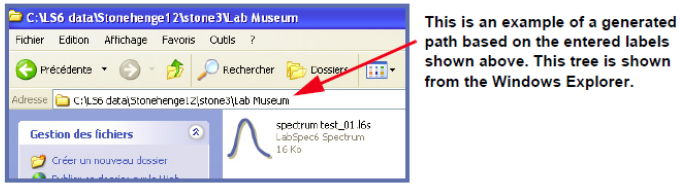
d) Once the sample adjustment is done, stop the Video by pressing the icon. [Remove the visualization optics, if this function is not automated.]

e) Select **Spectra** from the Data Tabs menu, then press the button to launch the **Real Time Display** (RTD). According to the selected parameters, real time acquired spectra will be continuously displayed. This will help you to improve the sample positioning or acquisition parameters like the Acquisition time(s) and other System settings.

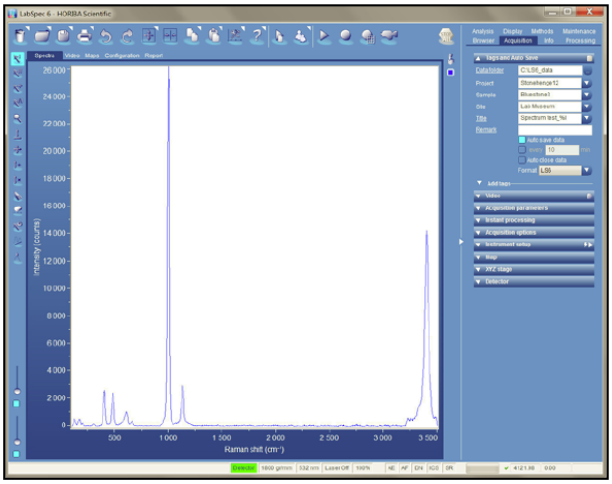
f) Once optimization is complete through the RTD acquisition click on the icon to stop the RTD. The final acquisition can now be performed, but before launching it, you may want to define the project. The figure below shows the labels which will be added from the root folder to the sample name. If the [Auto save data] tick box is not ticked, then these project tags will simply be added to the data information shell.

g) If the [Auto save data] tick box is ticketed and active, then the data will be saved automatically into the corresponding folder structure.





h) Then, press the Spectrum Acquisition icon: this action launches the acquisition according to the defined parameters. The results are automatically displayed and data recorded on the hard disk (if *Auto save data* box is enabled).

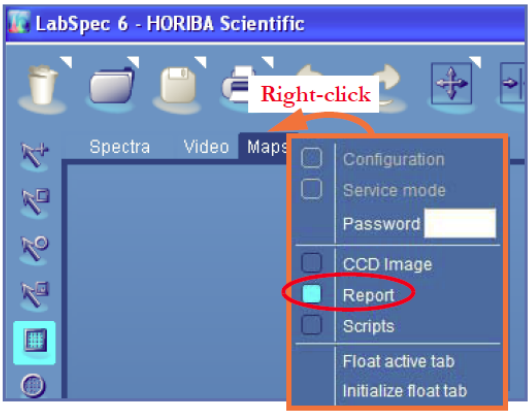


2.4.2 Generating a Report

When the acquisitions have been done, it is important to print/save the results including a graph or image with the measured parameters. To create a report page, follow the procedure describe below:

a) If not yet done, add the **Report** tab to the **Data Tabs Menu**: Right-click on the Data Tabs Menu to display the options window. Tick on the **Report** choice. The Report Tab is then created.

