


NF PROFESSION V2 SOFTWARE GUIDE

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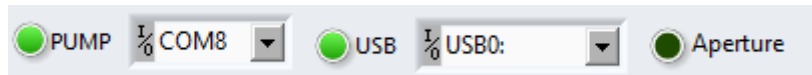
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1 LAUNCHING THE SOFTWARE

Double click the  **NF Profession V2** software shortcut on computer desktop.

To indicate that the NanoAnalyzer is correctly connected to the software:

1. Two beeps will sound from the NanoAnalyzer.
2. The green lights for PUMP and USB in the **Status Bar** (section 6) will be illuminated.



2 SOFTWARE SCREEN



1. **Navigation Bar** – Provides access to acquisition and analysis modes.
 - a. Upon launch, the software will default to acquisition mode.
2. **CCD Camera Window** – Real-time image of the flow cell, including the laser and sample injection capillary.
3. **Cytometer Controls** – Section 3. Allows control over data acquisition.
4. **Plot Area** – Section 4. Shows sample burst traces, histograms and dot plots.
5. **Status Bar** – Section 5. NanoAnalyzer connection and aperture status.
6. **Workspace Toolbar** – Section 6. Allows configuration of settings for data acquisition and analysis.
7. **Channel Information** – Section 7. Information for each detection channel.
8. **Gate Information** – Section 9. Information for each gated population.

3 CYTOMETER CONTROLS

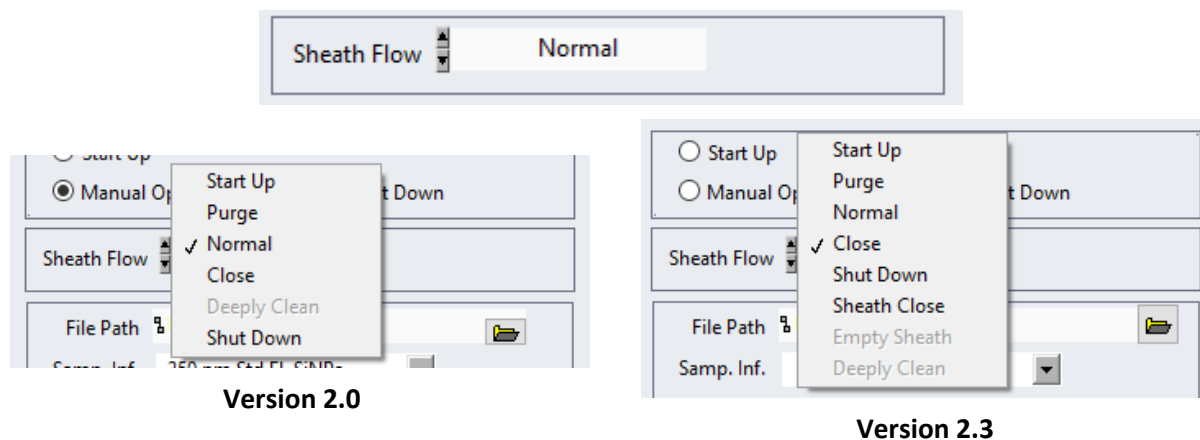
1. **NanoAnalyzer Power Control** – Section 3.1.
2. **Sheath Flow Control** – Section 3.2.
3. **Sample Information and Settings** – Section 3.3.
4. **Acquisition Control** – Section 3.4.
5. **Laser Control** – Section 3.5.
6. **Detector Settings** – Section 3.6.
7. **Sample Flow Control** – Section 3.7.

3.1 NanoAnalyzer Power Control

Three different modes are accessible through the NF Profession V2 software.

1. **Start Up** – Launches system, CCD camera, lasers and air pump.
2. **Manual Operation** – Used for data acquisition.
3. **Shut Down** – Turns off lasers and detectors to allow software and instrument shut down.

3.2 Sheath Flow Control



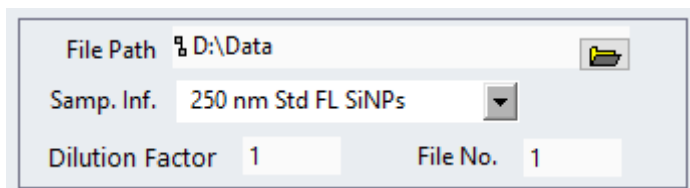
Note: in NF Profession, select the text to open the drop-down menu. Selecting the arrows will scroll through the menu.

Drop-down menu of Sheath Flow modes:

1. **Start Up** – Initializes the fluidics system, used in daily start-up procedure.
 - Once selected, procedure takes roughly 4 minutes, and cannot be cancelled.
2. **Purge** – Flushes the fluidics system with sheath fluid.
 - Once selected, procedure takes roughly 3 minutes, and cannot be cancelled.
3. **Normal** – Indicates the fluidics system is primed for sample stream focussing. Used for sample acquisition.
4. **Close** – Indicates the fluidics system is closed.
5. **Shut Down** – Deep cleaning of the fluidics system, used in daily shut-down procedure.
 - Once selected, procedure takes 4-8 minutes, and cannot be cancelled.
6. **Sheath Close** – Indicates that the sheath flow is closed, but not the waste flow.

When a Start Up, Purge, or Shut Down procedure is initiated, a timer will appear on the right-hand side of the box showing how many seconds it has left to run.

