



# LabSpec 6

## ParticleFinder Application V2

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Doc. UM-LabSpec6/En  
Part Number: 1131089032



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## Chapter 1

# ParticleFinder Application V2

## 1.1 Description

HORIBA Scientific's ParticleFinder Application for LabSpec6 provides an automated particle location and Raman analysis function, allowing fast chemical characterization of discrete particles.

The Application uses manually or automated adjusted thresholds to segment particles from background on a microscope image acquired with the Raman instrument. Morphological filtering operations can be applied to improve segmentation and remove unwanted features (such as dust particles, edge particles etc). Analysis of key particle statistics are calculated, and the located particles can be further refined according to statistical thresholds (for example, to only locate particles within a certain size range). Once the particles have been located, automated Raman analysis can be started - each particle is moved beneath the laser spot, and analyzed sequentially, using the full capabilities of the Raman instrument.

**NOTICE:** ParticleFinder is a plug-in Application for LabSpec6 Spectroscopy Suite; it cannot be used without LabSpec6. For basic image analysis and particle location, ParticleFinder can be used with LabSpec6 on any PC. For automated Raman characterization, ParticleFinder must be used in conjunction with a HORIBA Scientific Raman instrument controlled by LabSpec6 - the instrument must include a motorized mapping stage.

## 1.2 Installation and Licensing

The ParticleFinder Application is included in the basic LabSpec6 installer. However, it is a commercial Application, which must be individually licensed before it can be used. However, a 30 days trial period can be activated to test the application. This trial version can also be activated for all the applications with license fee.

During 30 days, the tested Application displays the remaining trial days in the LabStore area. This information is also displayed under each trial application.

At the end of the trial period, if the Application has not been activated, it will be switched automatically to a locked state.

**IMPORTANT NOTE:**

If you are running on Windows 7 or 10, it is recommended to run LabSpec6 with full administrator rights when you carry out the following licensing procedure. To do this, right click on the LabSpec6 shortcut and select «run as administrator».

If this is not done, it is possible that the license will only be temporary, and will be requested again when you start the next session.

This only needs to be done during licensing. For normal use the software can be started by running LabSpec6 in the normal way.

### 1.2.1 Procedure

LabSpec6 requires an activated license. LabSpec6 delivered with an instrument requires an additional license activation to use the **Acquisition** application (included in LabSpec6).

The **ParticleFinder V2 application** is included in the LabSpec 6.4.4 software and requires a fee-based license registration. Please take into account that every additional application with license fee requires that LabSpec6 license has been previously activated.

A form must be filled in LabSpec6 and the generated request license activation file with the extension **.l6l** must be sent to the local HORIBA service team by email.

The data contained in this file is encrypted and will be used for generating the license activation/unlock file with the extension **.l6u**. At HORIBA, it will be checked to verify the purchase order conformity in our database system. Once done, the license activation/unlock file with the extension **.l6u** will be generated and sent back to the local HORIBA Service Team that will send the generated file to the requester.

Each license will have an unique License Identification Number (LIN) and will be linked to an unique Personal Computer and its reference user. It will be possible to transfer this LIN to another computer by first revoking it.

In order to activate an application and request a license unlock/activation file, a form must be filled. Click first on the **Shopping Cart** icon  located below the application in the LabStore area:

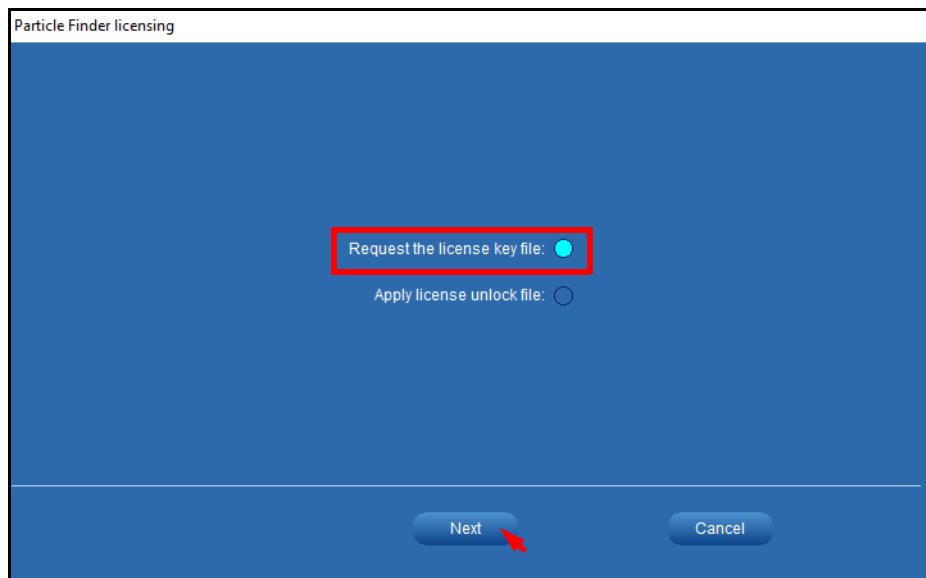
1



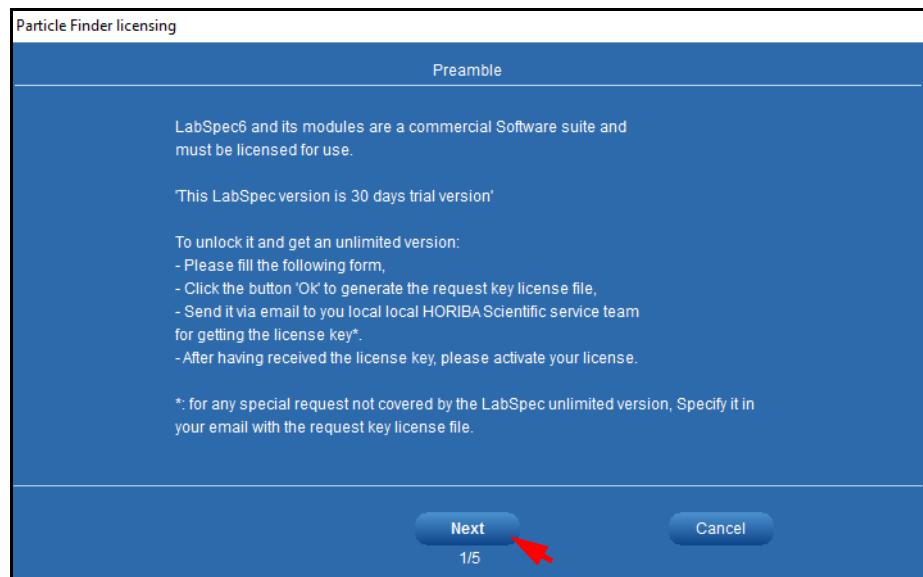
Several form windows will be displayed and all the mandatory fields must be filled.

Select the «**Request the license key file**» option, then click on **[Next]** button.

2

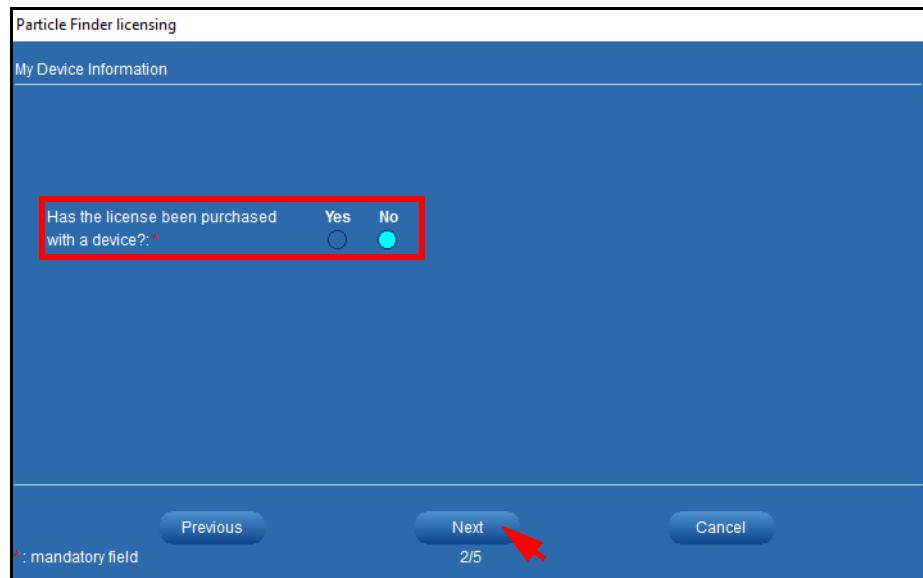


Step3 displays the preamble: read it then press on [Next] button.

**3**

Step4 displays «My Device information» window.

If the LabSpec license has not been purchased with an instrument, select the {No} answer.

**4**

If the LabSpec license has been purchased with an instrument linked to the current computer or if an additional license is requested for an off-line use (standalone use for data processing), please enter the instrument information like **Equipment ID** or **Serial Number** with equipment model.

5

Particle Finder licensing

My Device Information

Has the license been purchased with a device?\*:  Yes  No

Equipment ID:  S/N\*:

Equipment Model\*: LABRAM HR EVOLUTION

Previous Next 2/5 Cancel

\*: mandatory field

Step6 displays «**My Personal information**» window.

Some personal information is requested in order to link the LIN to a License Reference Person.

6

Particle Finder licensing

My Personal Information

Title\*: Mr.  Mrs.  Ms.  Dr.  Pr.

First Name\*: Donald

Middle Name:

Last Name\*: Dupont

Country\*: FRANCE

Email\*: donald.dupont@horiba.com

Organization\*: HORIBA

Department:

Organization Type\*: Industry

Job Title\*: MANUFACTURING/PROCESSING

Application Field\*: MATERIAL SCIENCE

Confirmation Email\*: donald.dupont@horiba.com

Previous Next 3/5 Cancel

\*: mandatory field

Step7 displays «**My LabSpec/HORIBA information**» window:

Three questions that could help HORIBA to know better its LabSpec6 users are asked. Do not hesitate to give some remarks or suggestions for improving your experience with LabSpec6. Giving your number of years with LabSpec6 will help the service team in case of assistance.

7

Particle Finder licensing

My LabSpec/Horiba Information

Years of experience with LabSpec Software\*: <1

Do you want to receive regular news about our product?: Yes  No

Remarks-Suggestions:

Previous Next 4/5 Cancel

\*: mandatory field

Once done, click on the [Next] button to display the final summary window (step8).  
The generated .l61 file is composed of the following 4 parts:

- A 6-character code to identify easily the request,
- The reference license user,
- The country where the license will be used,
- The name of the license request; LabSpec6 or a related application.

**Example:** KOYFKG\_Dupont\_FRANCE\_Particle Finder.l61

Read the privacy rules then select {YES} and click [OK] button to validate.

8

Particle Finder licensing

Generate the request key license file

Filename: KOYFKG\_Dupont\_FRANCE\_Particle Finder.l61

Reference License Person: Donald Dupont

"Respecting your privacy is important to us. The information about yourself is exclusively reserved for Horiba Jobin Yvon. You have the right to access, read, preserve, modify and disclose any information about yourself. To apply it, please contact Horiba Jobin Yvon, Avenue de la Vauve, Passage Jobin Yvon,

I have read the above condition\*: Yes  No

Previous OK 5/5 Cancel

\*: mandatory field

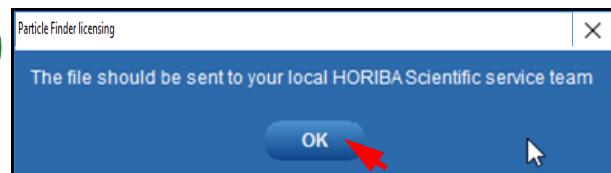
The file is automatically generated and saved on the Windows® desktop. The step9 shows the displayed window with the name and the location of the file.

9



Clicking [OK] will display another window (step10) informing the user to send the generated file to the local HORIBA Scientific Service Team in order to proceed with the request.

10



At HORIBA Scientific, we will check that the license is valid and we will send the activation/unlock file to the local HORIBA Scientific Service Team which will send the file to the user.

### > License Activation

The following procedure explains how to unlock and activate an application with license fee. Once the file with the **.l6l** extension has been sent (step10) to HORIBA Scientific Service Team, you will receive the unlock/activation file with the **.l6u** extension.