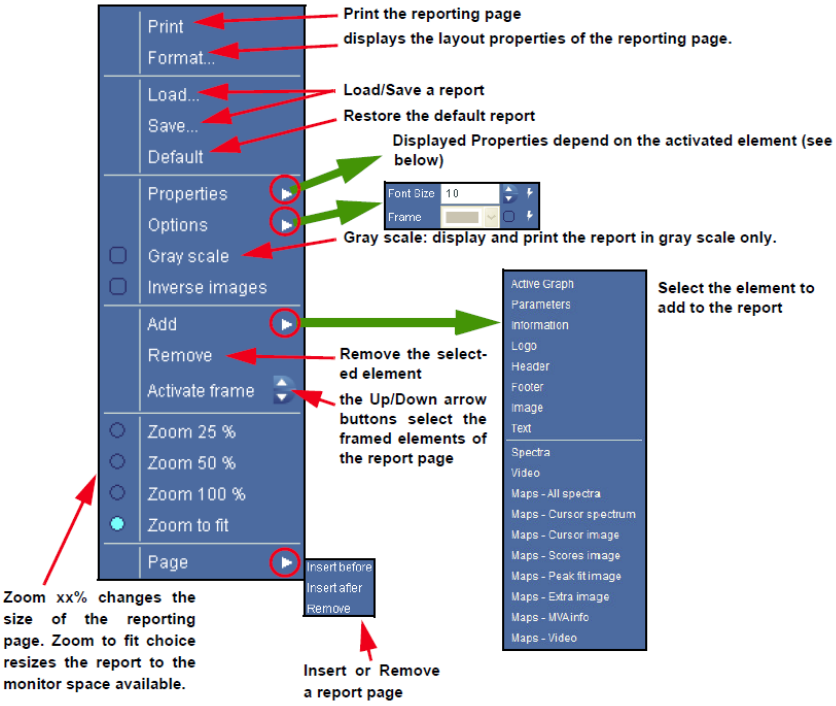
b)



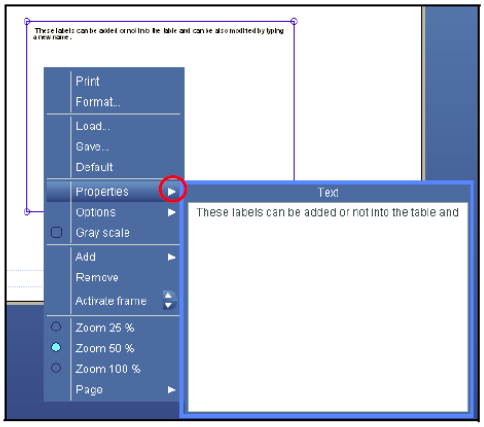
c) Using the mouse, select a data window.

d) Click on the **Report** Data Tabs Menu: the previously selected data window is automatically retrieved to the report area. The report template can be edited, saved, opened according to user requirements, as described below. Note that each data tab (Spectra, Video, Map) has its own individual report template.

e) Right-click to display the page properties. Figure below details the choices.

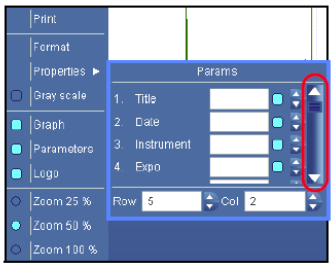


The displayed **Properties** depend on the activated element on the report page.



If the selected element is a text box, the Properties will show a text editor.

If the selected element is a spectra, a map or a 3D Image, the Properties will display two parameters: show or not the cursors and exclude or not empty space.



If the selected element is a table, the properties table list up to 50 parameter labels which can be added according to the used sample. These labels can be added or not into the table and can be also modified by typing a new name. Do not forget to re-dimension (row and column) for a better presentation.

IMPORTANT: When an image is added to the report, an empty frame is displayed on the report. Select it then click on **Properties** to launch the data browser to locate the image file which is to be inserted. A .jpg or .bmp format image is required.

f) **Print** the reporting page using the *Print button* in the main icon bar, or the right click option (Print) on the report template.

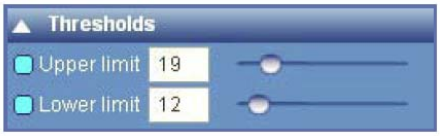
2.5 Working with ParticleFinder

ParticleFinder can be used to locate particles on any video image recorded through LabSpec6. This video can either be a single view image, or a montage video image covering a larger field of view.

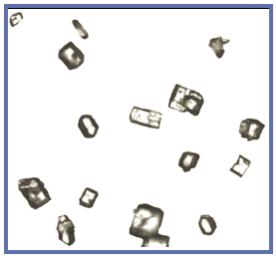
a) Acquire a video image in the standard way.

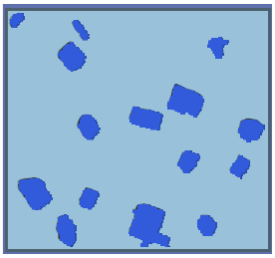
b) Then switch to ParticleFinder Module.

c) Use the thresholds section to segment the image and locate particles.