3.3 Sample Information and Settings



File Path D:\Data

Samp. Inf. 250 nm Std FL SiNPs

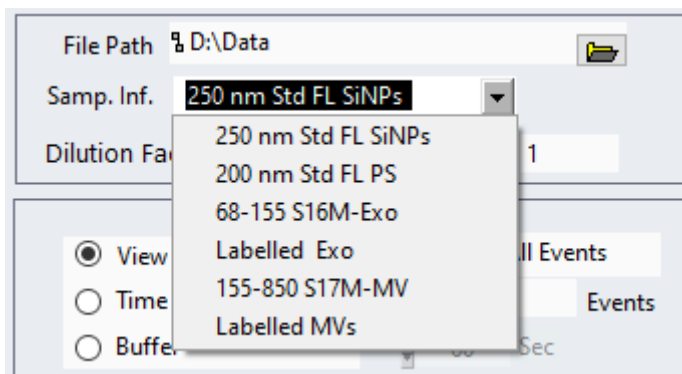
Dilution Factor 1 File No. 1

File path: A new folder named with today's date is automatically generated in the file path shown above.

- The default file path is D:\Data.
- All data and CCD images from today will be saved in this folder.

Samp. Inf.: Drop down list to load recommended laser and detector settings.

- To open this drop down, select the arrow.
- By selecting the text and typing in the box, the recording can be assigned a name.
 - This will not change the laser or detector settings.
 - This name will automatically be used when saving the file.



File Path D:\Data

Samp. Inf. 250 nm Std FL SiNPs

Dilution Factor 1 File No. 1

View

Time

Buffer

All Events

Events

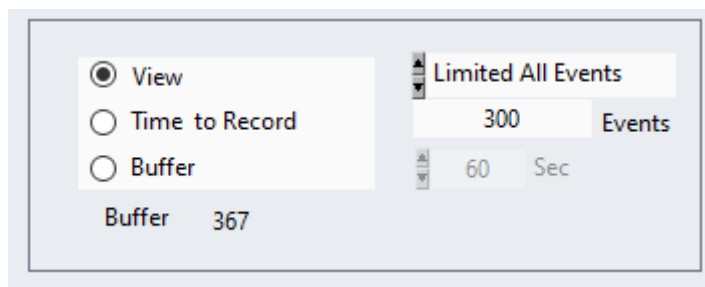
Sec

Dilution factor: The dilution factor applied to the sample before analysis. This is factored into concentration calculations. E.g. for a 1:100 dilution, enter 100.

File No.: Defined by the software at start-up. File number begins at "1" and increments with each recording.

- The File No. can be manually altered if necessary.

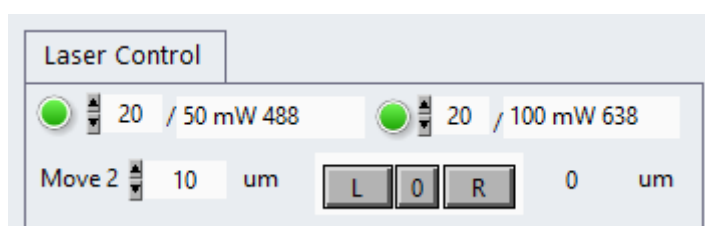
3.4 Acquisition Control



There are three modes within the acquisition control:

1. **View** – Aperture open. View particles passing the laser in real time.
 - a. By default, View shows the most recent 300 particles recorded in the dot plot and histograms.
 - b. To change this, change the number to the right of the selection panel.
2. **Time to Record** – Aperture open. Records the particles passing the laser in a defined time frame.
 - a. The default is 60 seconds (120 x 500 ms), this can be changed using the box to the right.
3. **Buffer** – Aperture closed. Holds the last recording in the plot area, allowing it to be saved in a different location or format.
 - a. Moving to the analysis tab, or selecting View will remove the recording from memory, so ensure that the recording is saved before leaving this mode.

3.5 Laser Control



The green button next to each laser indicates whether the laser is on or off.

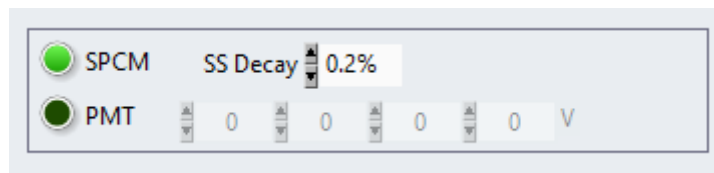
The value for blue (488 nm) laser power is set by the **Samp. Inf.** setting.

The red (638 nm) laser should be set to 20 mW.

The focus of the laser can be adjusted by changing the horizontal position of the laser focusing lens, left [L button] or right [R button]. The 0 button sets the current position of the focusing lens to 0 μm .

- The default step distance is set at 10 μm .
- Refer to the NanoAnalyzer User Guide section on alignment for more information on laser focus adjustment.

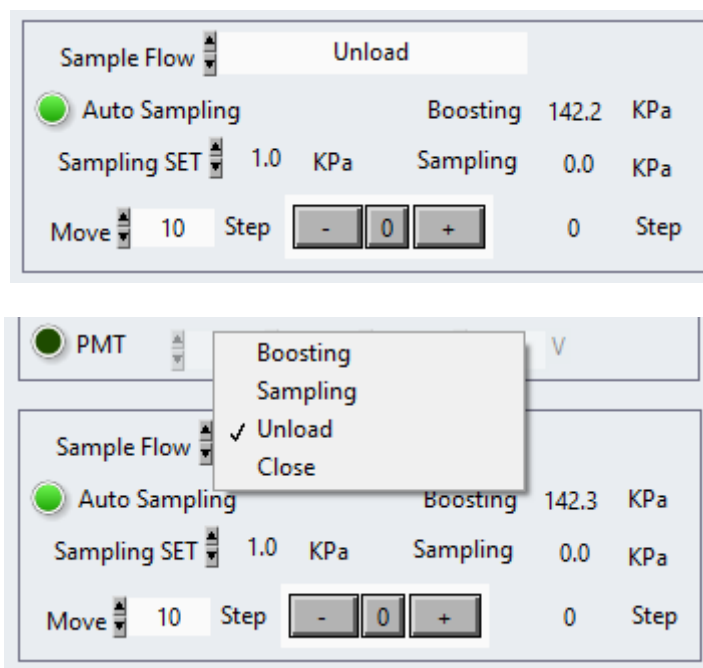
3.6 Detector Settings



Once Manual Operation mode has been selected, the detector settings allow modulation of detector parameters:

- The SPCM detectors can be turned on (indicated by the illuminated green button)
 - This is automatic when switching to Manual Operation in later versions.
- The SS Decay is set by the **Samp. Inf.** settings, but can be changed manually to 0.2%, 2%, 10%, or 100%.
 - The SS Decay is an attenuation factor, to avoid detector saturation.
- PMT voltage control is redundant for newer NanoAnalyzer models, as PMTs are not installed.

3.7 Sample Flow Control



Note: in NF Profession, select the text to open the drop-down menu. Selecting the arrows will scroll through the menu.

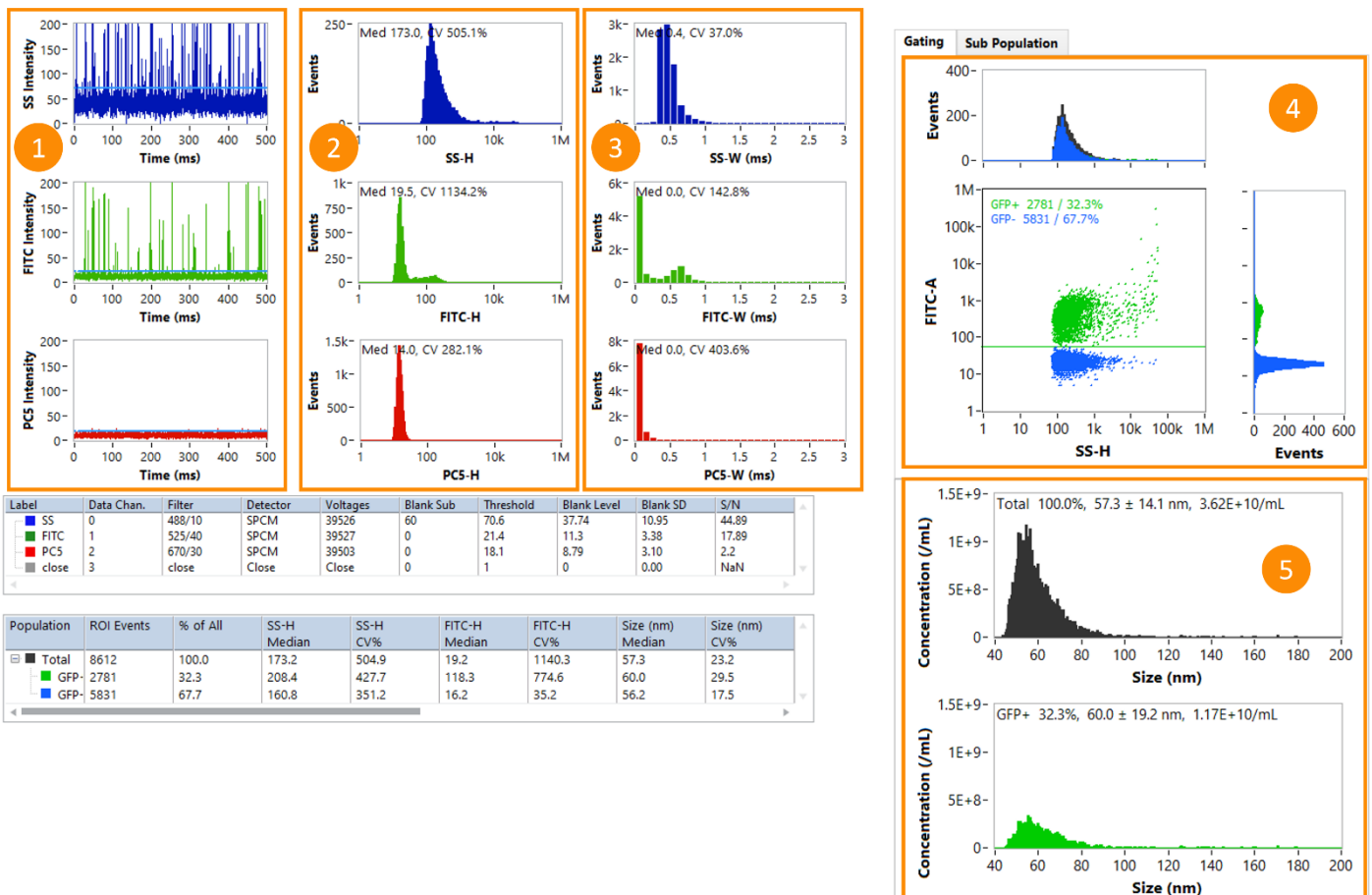
Sample Flow modes:

1. **Boosting** – Loads the sample tube and injects the sample into the flow cell at high pressure.
2. **Sampling** – Injects the sample into the flow cell at low pressure. Used for sample acquisition.
3. **Unload** – Unloads the sample tube.
4. **Close** – Closes the air pump and keeps the loading bay in its current position.

Auto Sampling should be switched on (green button illuminated) for sample analysis, with the sampling pressure set to 1.0 kPa.