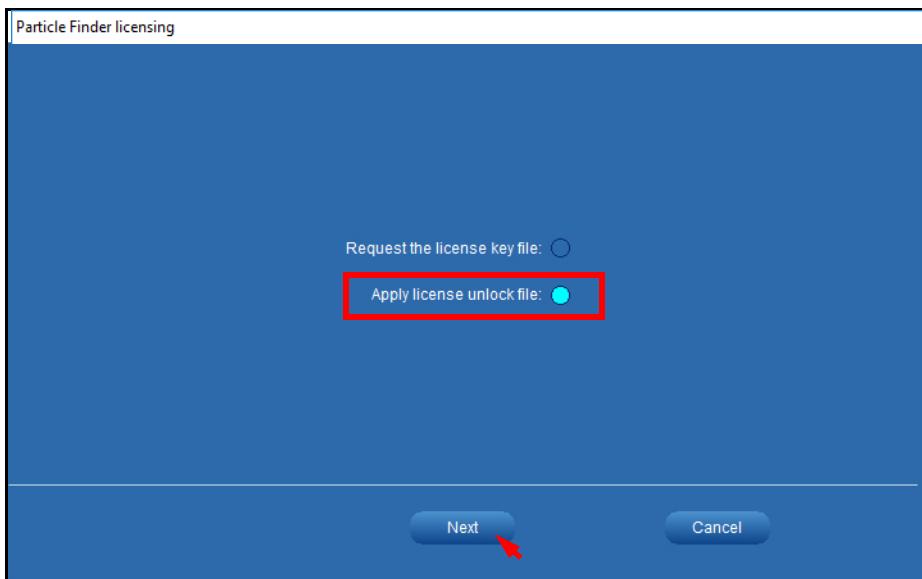
**Example:** KOYFKG\_Dupont\_FRANCE\_Particle Finder.l6u

To start the Particle Finder validation procedure, click on the **Shopping Cart** icon  located below the Particle Finder application in the LabStore (step11b).

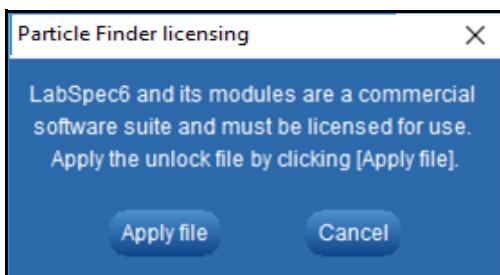
11



The window step12 will be then displayed. Tick the «**Apply license unlock**» option.

**12**

Once done, click on the *[Next]* button. The following pop-up window will be displayed:

**13**

Click on *[Apply file]* button to launch the Windows® Explorer application. Find and open the file you have received with the **.16u** extension. The name of the file is the same which has been sent to HORIBA Scientific for validation.

Opening the file will immediately validate the Application.

Once ParticleFinder application is activated, the **Shopping Cart** icon and is replaced by a new icon . This new icon could be used for the revocation/transfer of the license.

## > Revocation

If for some reason, a license should be transferred from one computer to another, a revocation/transfer procedure can be done.

Follow the procedure describes below:

- Click on the located in the LabStore under the Application logo,
- Follow the instructions which will generate a file with a **.16r** extension,
- From the new computer, proceed as for a new license activation, from step1 to step10,

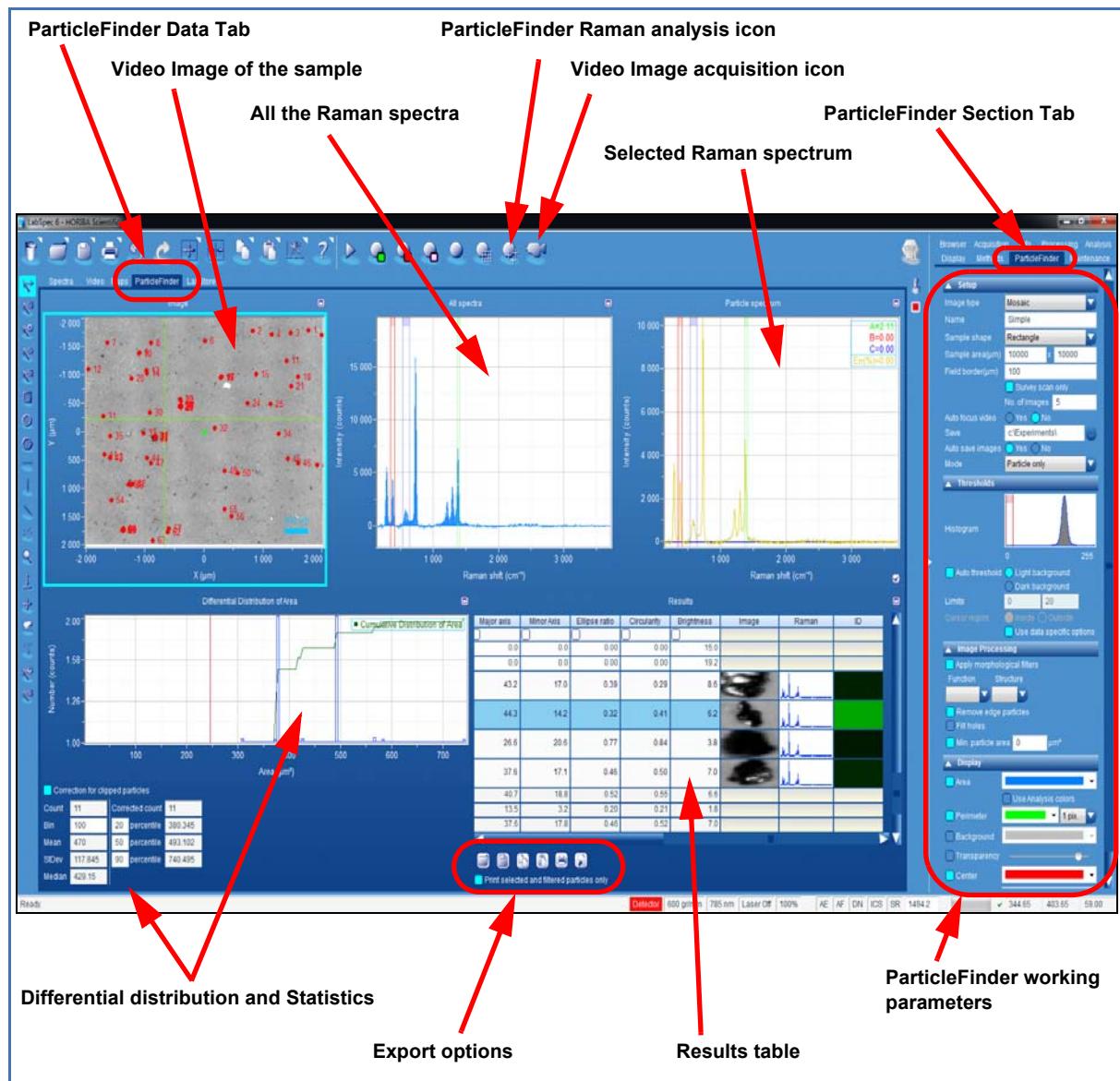
- Once the «license request file» with a **.l6l** extension has been generated, send **both**, this file and the «revocation» file, to the HORIBA Scientific Service Team,
- You will receive the **.l6u** file to unlock the application. Proceed to the previous steps 11 to 13 to activate the application.

The revocation file will prove that your application has been removed from the initial computer.

For a complete transfer of a LabSpec6 software + application(s) licensed package to a new computer, read the instructions detailed in the LabSpec6 Reference Manual, p/n: 1131089151.

## 1.3 ParticleFinder Interface

Once the ParticleFinder Application has been installed, an additional ParticleFinder Data Tab is added to the general interface. The figure below shows these new ParticleFinder elements.



## Chapter 2

# 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 Mosaic (montaged) video Image covering a larger field to analyze.

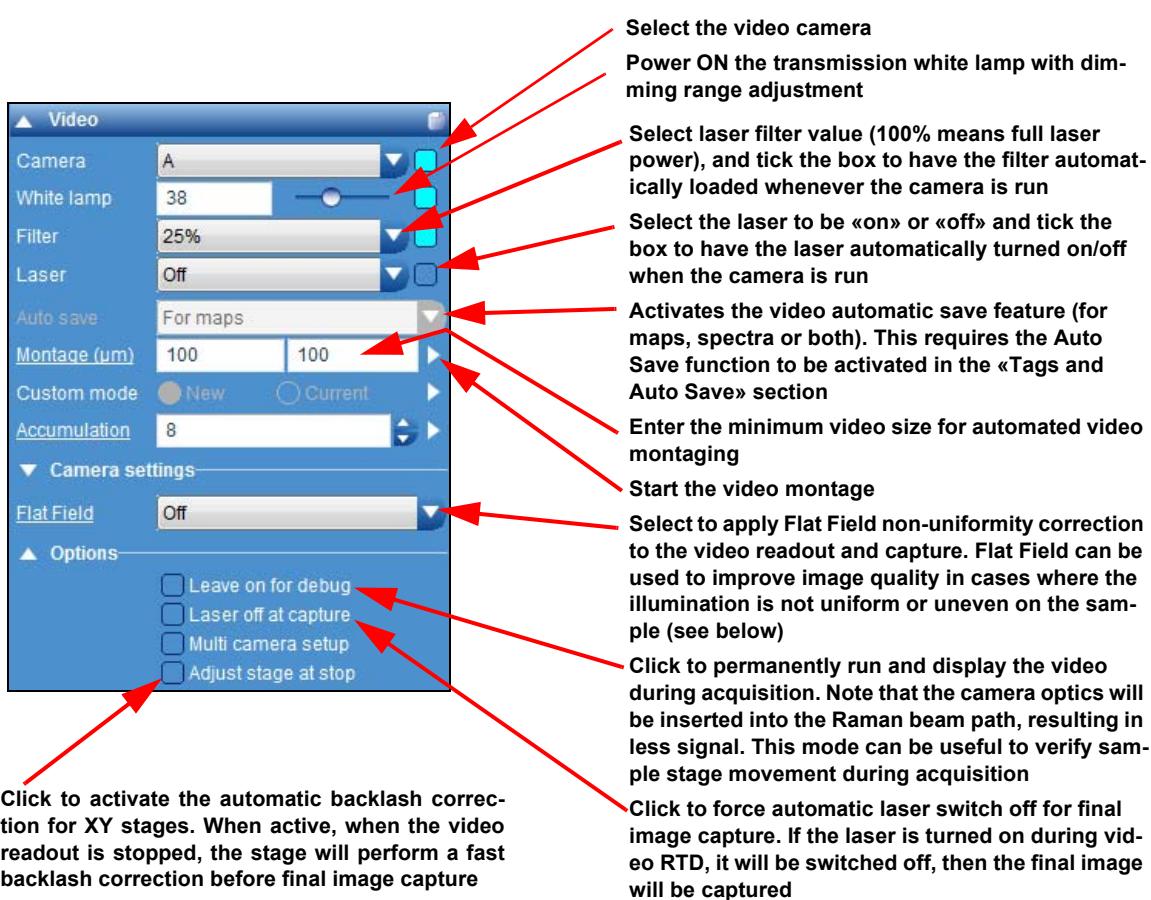
## 2.1 Video Image Capture

- 1 Select [Video] from the Data Tabs



The Video Data Tab is used to display optical video images, either in live readout of the camera, or static, captured images.

- 2 From Acquisition > Video, adjust the parameters for an optimal video Image



### Using the Flat Field Correction

The **Flat Field** corrector file must be saved before use. To do this, use a reference sample which has no visible features.



If a real sample with particles is used and to improve the Flat Field Correction, slightly defocus the video image to lower the particles contrast which can interfere on the global Flat Field correction.

Acquire the video image, making sure that Flat Field is turned off; then click on **Flat Field > Save reference**. Reference files are stored individually for each objective and camera on the system.