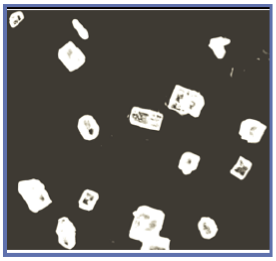


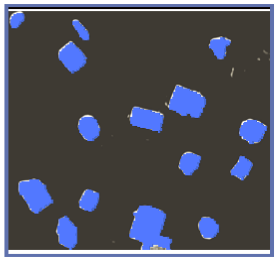
a. The upper threshold is used to locate particles which appear dark on a light color background. As the upper limit threshold value is reduced, only the very darkest particles will be located; increasing the Upper limit threshold value will allow less dark particles to be located.





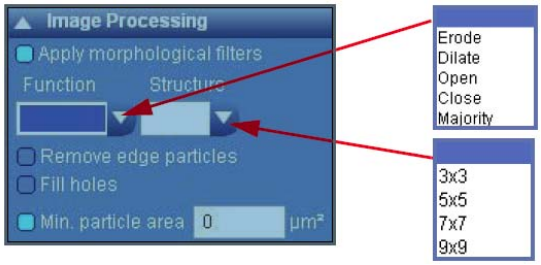
b. The lower threshold is used to locate particles which appear bright on a dark colored background. As the Lower limit threshold value is increased, only the very brightest particles will be located; reducing the Lower limit threshold value will allow less bright particles to be located.



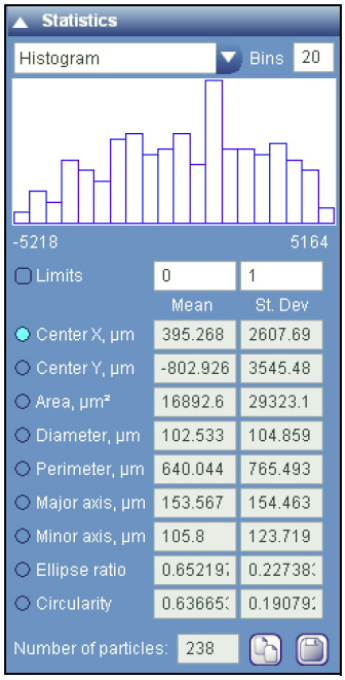


d) Manually adjust the Upper limit and Lower limit thresholds to achieve the desired segmentation of the video image. If unexpected particle location is observed please check the Image Processing, Statistics, and Display section.s, and if necessary turn off all Image Processing options and turn off the Statistics <<Limits>> functions. The Display of located particles can be configured in the Display section, including options for display of particle area, perimeter, and the background (non-particle) colors.

e) Use Image Processing section to provide morphological filters and other tools to improve and enhance the particle segmentation. Select the desired filter from the drop down box, and the corresponding structural element size (in pixels). The structural element is the unit in which a morphological filter is performed. A typical structure element is a square of an odd number of pixels (e.g., 3x3, 5x5, 7x7, 9x9), and the operation results is applied to the center pixel.



f) Use the statistics section to display the statistics of key particle size and shape descriptors as defined by the current ISO 9276-6:2008, for the particles located in the video image. The mean and standard deviation values for the following parameters are calculated and displayed.

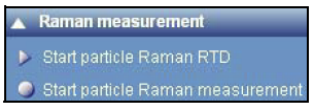


g) The Histogram displays results for the selected statistic parameter. Select the statistic parameter using the adjacent radio buttons. The Histogram displays results for the selected to show the histogram and the cumulative particle count. The number of bins (channels) can be specified in the <<Bins>> text box – the number of bins must range from 3-100. The located particles can be further filtered based on a sizes or shape descriptor.

a. Select the descriptor (e.g.diameter) by which you want to filter,
b. Select the minimum and maximum threshold values for the filtering (e.g. 25-50 µm),
c. Tick the <<Limits>> box.
d. The particle displays on the video image, and the statistics will be updated to locate particles that meet the criterion. Other particles are ignored.
e. Click on the <<Copy full statistics>> to copy (as text) the full statistics table, with all parameters for all particles. Then paste the data into external software (text reader, spreadsheet, excel, etc.) for further processing.