- Sampling pressure can be set to the right of **Sampling SET**.
 - This should always be between 0.3 and 1.5 kPa, 1.0kPa recommended.
- The with **Auto Sampling** switched on, **Step** count is automatically adjusted by the system to maintain correct pressure.
- The pressure beside **Boosting** and **Sampling** show the current pressure achieved.

4 PLOT AREA

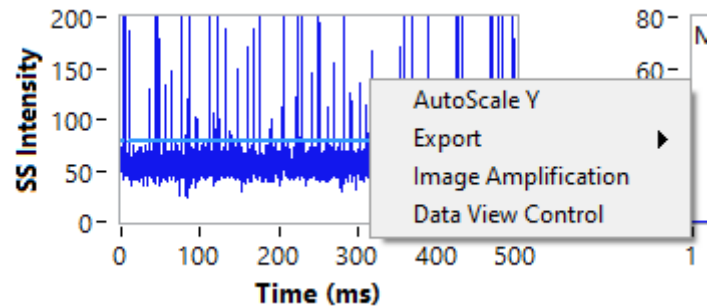


- Event Burst Trace Settings** – Section 4.1. Real-time burst traces for each detection channel as particles pass through the laser beam.
- Peak Height/Area Histograms** – Section 4.2. Accumulated histogram of peak height/area for each detection channel.
 - To switch between height and area units, use the **Histogram axis units** button.
- Peak Width Histograms** – Section 4.2. Accumulated histogram of peak width for each detection channel.
- Dot Plot** – Section 4.3. Bivariate dot plot of two selected channels, including histograms for both channels.
- Size Distributions** – Section 4.4. Size distribution histograms of total population and each gated population.

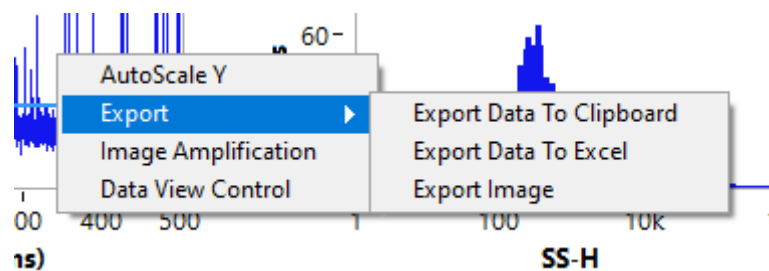
4.1 Event Burst Trace Settings

The event burst trace displays a dynamic discrete 500ms window of live data during acquisition or a static 500ms window of record data in analysis mode.

Right clicking an event burst trace brings up a list of options.

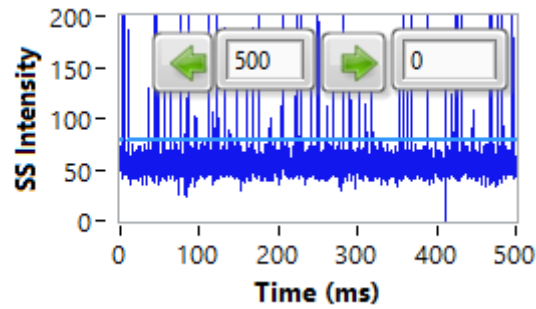


- **AutoScale Y** – Automatically set the upper limit of the y-axis to match the data.
 - This is not recommended, as often the automatic scale will not allow for clear visualisation of the baseline.
- **Export** – Allows the event burst trace recordings to be exported as a data file or image.
 - **Export Data to Clipboard** copies the data to the PC clipboard.
 - **Export Data to Excel** creates an Excel spreadsheet containing the data.
 - **Export Image** saves an image of the event burst trace.



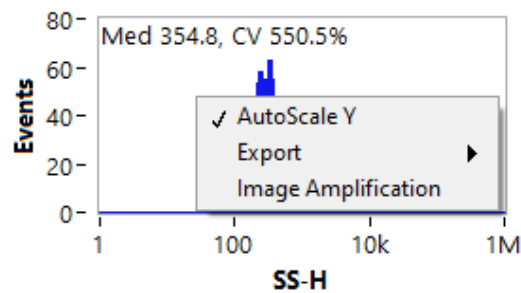
- **Image Amplification** – Opens the image amplification window, allowing for export of high-resolution images of the event burst trace.
 - See Section 9 [Image Amplification Window](#).
- **Data View Control** – Brings up controls for moving through the event burst trace.
 - Click the right and left arrows to move through the recorded event burst trace.
 - The left box (500) is the increment by which the arrows move the event burst trace.

- The right box (default 0) is the time (in ms) at the start of the current 500 ms window.



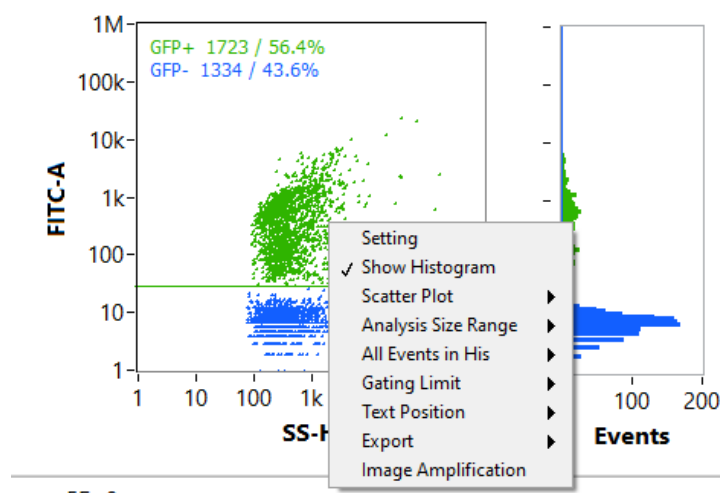
4.2 Peak Histogram Settings

Right clicking any of the peak histograms (either height/area or width) brings up a list of options. These are the same as the event burst trace options, minus the Data View Control.