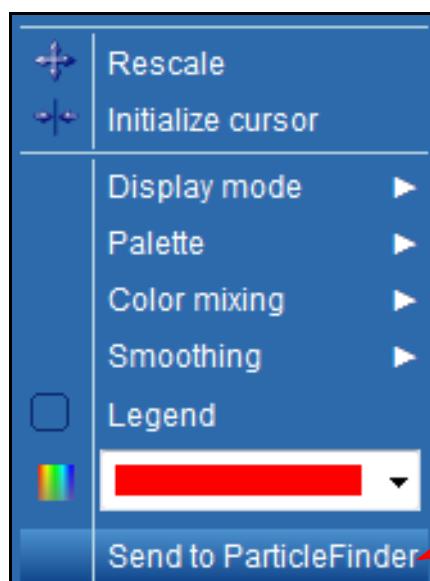
Flat Field correction can be applied to non-corrected data after acquisition by clicking on **Flat Field > Apply correction**.

Flat Field example:



**3** Click on the [Video Icon] to capture the Image of the sample

**4** Right-click on the Video Image, then select [**ParticleFinder**] choice. The ParticleFinder tab is opened and the Video Image is transferred to the [Image] area.



Send the Video Image to  
the ParticleFinder Tab

**5 IMPORTANT:** As soon as a Video Image is transferred to the **ParticleFinder** tab, the practical search of the particles starts immediately with the default parameters of Thresholds, filters etc.). Read the “Defining Thresholds” and “Image Processing” chapters to find the best settings.

## 2.2 Defining Thresholds

This step is the most important part for a good particle selection. There are two ways for a fine discrimination of the particles:

### 2.2.1 Auto threshold ON

Tick on **[Auto threshold]** box to activate the feature. Once done two choices are available: **Light background** and **Dark background**; read the explanation detailed below. If the sample background is not clearly identified, test both settings and choose the best one.

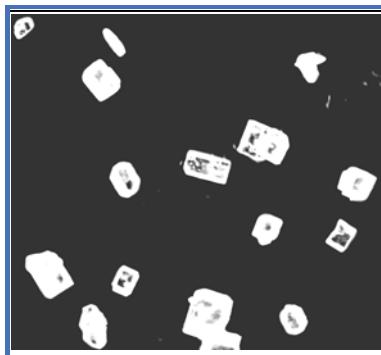
#### > Light background

This threshold is used to locate particles which appear dark on a light colored background.



#### > Dark background

This threshold is used to locate particles which appear bright on a dark colored background.





**Notice:** take into account that if too many particles are detected, it will become possible to filter them later by using specific parameters like area, diameter, perimeter, brightness and by applying morphological filters.

## 2.2.2 Auto threshold OFF

If the [Auto threshold] is deselected, it becomes possible to choose an area histogram and the detection limits (lower and upper). It is also possible to choose the selected region on the histogram or outside it.

The upper limit and lower limit thresholds can be adjusted together, to provide a notch-type threshold. In this case, the lower limit threshold slider should be positioned at a lower value than the upper limit threshold slider. Particles will be located which are brighter than the lower limit threshold, but are darker than the upper limit threshold.

Manually adjust the upper limit and lower limit thresholds to achieve the desired segmentation of the video image. Note that the particle location display on the video image will be affected by settings in the [Image Processing](#), [Statistics](#) and [Display](#) sections. If unexpected particle location is observed please check the settings in these sections, and if necessary turn off all Image Processing options and turn off the «Limits» functions of the table [Results](#).

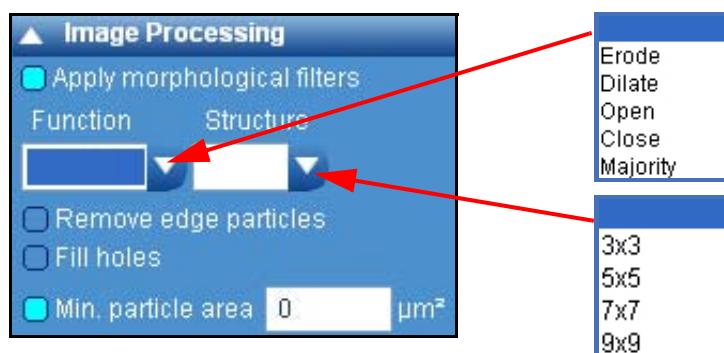
The display of located particles can be configured in the [Display](#) section, including options for display of particle area, perimeter, background (non-particle), transparency, center colors and the particles numbering.

## 2.2.3 Use data specific options

Tick this box to include the complete configuration (including the thresholds) when the data are saved and opened.

## 2.3 Image Processing

The Image Processing section provides morphological filters and other tools to improve and enhance the particle segmentation.



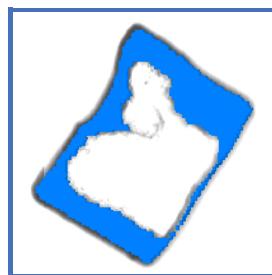
## > Morphological Filters

Select the desired filter from the drop down box, and the corresponding structural element size (in pixels). The structural element is the unit on 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 result is applied to the center pixel.

Multiple filters can be applied. Note that filters are applied in a top to bottom order as listed in this section. The complete filter result can be toggled on/off using the «Apply morphological filters» tick box.

### > Erode

Replace the center pixel with the minimum value of the structure element. The typical result of Erode is to remove edge pixels of the particle area.



### > Dilate

Replace the center pixel with the maximum value of the structure element. The typical result of Dilate is to add pixels to the edge of the particle area.



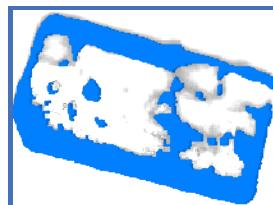
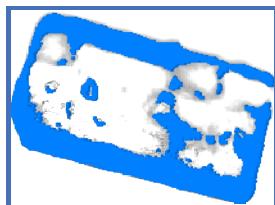
### > Open

Perform an Erode followed by a Dilate. The typical result of Open is to smooth the perimeter, remove very small particles, and break a thin line into segments.



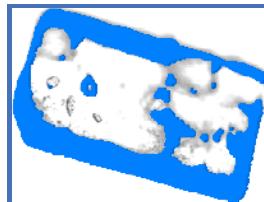
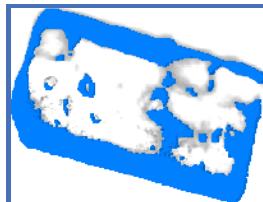
### > Close

Perform a Dilate followed by Erode. The typical result of Close is to smooth the perimeter, fill very small holes, and connect neighboring segments into a line.



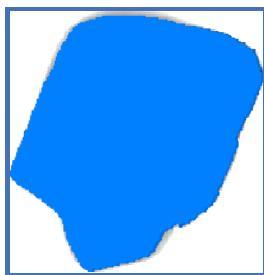
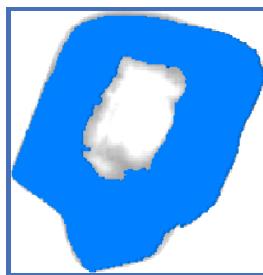
### > Majority

Replace the center pixel with the minimum value, if half or more pixels in the structure element are background. Replace the center pixel with the maximum value, if more than half the pixels in the structure element are particles. The typical result of Majority is to smooth the perimeter, and remove very small particles.



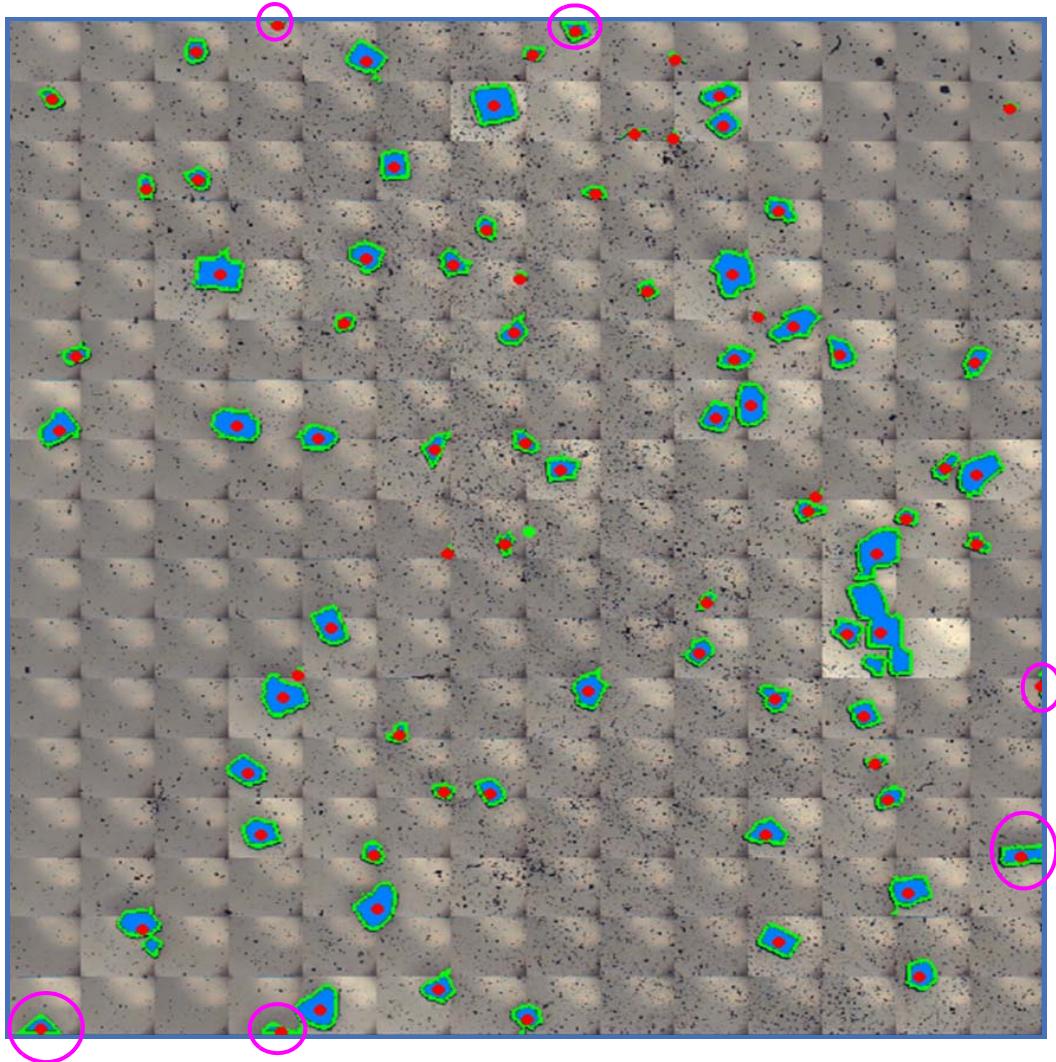
### > Fill Holes

Replace background pixels enclosed by particle pixels with particle pixels. Effective in correcting an extraordinarily bright area (for dark particles) or dark area (for bright particles) within particles.



### > Remove edge particles

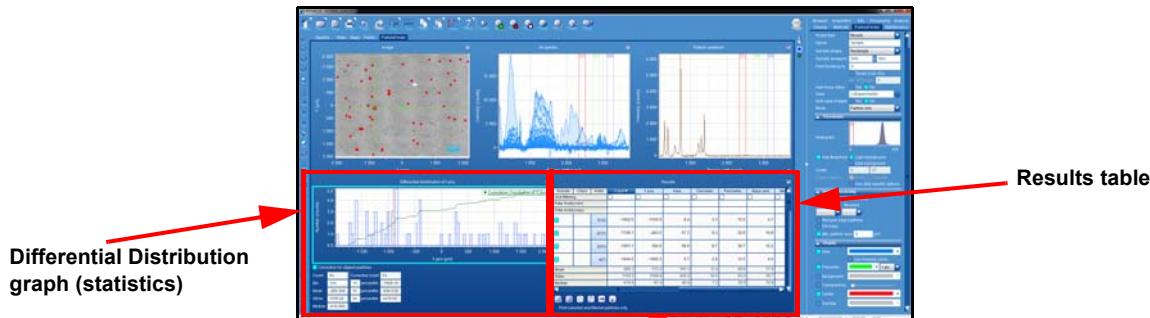
Tick the box to exclude all particles which are touching the edge of the video image. All excluded particles will not be marked on the video image, and will not be included in the statistical analysis. The figure below shows magenta circles around particles which will be removed by activating this filter.