	A	B	C	D	E	F	G	H	I	J	K	L
1		X	Y	Area	Diameter	Perimeter	MajorAxis	MinorAxis	Ratio	Circularity		
2	1	-2534.84	-5968.55	7205.07	95.7759	671.021	152.199	81.8351	0.537683	0.448423		
3	2	346.432	-5974.22	1385.59	42.0022	265.971	83.1714	29.4524	0.354117	0.496122		
4	3	512.863	-5865.01	17736.6	150.272	1037.8	264.435	195.136	0.737935	0.464896		
5	4	4322.22	-5984.3	969.913	35.1416	147.535	36.6721	32.2815	0.894916	0.748302		
6	5	-475.121	-5860.83	692.795	29.7001	95.7635	51.2669	20.9858	0.409344	0.974333		
7	6	3131.44	-5772.42	1524.15	44.0523	259.418	87.7844	33.265	0.37854	0.462211		
8	7	-3355.72	-5651.99	53206.7	260.278	949.119	289.323	236.921	0.818879	0.861524		
9	8	-1640.69	-5571.47	100317	357.39	1336.64	377.6	350.32	0.928001	0.840062		
10	9	3085.86	-5689.29	1662.71	46.0112	342.263	73.2001	73.2001	1	0.422331		
11	10	87.9764	-5654.53	692.795	29.7001	54.163	62.2143	0	0	0.956893		
12	11	4668.83	-5664.3	1385.59	42.0022	270.056	81.8135	39.1951	0.479078	0.488545		
13	12	-7.67893	-5594.62	5958.04	87.0977	750.737	197.267	138.069	0.69969	0.364476		
14	13	134.72	-5678.79	554.236	26.5646	101.489	36.5793	21.0106	0.574386	0.822305		
15	14	149.567	-5636.58	692.795	29.7001	132.412	50.6017	21.6921	0.428683	0.704662		
16	15	1498.7	-5580.81	5126.69	80.7929	627.713	174.761	148.855	0.851763	0.306651		
17	16	866.09	-5565.4	554.236	26.5646	86.366	41.3873	19.1674	0.463123	0.966295		
18	17	3534.46	-5542.18	5958.04	87.0977	375.71	92.3726	86.0084	0.931103	0.728289		
19	18	4172.65	-5549.65	1385.59	42.0022	262.77	66.3702	47.1596	0.710554	0.502165		
20	19	1439.36	-5530.75	1108.47	37.568	229.776	61.5545	26.3128	0.427472	0.513645		
21	20	2543.68	-5500.16	969.913	35.1416	209.851	45.2329	24.0129	0.530672	0.52609		
22	21	1519.92	-5359.73	692.795	29.7001	141.809	33.0569	23.117	0.699311	0.657965		
23	22	-2276.6	-5341.78	692.795	29.7001	93.3065	71.4284	0	0	1		
24	23	-131.982	-5360.67	831.354	32.5348	182.129	35.9192	23.5886	0.656711	0.5612		
25	24	-789.115	-5300.83	554.236	26.5646	86.366	42.4775	18.914	0.44527	0.966295		
26	25	2000.92	-5151.58	29928.8	195.209	983.739	224.957	199.216	0.885573	0.623404		
27	26	4191.2	-5211.68	6373.72	90.0848	369.531	96.2615	89.477	0.92952	0.765861		
28	27	1462.73	-5237.84	554.236	26.5646	101.489	31.1171	20.4819	0.65822	0.822305		
29	28	-331.397	-4977.77	82996.9	326.077	3266.17	639.27	504.209	0.934983	0.312677		
30	29	1767.61	-5144.46	9422.02	109.528	654.056	184.423	118.298	0.641452	0.526092		
31	30	-4805.81	-5080.86	18151.2	152.023	1904.9	250.511	245.953	0.981804	0.250719		
32	31	1516.15	-5172.6	969.913	35.1416	180.529	54.7517	24.4525	0.446607	0.611539		
33	32	998.616	-5167.84	692.795	29.7001	141.809	33.0649	24.0813	0.728303	0.657965		
34	33	4657.66	-5121.3	554.236	26.5646	114.088	25.731	21.6914	0.843005	0.731499		
35	34	4654.6	-4950.59	8857.78	106.258	392.905	126.251	96.8836	0.767388	0.84962		
36	35	1943.18	-4843.18	20229.6	160.49	1656.34	393.784	275.624	0.699936	0.304404		
37	36	-1044.82	-4944.92	554.236	26.5646	101.489	31.483	24.6954	0.784404	0.822305		
38	37	3601.07	-4904.43	969.913	35.1416	213.052	51.9268	21.3818	0.411696	0.518186		
39	38	3861.14	-4902.93	831.354	32.5348	123.938	34.2928	30.0984	0.877688	0.824693		
40	39	-5218.58	-4875.63	554.236	26.5646	103.09	36.2095	21.5908	0.596274	0.809539		
41	40	4302.98	-4800.04	554.236	26.5646	119.813	35.0992	18.9744	0.540594	0.696543		
42	41	1017.31	-4787.44	554.236	26.5646	83.455	49.1782	1.45604	0.0296075	1		
43	42	1089.99	-4719	6126.69	80.7929	913.172	222.62	49.7228	0.223353	0.277962		
44	43	1457.23	-4688.23	1108.47	37.568	175.257	39.6199	37.3438	0.942552	0.673431		
45	44	1987.89	-4645.71	554.236	26.5646	101.489	36.3368	22.1512	0.609608	0.822305		
46	45	2998.06	-4632.48	1385.59	42.0022	228.175	54.9289	40.4481	0.736371	0.578301		
47	46	-389.336	-4570.75	692.795	29.7001	143.41	36.8015	21.6606	0.588578	0.650622		
48	47	1608.93	-4522.87	692.795	29.7001	130.811	33.7542	30.899	0.915412	0.713284		
49	48	-1349.1	-4459.88	1662.71	46.0112	226.575	51.804	45.5088	0.878481	0.637972		
50	49	1571.14	-4474.28	969.913	35.1416	169.531	54.6515	33.2278	0.605777	0.651212		