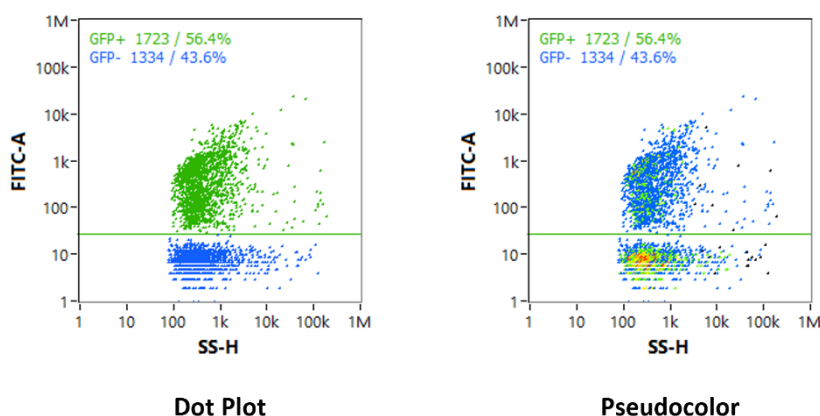


4.3 Dot Plot Settings

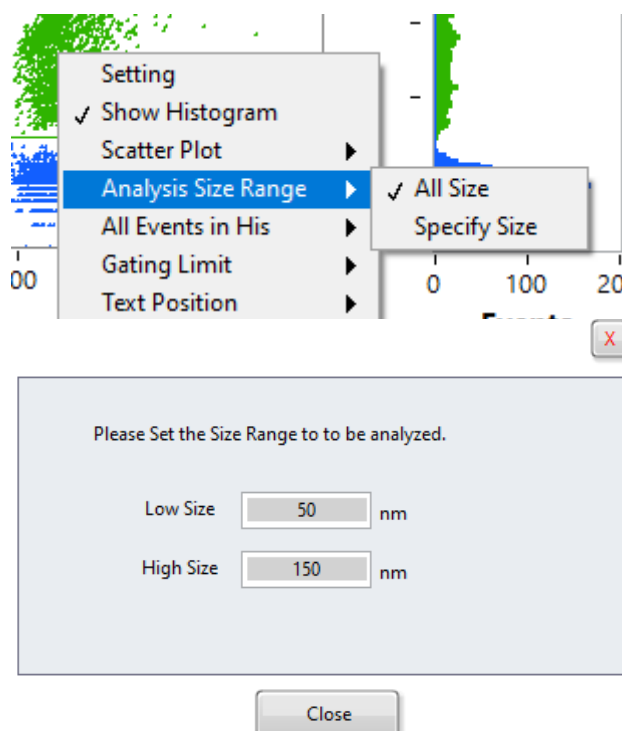
Right clicking the dot plot brings up a list of options.



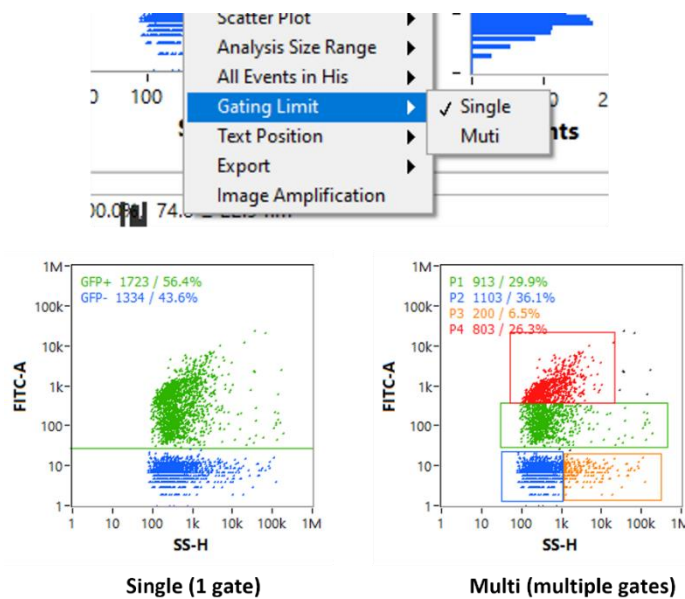
- **Setting** – Brings up a settings panel to change the display settings of the dot plot.
 - If wanting to export an image of the dot plot, image amplification window (see Section 9 Image Amplification Window) gives more control over dot plot display settings and allows for higher resolution images.
- **Show Histogram** – Toggle to show or hide the histograms surrounding the dot plot.
- **Scatter Plot** – Change the display mode of the dot plot.
 - **Dot plot** – Particles shown as dots, coloured by population.
 - **Pseudocolour** – Particles shown as dots, coloured by density.



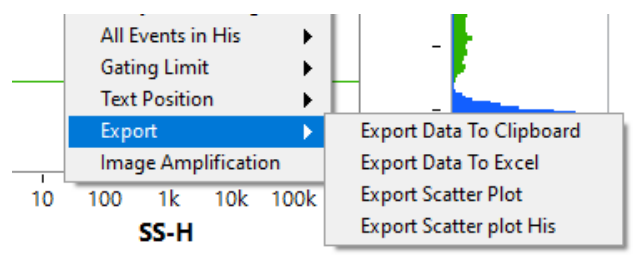
- **Analysis Size Range** – Set the size range of particles to be included on the dot plot.
 - **All size** – Plot all particles on the dot plot.
 - **Specify size** – Input lower and upper bounds on size to be displayed on the dot plot.