#### > Minimum particle area

Tick the box and specify a minimum particle area (in  $\mu\text{m}^2$ ). All particles which have an area less than the specified minimum will be excluded. All excluded particles will not be marked on the video image, and will not be included in the statistical analysis.

## 2.4 Results & Statistics



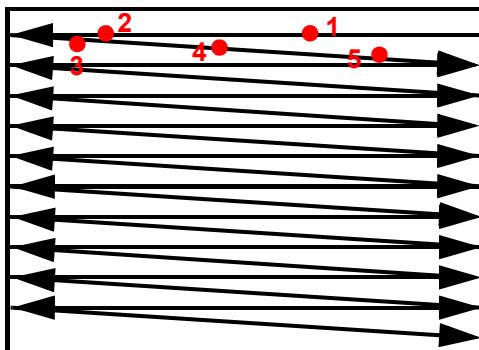
As shown on the figure above, The ParticleFinder results are listed in a table located on the bottom hand right area of the LS6 interface and the statistics on the bottom left.

### 2.4.1 Results table

According to the selected **Threshold** and the **Image Processing** parameters, particles will be detected. These detected particles are displayed on the sample **Image** and listed in the **Results** table.

#### How is numbered the particles on the Image and in the table?

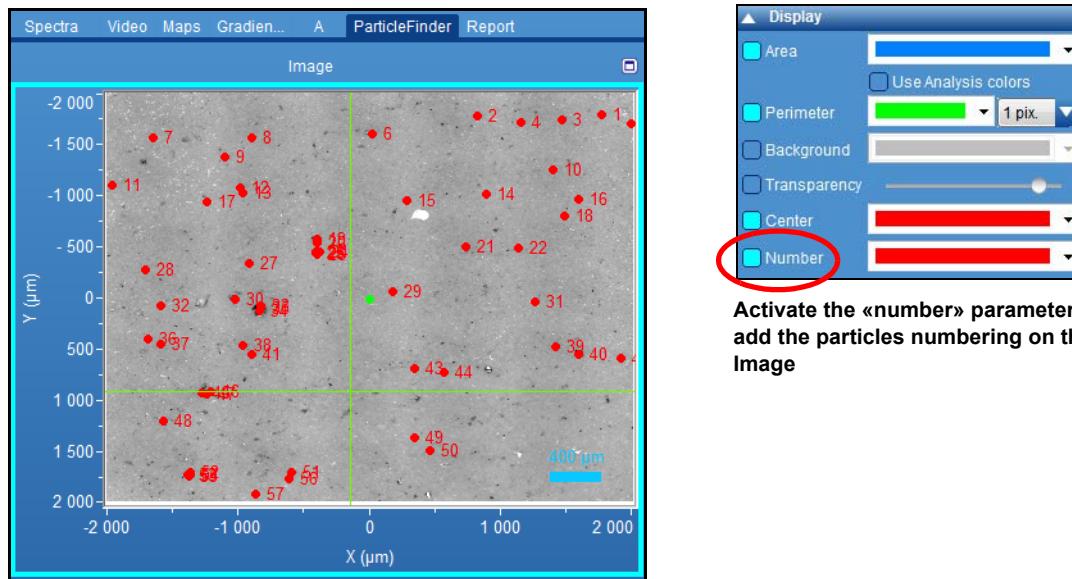
On the Image, the particles number starts from the upper right hand side and move to the bottom using a horizontal zigzag shape.



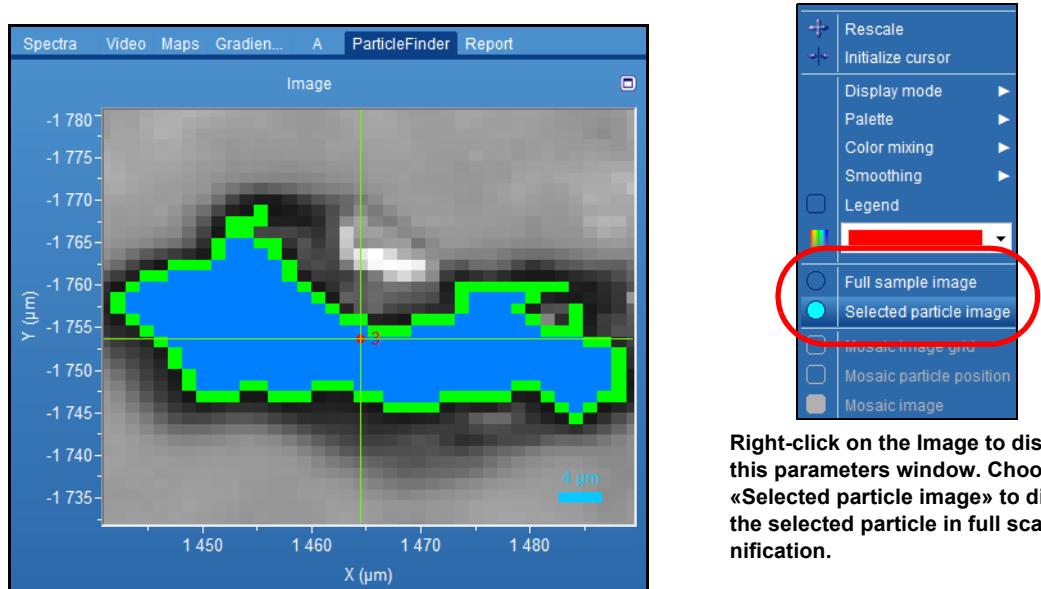
Particles number on the sample **Image**

**IMPORTANT:** All the particles founds on this Image receives an incremental numbering which will never be changed as long as the **Threshold** or/and **Image Processing** will not be modified even if the filters are activated in the Results table (see below).





**Figure 1-1 Example of detected particles found on a sample**



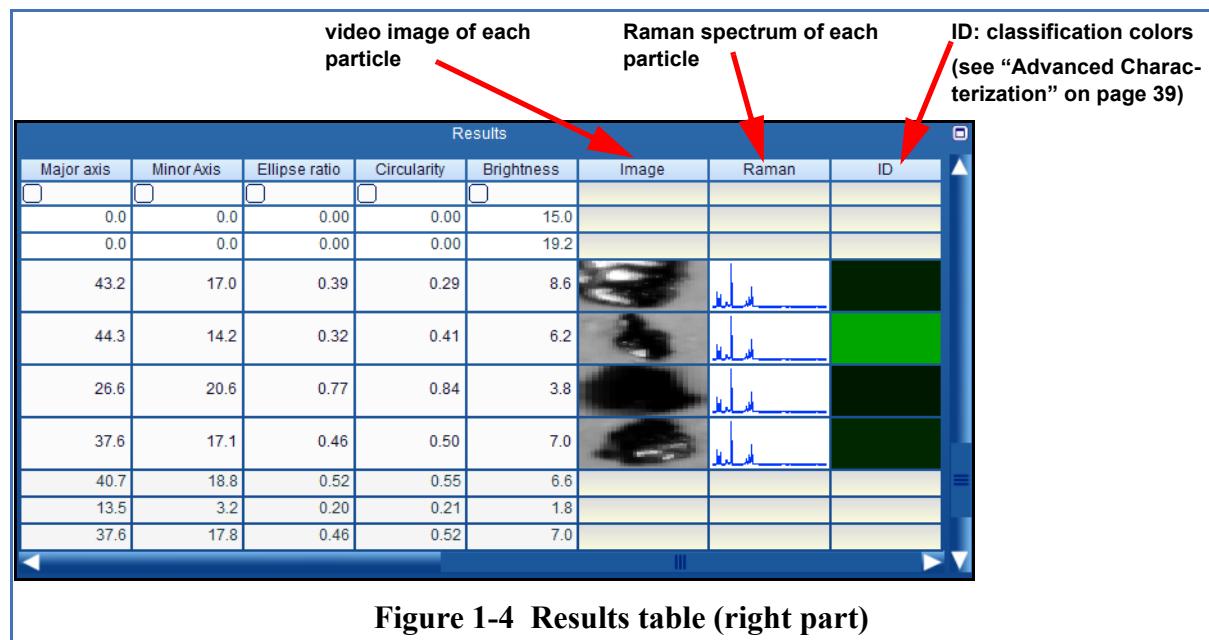
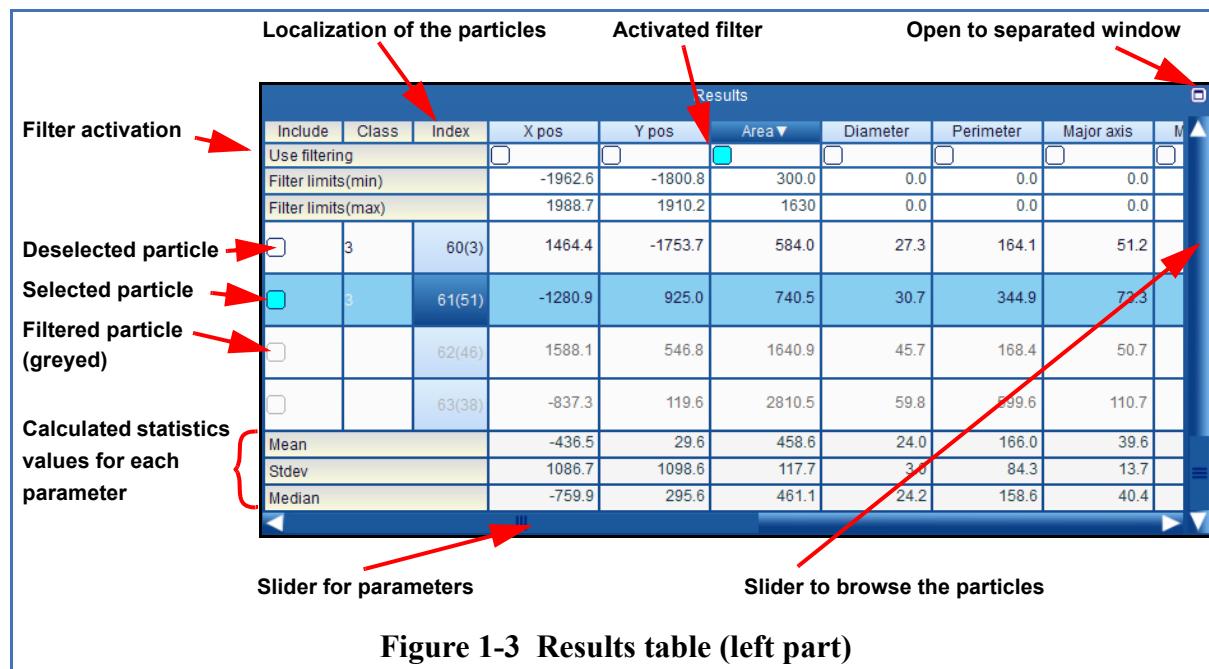
**Figure 1-2 Selected particles in full screen magnification**

The Results table contains all the detected particles and the associated parameters like area, perimeter, circularity and many others which can be used as filter. Each detected particle can be separately removed and thus will not be taken into account in the statistics and later for Raman analysis. In this table, specific calculations like [Mean], [Stddev] and [Median] are displayed for all the detected and selected particles.

The column [Index] contains 2 numbers:

- The number in brackets is linked to the detected particles and will never change as the **Threshold** or/and **Image Processing** will not be modified,

- The number in front changes according to the selected parameters (Xpos, Ypos, Area, diameter, etc.) and the numbering is incremental from the lowest value to highest.



### How to see where is a particle listed in the Results table?

Just click on the particle «Index number» and the cross-target will of the Image will move to show the location of the particle on the sample.