f. Click on <<Save full statistics>> to save (to a tab separated values text file) the full statistics table, with all parameters for all particles. The text file can be read by standard text readers, spreasheets softwares, etc.
g. Start Raman RTD by opening the **Raman measurement** tab. This function will open the standard LabSpec6 <<Move stage/Acquire spectrum>> tool. Click anywhere on the image to move the point underneath the laser spot.
h. Click on the run arrow in the inset window to start acquiring RTD data, according to the parameters set in the Acquisition tab.
i. To stop the RTD measurement, click on the [Stop All] button.
j. Start Raman Measurement by opening the **Raman measurement** tab. This function will use the LabSpec6 multipoint spectral array acquisition tool . Once this icon is clicked, the system will automatically move to the center of each particle, and acquire a single spectrum according to the parameters set in the Acquisition tab. The spectra acquired by ParticleFinder will be grouped ina standard LabSpec6 spectral array file, which can be further processed and analyzed using the standard LabSpec6 functions.



Note: for the ParticleFinder <<Start particle Raman measurements>> function to perform correctly, please ensure that the video window is active when the measurement is started.

k. Label the particles on the video image with their acquisition number after spectrum acquisition, allowing them to directly be related to the data in the spectrum array file. To do this, use the Display > Map display options section in the main LabSpec6 interface, and teck the boxes for <<Positions>> + <<Index>>, and <<Limits>>.

2.6 Maintenance

The raman microscope shall be stored with the enclosure closed at all times, so as to minimize the exposure to dusty, corrosive, or excessively humid environments, temperatures outside the range of 0--40ºC, and mechanical shock and vibration. If the raman microscope cannot meet the acceptance criteria (see Section 2.3), the troubleshooting from the reference manual will be performed. If the raman microscope still does not meet the acceptance criteria, the Horiba Service technician will be contacted. Until the raman microscope can meet the acceptance criteria, the microscope will be labeled accordingly.

2.7 References

Horiba Jobin Yvon, 2015, Doc UM-XpLabSpec6/Eu, *LabSpec6 Spectroscopy Suite.*

Horiba Jobin Yvon, 2014, Doc UM-XploRA PLUS/En, *XploRA PLUS System User Installation and Operation Manual*.

Horiba Jobin Yvon, 2013, Doc UM-LabSpec6/En, *LapSpec6 Particle Finder Module.*

ISO 9276-6:2008-09 (E). Representation of results of particle size analysis - Part 6: Descriptive and quantitative representation of particle shape and morphology.

3.0 Records

The following records generated through implementation of this procedure shall be prepared and submitted to the WIPP Records Center in accordance with NP 17-1 “Records”.

QA Record

- Scientific Notebook

4.0 Appendices

Not applicable.

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