- **All Events in His** – Choose whether to show or hide the total population within the histograms surrounding the dot plot.
- **Gating limit** – Set the limit for number of gates applied to the dot plot.
 - **Single** – Only one gate can be set.
 - **Multi** – Multiple gates can be set. To remove gates set in Multi mode, change the gating limit back to single.
 - Note: On some software versions, this is simply a toggle option labelled 'Multi Gating'.



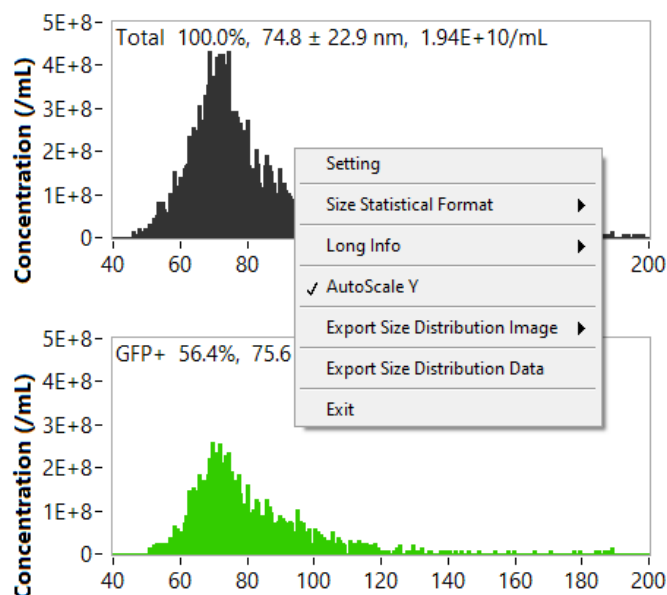
- **Text Position** – Changes the position of the population name texts within the dot plot.
- **Export** – Allows the event burst trace recordings to be exported as a CSV file or image.
 - **Export Data to Clipboard** copies the dot plot data to the PC clipboard.
 - **Export Data to Excel** creates an Excel spreadsheet containing the dot plot data.
 - **Export Scatter Plot** saves an image of the dot plot.
 - **Export Scatter Plot His** saves an image of the dot plot and surrounding histograms.



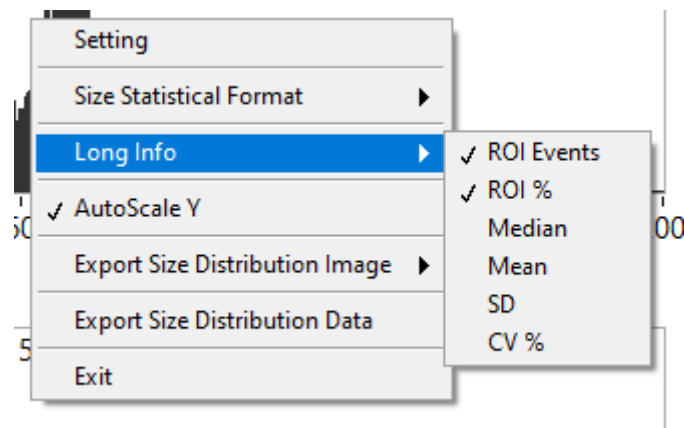
- **Image Amplification** – Opens the image amplification window (see Section 5 [Image Amplification Window](#)).

4.4 Size Distributions Settings

Right clicking the dot plot brings up a list of options. Some are the same as the event burst trace or dot plot options, while some are different.

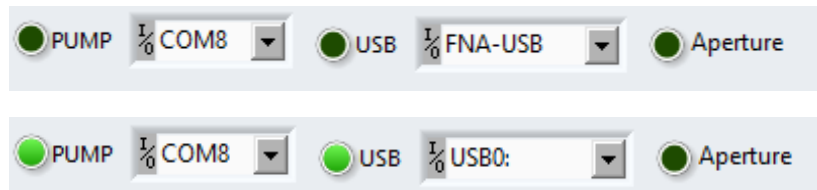


- **Setting** – Brings up a settings panel to change the display settings of the size distributions.
 - The image amplification window (see Section 9 [Image Amplification Window](#)) gives more control over size distribution display settings.
- **Size Statistical Format** – Choose statistical information to be shown above the distributions.
 - **Short** – Displays name, percent of total, mean ± SD, and concentration.
 - **Long** – Displays custom statistics.
- **Long Info** – Choose the statistical information to display when Long is selected as the Size Statistical Format.
 - **ROI events** – Number of events within the population.
 - **ROI%** – % of total events within the population.
 - **Median, mean, SD** – Median, mean, SD size, respectively.
 - **CV%** – CV (coefficient of variance) of size.



- **AutoScale Y** – Automatically set the upper limit of the y-axis to match the data.
- **Export Size Distribution Image** – Exports an image of the chosen size distribution.
 - This can be better achieved using the image amplification window (see Section 9 [Image Amplification Window](#)).
- **Export Size Distribution Data** – Exports data from the size distributions.

5 STATUS BAR



The green lights for PUMP and USB should be illuminated once the NF Profession 2.0 software has been launched to show correct connection between the air pump, cytometer, and PC.

Ensure that the green light for Aperture is not on whilst Boosting samples.