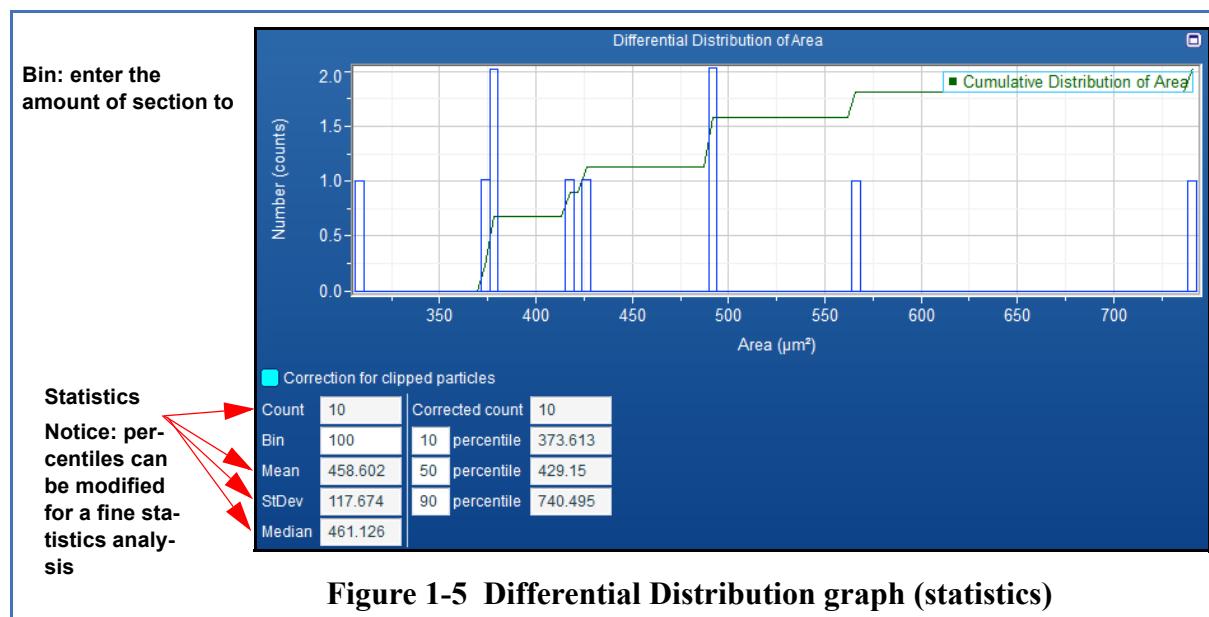
### How to use the Results table?

According to the modifications and filters you will apply in to the parameters, some particles will be excluded and greyed in the Results table. The [Differential Distribution] graph shows the statistics Histogram which is real time updated.

#### Example:

In the Results table, select a descriptor - parameter - that seems to be most appropriate to refine your searches. For example, on the Figure 1-3, we have chosen to filter the [Area] parameter from a minimum of  $300\mu\text{m}^2$  to a maximum of  $1630\mu\text{m}^2$ . The statistics will be updated to locate particles that meet the criterion. Other particles are ignored. In our example, the particle 61(51) has been found with an area of  $740.5\mu\text{m}^2$ . The calculated statistics show that the found filtered particles have a [Mean] of  $458.6\mu\text{m}^2$ , [Stdev] of  $117.7\mu\text{m}^2$  and [Median] of  $461.1\mu\text{m}^2$ .

Double-click on [Area] name of the table. On the left hand side of the results table, the statistic graph shows then the Differential Distribution of Area (see figure below).



The Histogram of the Differential Distribution displays results for the selected statistic parameter [Area] (example above). A superimposed graph shows the cumulative distribution of area. These statistics can be displayed as a function of any of one of the parameters of the result table.

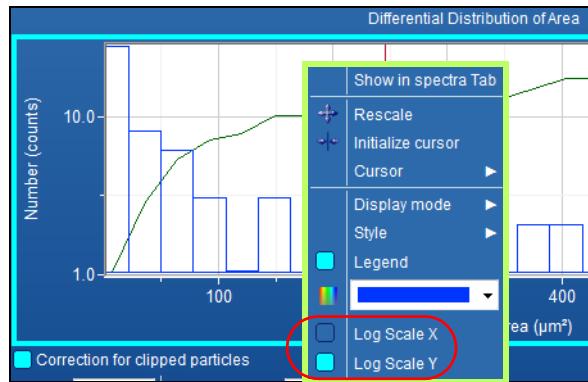
### 2.4.2 Histogram/graph statistics setup

The number of bins (channels) can be specified in the [Bin] text box located below the Histogram/graph - the number of bins must range from 3 - 100.

Improved statistics can be done by entering a % value of the selected parameter. In our example 10% of area are within  $300\mu\text{m}^2$  and  $373.613\mu\text{m}^2$ .



Right-click on the graph to display the parameters window: Log scale X or Log scale Y could be a better choice to highlight some results.



**NOTICE:** The statistics section displays the statistics of key particle size and shape descriptors as defined by the current ISO document (ISO 9276-6:2008 - Representation of results of particle size analysis -- Part 6: Descriptive and quantitative representation of particle shape and morphology) for the particles located in the video image.

- **List of the parameters used as filters**

**> X pos**

X coordinate of center of mass with respect to video origin (in  $\mu\text{m}$ )

**> Y pos**

X coordinate of center of mass with respect to video origin (in  $\mu\text{m}$ )

**> Area**

Area,  $A$  ( $\mu\text{m}^2$ )

**> Diameter**

Area-equivalent diameter,  $X_A$  ( $\mu\text{m}$ )

**> Perimeter**

Perimeter ( $\mu\text{m}$ )

**> Major axis**

Major axis of the Legendre ellipse,  $X_{L,\max}$  ( $\mu\text{m}$ )

**> Minor axis**

Minor axis of the Legendre ellipse,  $X_{L,\min}$  ( $\mu\text{m}$ )

**> Ellipse ratio**

Ratio of the length of the major axis over that of the minor axis,  $X_{L,\max} / X_{L,\min}$

## > Circularity

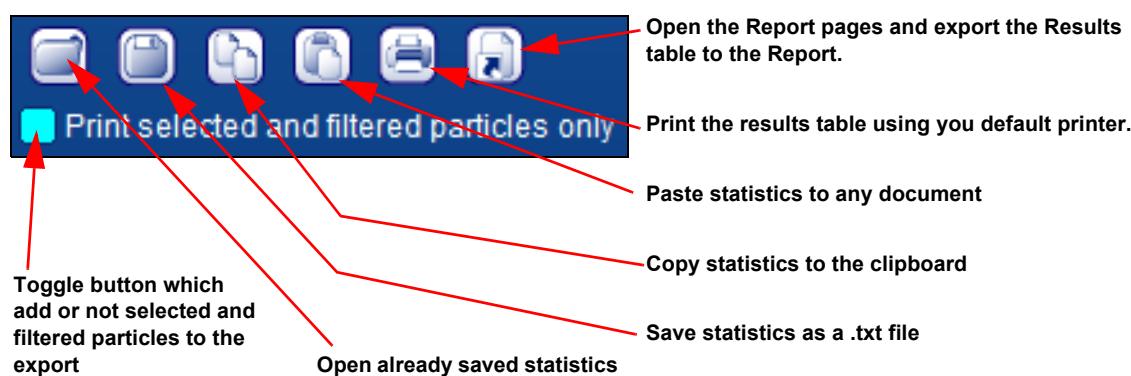
Ratio of the area equivalent diameter  $X_A$  ( $\mu\text{m}$ ) over the perimeter equivalent diameter,  $X_P$  ( $\mu\text{m}$ ).

## > Brightness

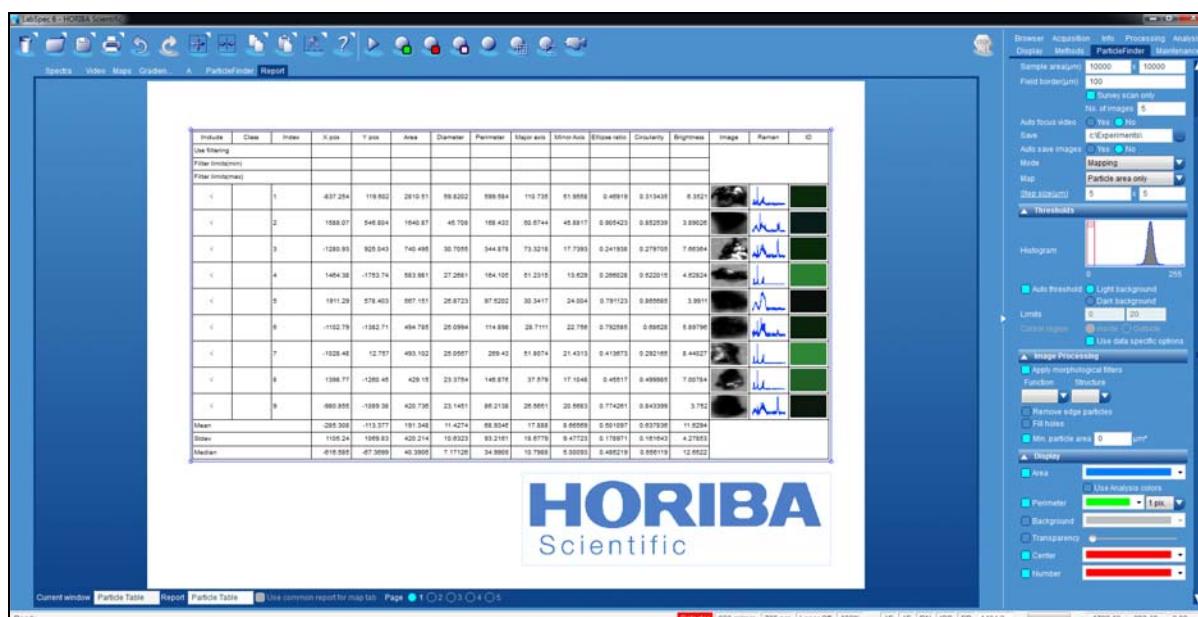
Show average intensity of all pixels for each particle. (For transmission illumination this value gives an indication of particle transparency, which can be a useful filtering tool).

### • Exporting Results

In addition to the regular saving project in LS6, several additional buttons are gathered below the Results table.



Report example of an exported Results table:



Click on «Copy 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 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	95.7799	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	17735.6	150.272	1037.8	264.435	195.136	0.737935	0.454896			
5	4 4322.22	-5984.3	969.913	35.1416	147.535	36.0721	32.2815	0.894916	0.748302			
6	5 -4712.121	-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	299.418	87.7844	33.265	0.37894	0.462211			
8	7 -3355.72	-5651.99	53206.7	260.278	949.119	289.323	236.921	0.618879	0.8616524			
9	8 -1640.69	-5571.47	100317	357.39	1336.54	377.5	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 87.9784	-5694.53	692.795	29.7001	94.163	62.2143		0	0 0.990893			
12	11 4866.83	-5664.3	1385.59	42.0022	270.096	81.8135	39.1951	0.479078	0.4886545			
13	12 -7.67893	-5594.62	5958.04	87.0977	750.737	197.357	138.069	0.6959	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	827.713	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	0.530872	0.52609			
22	21 1519.92	-5399.73	692.795	29.7001	141.809	33.0568	23.117	0.699311	0.657965			
23	22 -2276.6	-5341.78	692.795	29.7001	93.3055	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	-5191.58	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	325.077	3266.17	539.27	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	1815.12	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	-5157.84	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 4854.6	-4990.59	8867.78	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	51.9358	21.3818	0.411696	0.518186			
39	38 3851.14	-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	0.696643			
42	41 1017.31	-4787.44	83.455	49.1782	14.45604	0.0296075						
43	42 1089.99	-4719.5126.69	80.7929	913.172	222.62	49.7228	0.223353	0.277952				
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	36.8016	21.6606	0.588578	0.650622			
48	47 1508.93	-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	54.8515	33.2278	0.605777	0.651212			

Click on «Save 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, spreadsheet software etc.

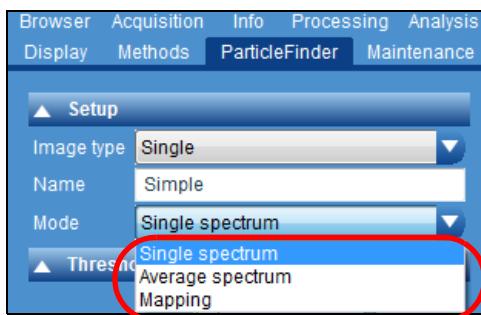
## 2.5 Experiment Setup

The Setup section allows the user to define the characteristics of the video image and the type of scanning. There are two main video images: **Single** or **Mosaic**. According to this choice, multiple parameters can be defined.

### 2.5.1 Single Image

A Single Image Video means that a video image is made using one of the following methods:

- Single field of view video which is a shot image captured by the Instrument,
- Single extended video montage: single video capture covering a large sample area using the «Montage» feature (see details in the LS6 Reference Manual),
- Single map image generated from the Acquisition > Map section (see details in the LS6 Reference Manual).



As shown on the figure above, three Raman acquisition modes are available: **Single spectrum**, **Average spectrum** and **Mapping**.

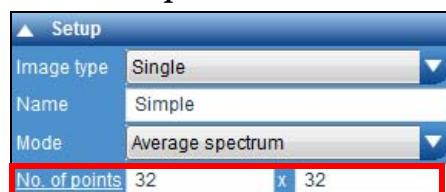
#### > Single spectrum

This mode will acquire one Raman spectra for each detected particle. The acquisition point is then located in the center of the particle.

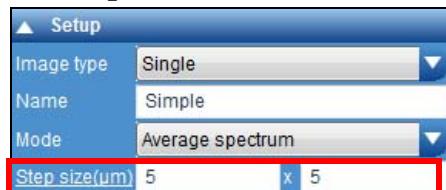
#### > Average spectrum

This mode will measure several points on each particle. Then, the user has the choice between 3 different acquisition points selections.

- **No. of points**

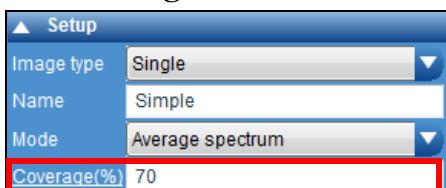


- Step size



The defined Step size will be applied inside each particle.  
An average Raman spectrum will be calculated from  
those performed at each step.

- Coverage

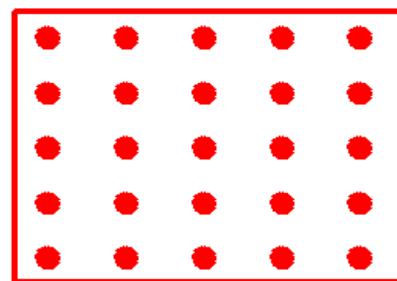
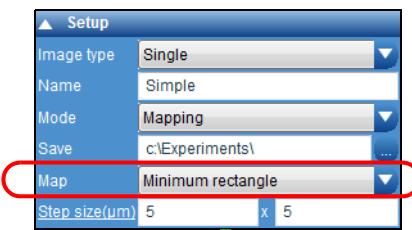
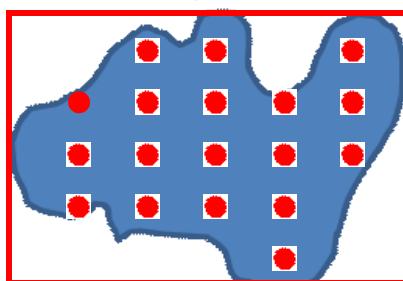
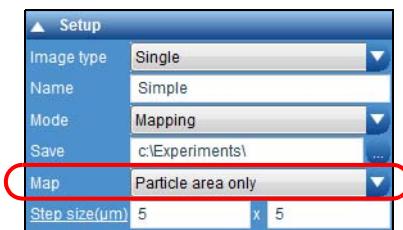


Enter the % of coverage which will be applied on the particles.

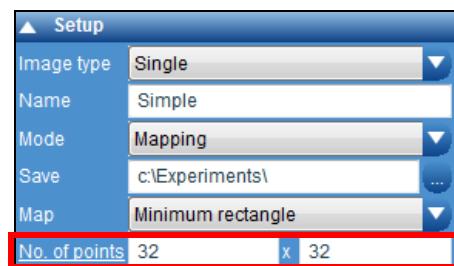
## > Mapping

This mode will acquire and save a Raman spectrum for each acquisition point. The map for each particle will be defined first by choosing a shape (minimum rectangle or data acquisition inside a particle) and acquisition point coverage.

- Map shape

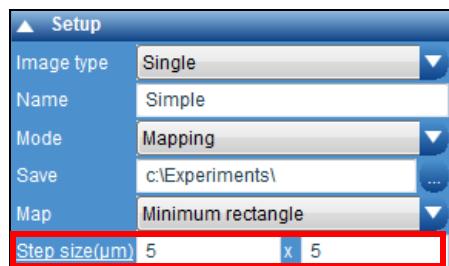


- No. of points

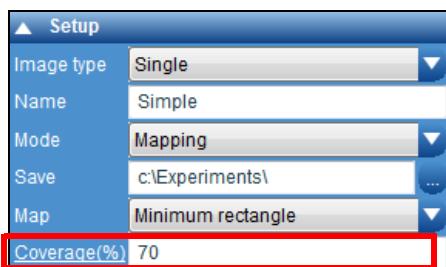


An average Raman spectrum will be calculated according to the entered number of points ( $X \times Y$ ). Thus the number of data acquisition will remain the same whatever the area of each particles. But the steps between data will be different.

- Step size

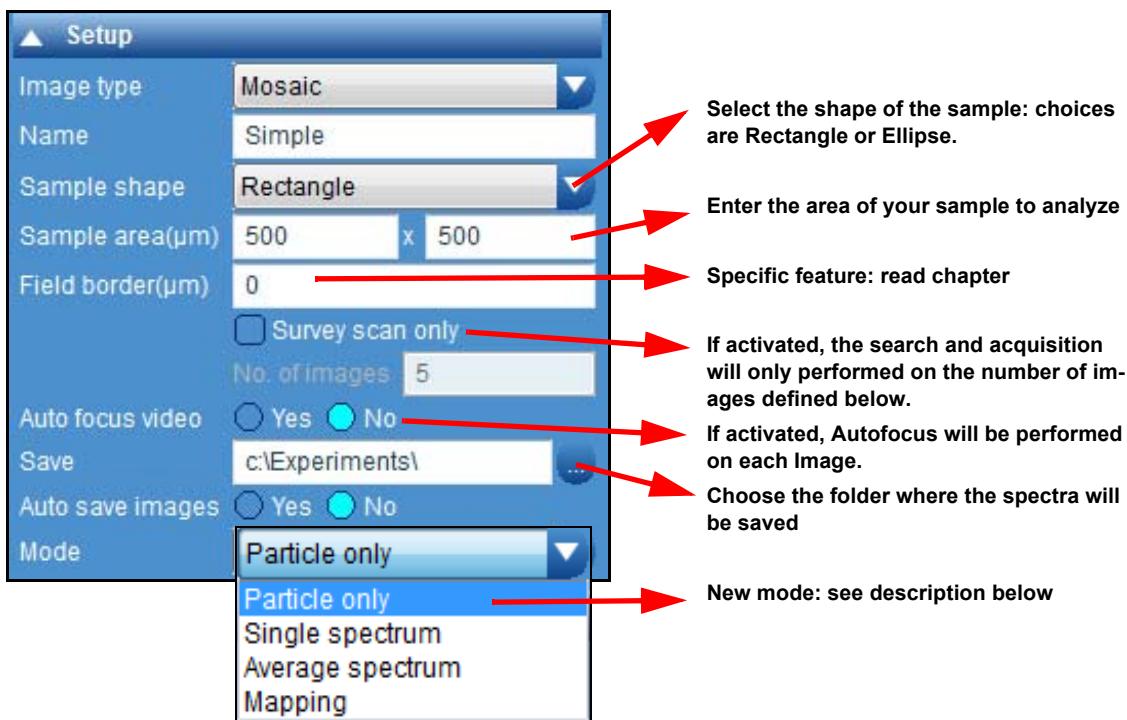


- Coverage



## 2.5.2 Mosaic

Mosaic Image type is the most advanced mode to perform the particles search and the Raman acquisition on a large sample. This mode includes unique feature to avoid the lost of particles located on the border between the fields of view.



**IMPORTANT:** before using the Mosaic Image, verify the [Instrument setup] section settings located in the Acquisition tab. It is very important to verify the selected values especially the Objective magnification which must be the same as really selected on the microscope.

### > Mosaic Modes

Using a Mosaic Image, four Modes are available. Three of them are the same as for the Single Image described above. The new [Particle only] Mode does not perform Raman acquisition; it launches the particles identification, image by image, on all the sample area (with the exception if the [Survey scan only] has been activated with a limited number of images).

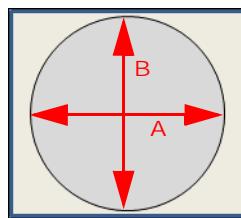
**CAUTION:** this mode requires to choose the right settings for **Thresholds** and **Image Processing** before starting the particles detection which could be a long process.

### > Setup Procedure

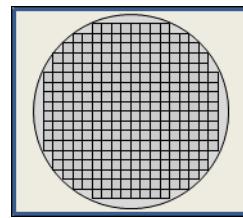
First enter the shape (Rectangle or Ellipse) and the area of your sample to analyze.

**IMPORTANT:** we strongly recommend you to calculate and then enter a sample shape size which be a multiple of the field of vision.

According to your Instrument composition and setup (selected microscope objective, video camera type, laser type), software will calculate the amount of Images necessary to cover the entire sample. The specific and useful **Field border** parameter will also be taken in account (see “[Field border](#)” on page 32).



Sample size



Calculated Images



The Mosaic mode is more precise than a «Montage» Image because the particles search and Raman acquisition is performed frame by frame to cover the entire sample.

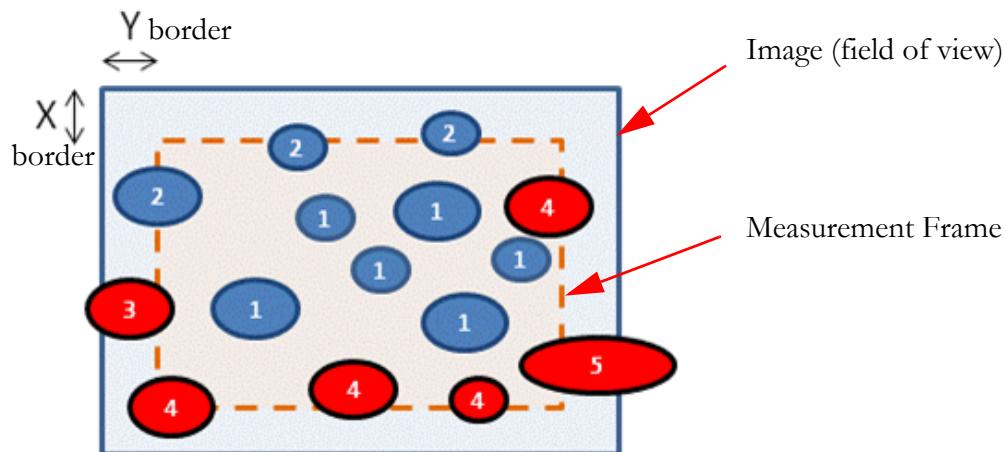
The mosaicking will only be applied for areas which are completely inside the sample. There should be no videos acquired over the edge of the sample.

### > Field border

This is a new and very powerful feature. It avoids to lost clipped particles. Indeed, there is a high probability that particles will be missed because they lie on an edge of the individual images.