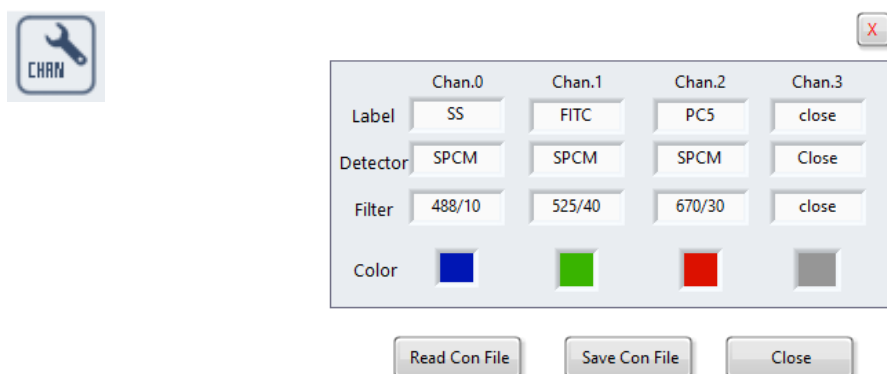
- The aperture is used to protect the detectors from strong signals and boosting may result in detector saturation if opened.
- If switching the filter for fluorescent channel 2, ensure that the aperture is closed (light off) first.

6 WORKSPACE TOOLBAR



- | | |
|--|--|
| <ol style="list-style-type: none"> 1. Detector Configuration – Section 7.1. 2. Baseline Adjustment – Section 7.2. 3. Threshold Adjustment – Section 7.3. 4. Data Display Settings – Section 7.4. 5. Histogram Gating – Section 7.5. 6. Histogram Axis Units – Section 7.6. 7. Trigger Channel Selection – Section 7.7. 8. Save Data – Section 7.8. | <ol style="list-style-type: none"> 9. CCD Camera Power Control – Section 7.9. 10. CCD Camera Settings – Section 7.10. 11. Save CCD Camera Image – Section 7.11. 12. Gating Options – Section 7.12. 13. Set Concentration Standard – Section 7.13. 14. Set Size Standard – Section 7.14. 15. Set Blank Control – Section 7.15. 16. Analysis Report (PDF Generation) – Section 7.16. |
|--|--|

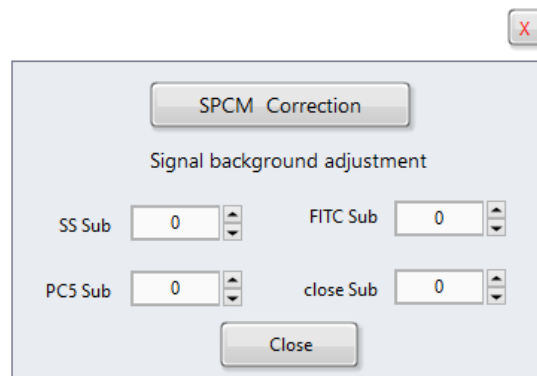
6.1 Detector Configuration



Select Detector Configuration to view and change detector settings.

- Click a Channel's Label, Filter, or Color to see a dropdown list of options.
- Changing a Label will automatically load a corresponding Filter and Color.
- Ensure the bandpass filters in the Detector Configuration match the bandpass filters in the NanoAnalyzer.

6.2 Baseline Adjustment (Signal Processing Control)

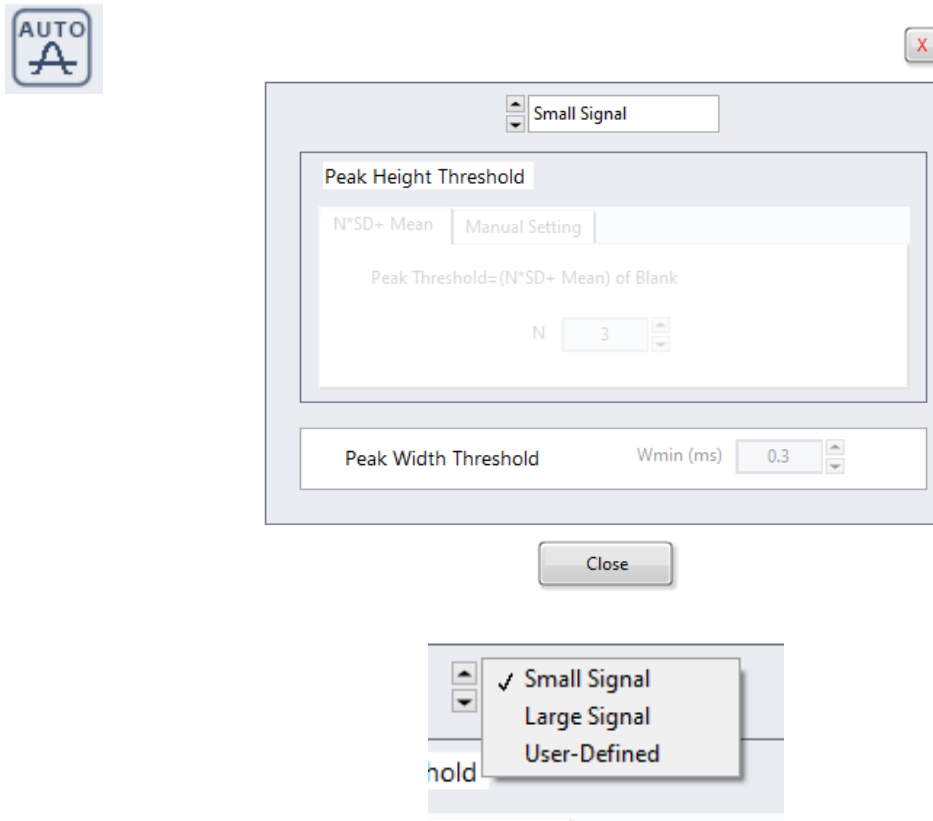


SPCM Correction is applied automatically in data analysis mode.