#### Principle of the feature

The sample covering will be made using the **Measurement Frame** rather than the full field of view:



**Measurement frame** inserted within the field of view

All particles totally inside the frame are included (e.g., 1)

All particles lying on TOP and/or LEFT borders of frame are included (e.g., 2)

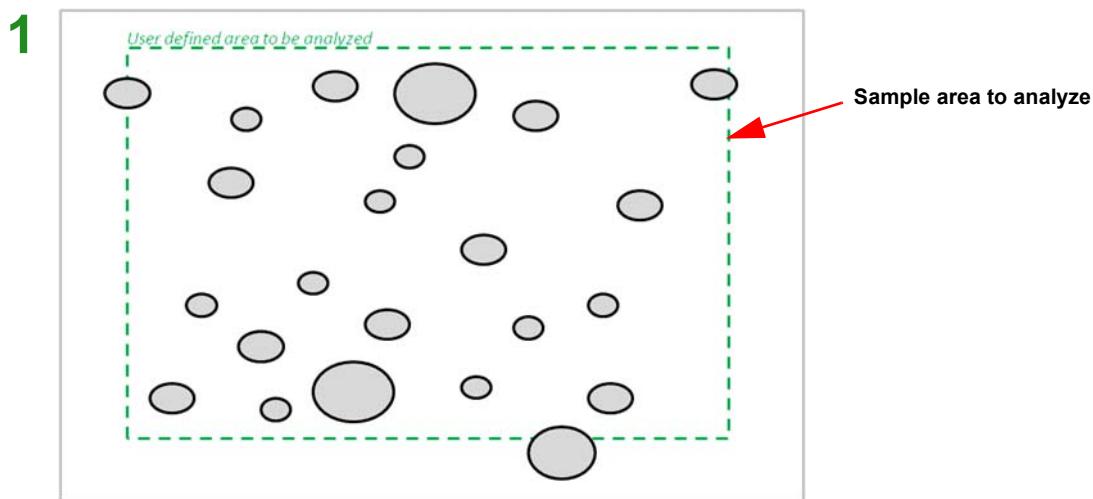
--> but particles which also lie on the image border are excluded (e.g., 3)

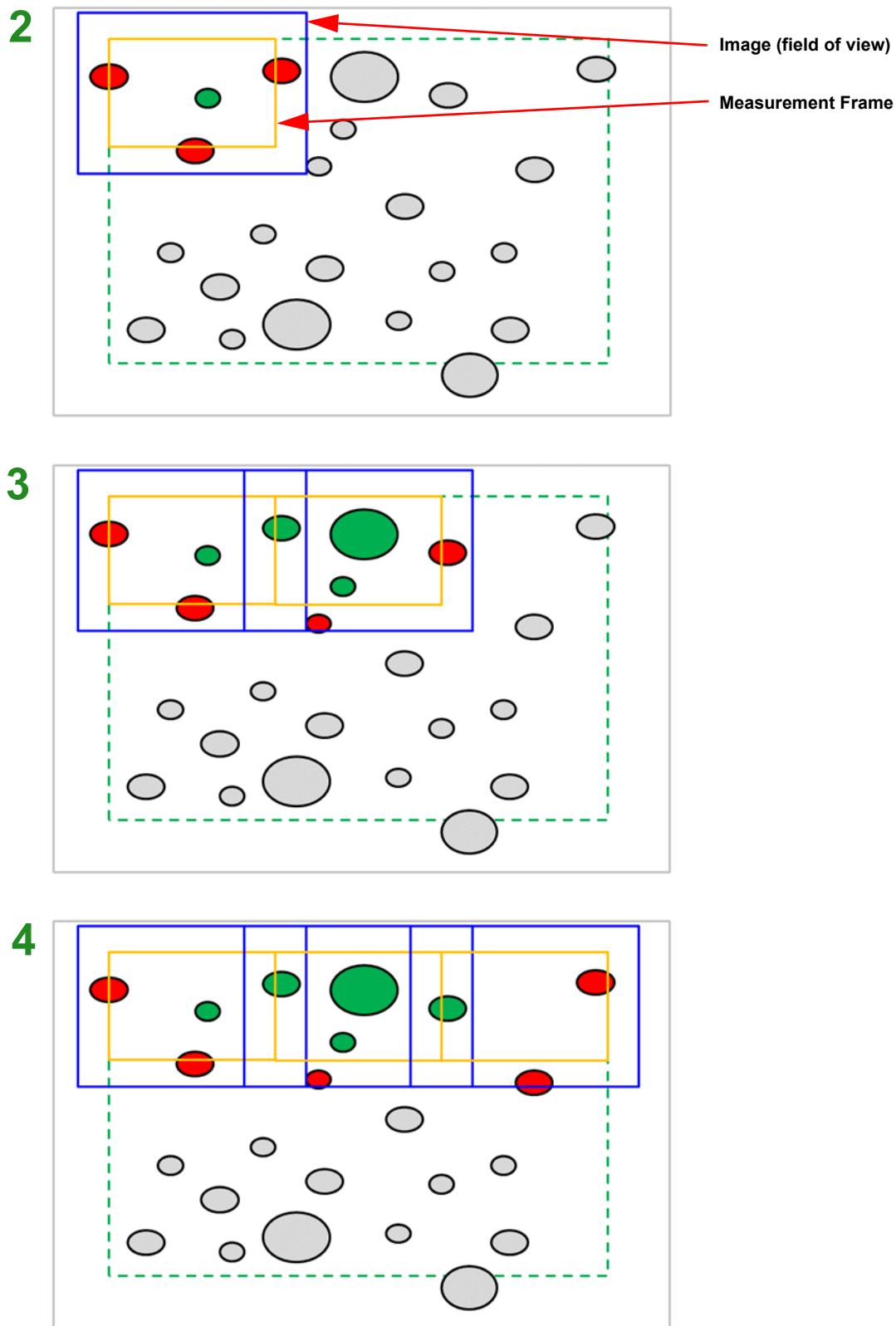
All particles lying on BOTTOM and/or RIGHT borders of frame are excluded (e.g., 4)

--> and any particles which also lie on the image border are still excluded (e.g., 5)

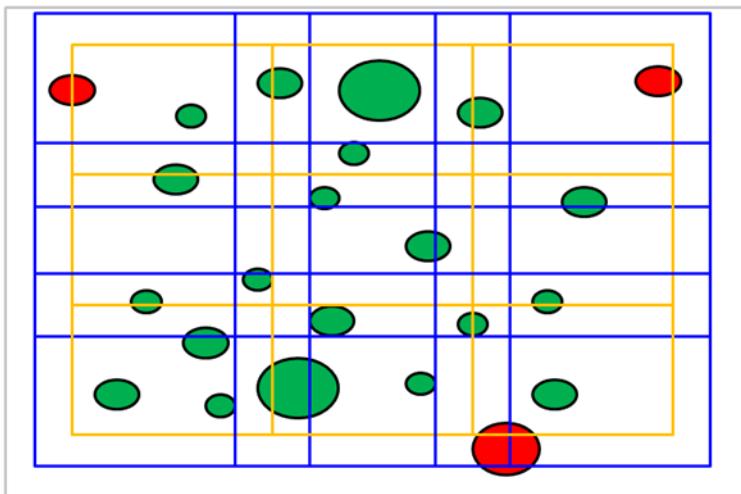
### Sequence example

= particle      = excluded particle      = validated particle





02/09/2017

**5**

At the end, all the clipped particles  
were reconstituted with precision

## 2.6 Raman Measurement

### > Start Real Time Display (RTD)

This function uses the standard LabSpec6 «Move stage/Acquire spectrum» tool. See the chapter “How can I acquire a spectrum?” on page 31 in the full LabSpec6 manual.

Click anywhere on the Video Image to move the point underneath the laser spot.

Click on the run arrow  in the inset window to start acquiring RTD data, according to the parameters set in the Acquisition tab.

To stop the RTD measurement, click on the [Stop All] button .

### > Start Raman measurement

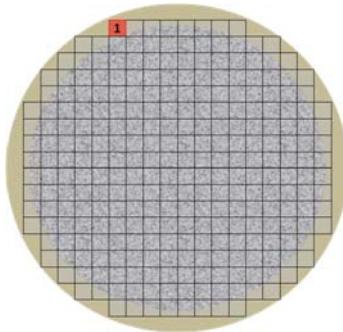
Once the particles, important for your experiment, have been detected and selected from the [Results] table, you can launch the Raman analysis. Take into account that each data point will be analyzed with all the acquisition parameters defined in the «Acquisition tab» (see the LS6 Reference Manual). In certain conditions, if thousands of data points have been set using the mapping feature, this could take a long time!

The procedure described below shows how the software proceeds when a Raman acquisition is started. This example is for Mosaic, the most advanced acquisition.

To start Raman Acquisition, click on the Start ParticleFinder button  . Once this icon is clicked, the system will automatically move from one data point to the next of each particle, and acquire the spectrum.

**NOTE:** to perform correctly, please ensure that the video window is active when the measurement is started.

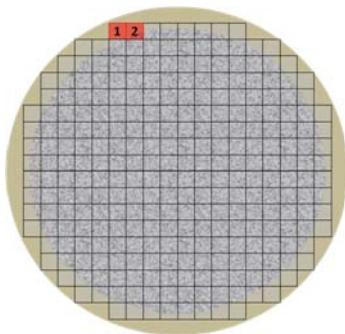
#### The software moves the stage to Image position 1



Then:

- Perform video autofocus (if activated)
- Acquire video image
- Save video image as {name}\_video\_X1\_Y1.l6v
- Locate particles using autothresholds (or using preset manual thresholds)
- Produce particle statistics

- Produce particle thumbnails
- Do Raman acquisition (single spectrum, average spectrum, map)
- Save Raman data project file

**Move to image position 2**

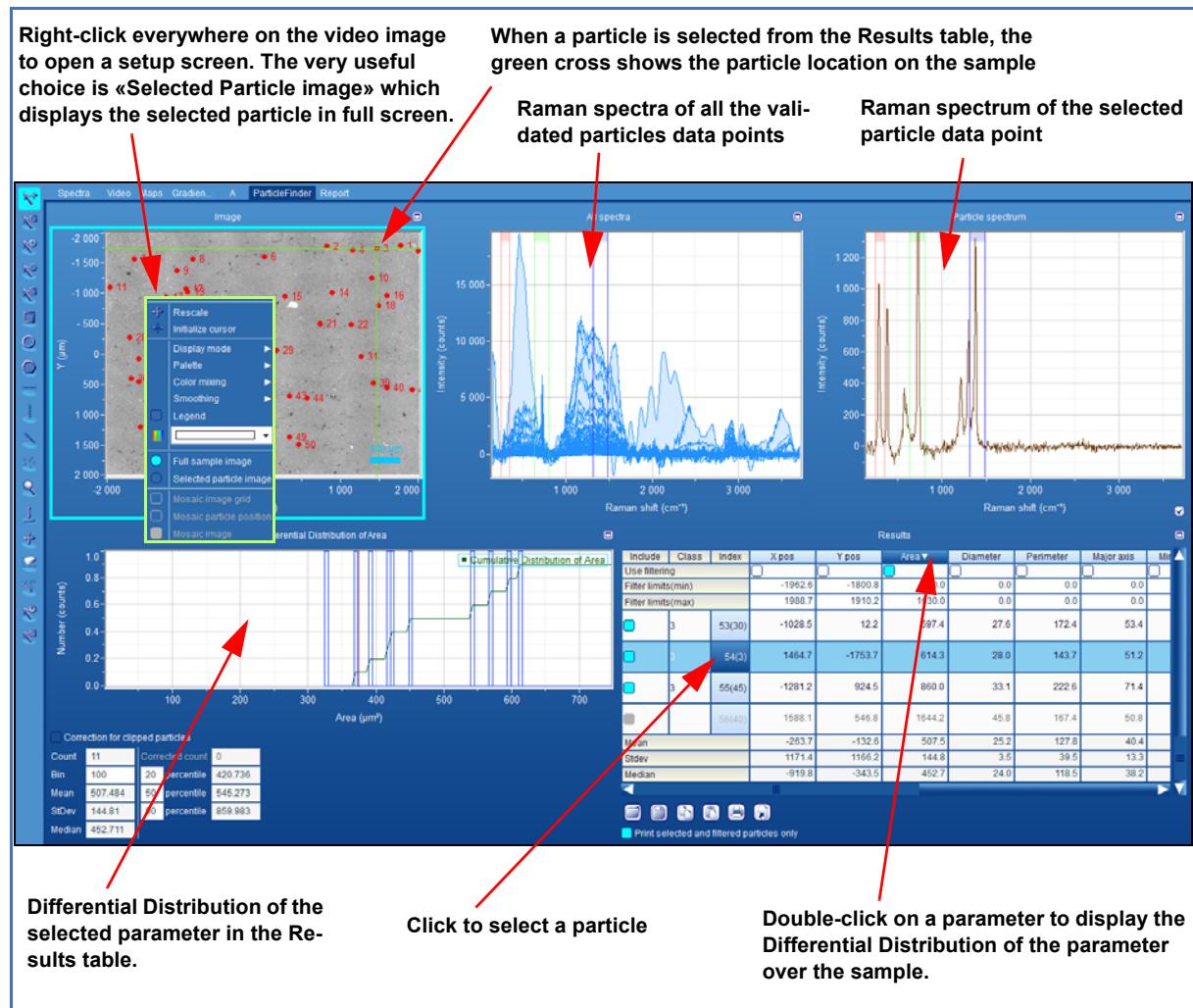
- Perform video autofocus (if activated)
- Acquire video image
- Save video image as {name}\_video\_X2\_Y1.l6v
- Locate particles using autothresholds (or using preset manual thresholds)
- Produce particle statistics
- Produce particle thumbnails
- Do Raman acquisition (single spectrum, average spectrum, map)
- Append data to project file
- Save Raman data project file

**The software continue until all image positions are covered and acquisitions done.**

All the spectra are saved in the [All spectra] graph or individually displayed in the [Results] table of the ParticleFinder tab. Moreover the spectrum of each data point can be displayed in the Particle spectrum graph of the ParticleFinder tab.

The spectra acquired by ParticleFinder will be grouped in the new LabSpec6 ParticleFinder file (.16pf), which can be further processed and analyzed using the standard LabSpec6 functions. Please consult the full LabSpec6 manual for information of available functions and how they are used.

The figure below shows how to visualize a result according to your sample:



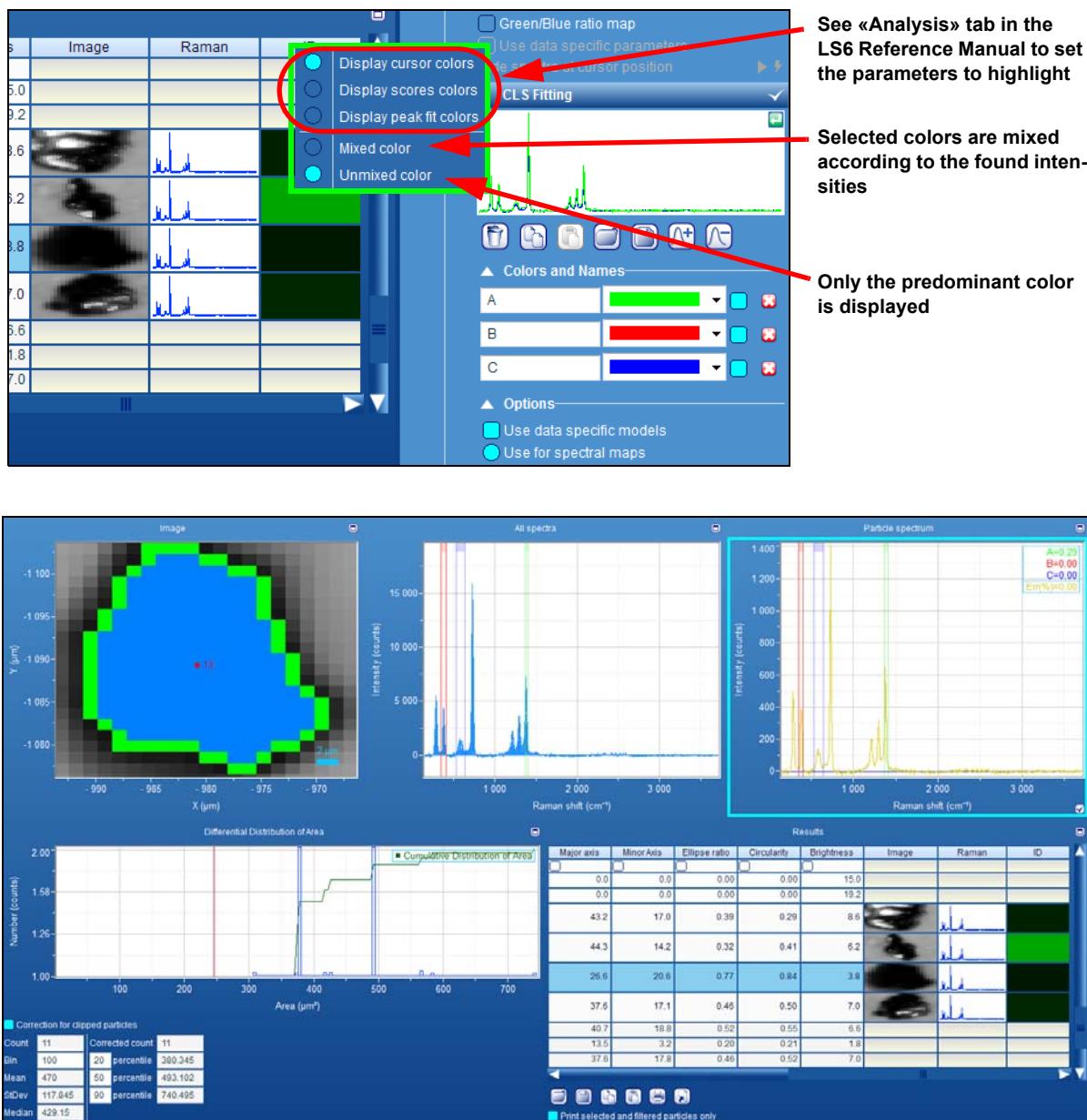
**IMPORTANT:** the Raman functionalities described in this manual concern the ParticleFinder Application. The use of this Application doesn't exclude all the standard features described in the LS6 Reference Manual.

For example, if you find an interesting particle, you can perform a traditional mapping on XYZ for a 3D Raman characterization of this particle.

## 2.7 Advanced Characterization

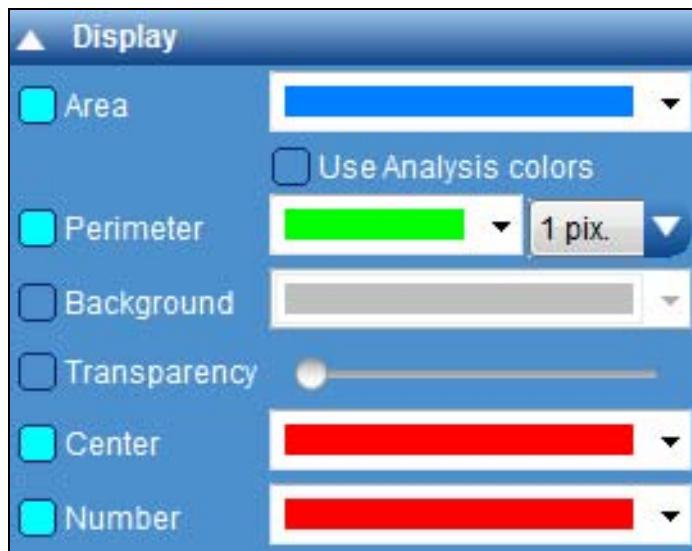
The ParticleFinder Application can use the powerful Map Characterization or CLS fitting features described in the LS6 Reference Manual.

The last column of the Results table is called ID. Right-click on the [ID] button to display a window with choices shown on the figure below. These choices allow to highlight, using colors, the particles with a specific spectral wavelength(s)



## 2.8 Display

The Display section allows control of the superimposed particle display on the video window. Superimposed particle display is only possible when one or both of the Thresholds are active. Note that in some cases settings in the Thresholds, Image Processing and Statistics sections may mean that no particles are located, and there will be no particle display visible.



### > Area

Turn on/off and select the color of the particle area fill. Turning on the area display will obscure the particles on the video. The transparency of the area display can be controlled with the Transparency slider.

### > Perimeter

Turn on/off and select the color and line thickness of the particle perimeter.

### > Background

Turn on/off and select the color of the background. The background area is any area which is not classified as a particle. Turning on the background display will obscure the non-particle region of the video. The transparency of the background display can be controlled with the Transparency slider.

### > Transparency

Turn on/off and select the degree of transparency for the display of particle Area, Perimeter and Background.

### > Center

Turn on/off and select the color of the particle center marker.

### > Number

Turn on/off the display of the numbers which are allocated for each detected particle.

## 2.9 Saving your data

### STEP 1

**VERY IMPORTANT NOTIFICATION:** before starting the standard Save action, it is very important to activate the [Use data specific options] button located in the Thresholds section of the ParticleFinder tab.

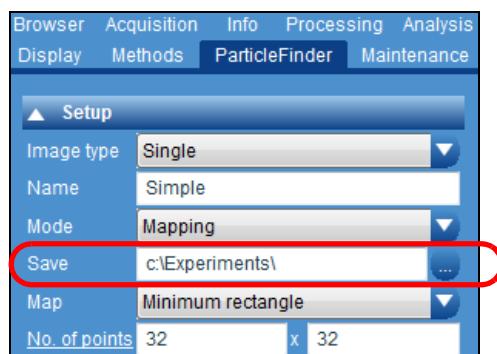
Moreover, this button **must be activated before a saving data action AND before you want to open an already saved ParticleFinder data.**

#### Why is it so important?

If the [Use data specific options] button is not activated, all the thresholds limits will not be saved and thus the particles numbering will then lost. That means also that the Raman measurements linked to the particles numbers and location will also be lost. So do not forget to verify that this box is always activated.

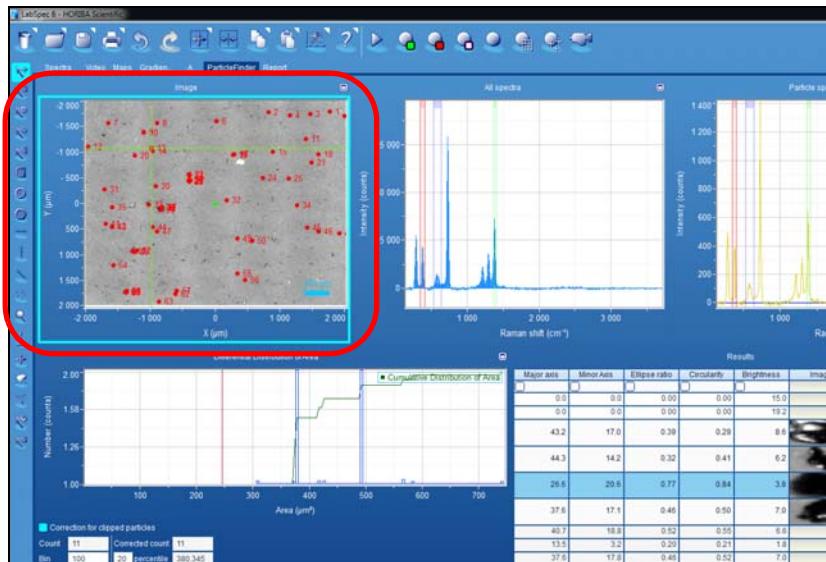
### STEP 2

Verify or change the location of the recipient folder for the spectra data which are individually saved with the particle number.



### STEP 3

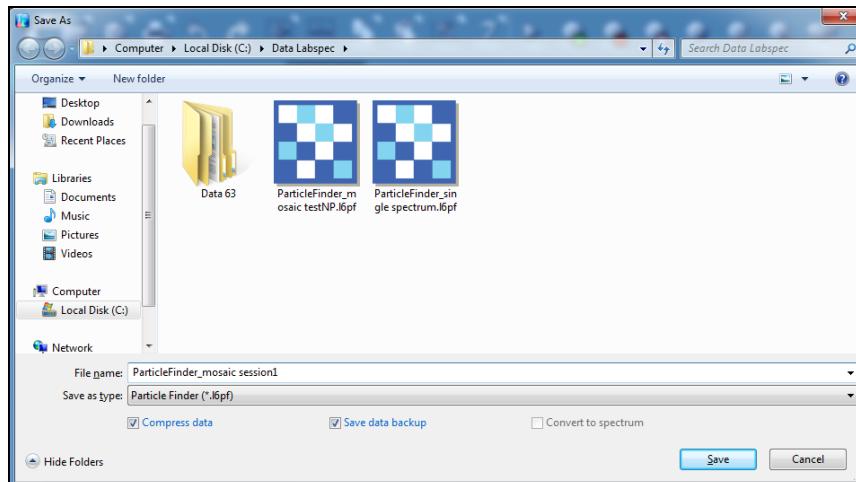
Click the Image part to select it as shown on the figure below:



### STEP 4

All your session can then be saved. All the acquisition data, Images, thumbnail images, particles numbering, thresholds, morphological filters, etc. will be saved. The ParticleFinder Application uses a new extension XXX.16pf.

Click on the standard Save icon located in the Icon bar. The following window will be opened:



Enter a name to your session then click on Save button to validate.