- Yellow = correction on; grey = correction off.
- Removing SPCM correction allows visualisation of the events as they were collected.
 - SPCM correction is applied to data using a dead-time-correction curve specific to each detector.

Signal background adjustment can be applied to reduce an elevated baseline in one of the detection channels (refer to the Data Analysis Guide for more information).

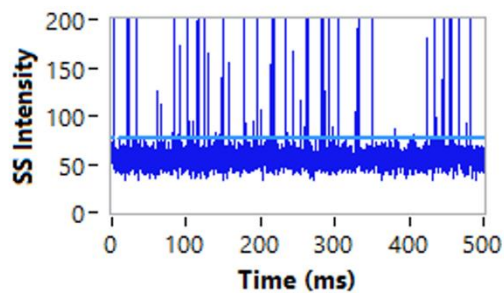
6.3 Threshold Adjustment



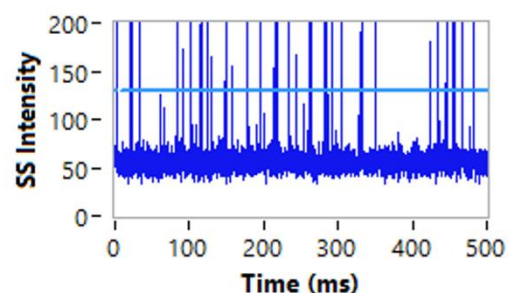
The threshold adjustment pop-up is used to set the thresholds of the detection channels. The threshold is the intensity value an event must exceed to be distinguished from the baseline.

The top dropdown box contains three settings:

- **Small Signal** – Automatically sets the threshold at mean + 3 St.Dev. of the baseline.
- **Large Signal** – Automatically sets the threshold at mean + 10 St.Dev. of the baseline.
- **User Defined** – Allows the user to manually set the thresholds, or choose a number of standard deviations above the mean.

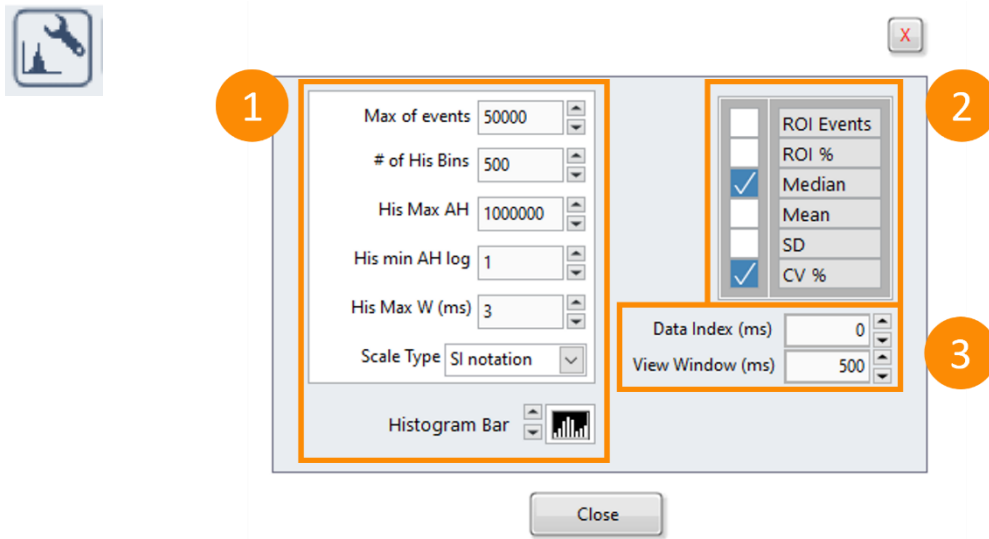


Small Signal



Large Signal

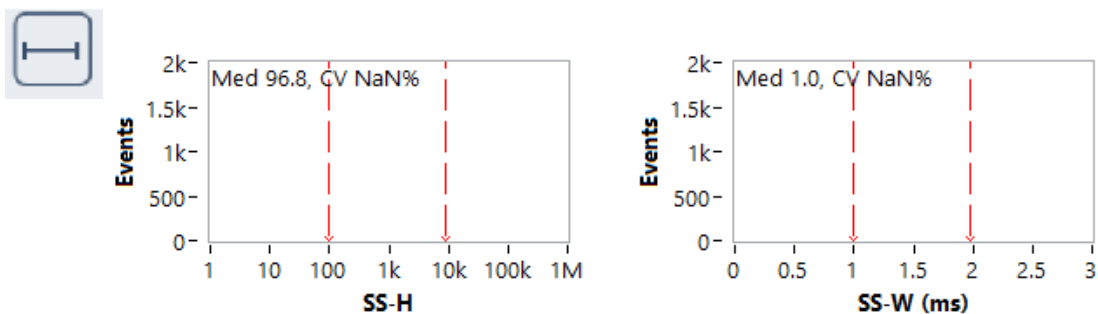
6.4 Data Display Settings



The data display settings are used to change the parameters of the event burst trace and peak histograms.

- Area 1 controls the display parameters of the histograms.
- Area 2 allows selection of statistics to be displayed on the histograms.
- Area 3 controls the width and position of the event burst trace within a recording.
 - **Data Index (ms)** – The start position of the event burst trace.
 - **View Window (ms)** – The width of the event burst trace.

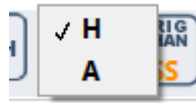
6.5 Histogram Gating



Brings up dashed red lines on the peak histograms to gate populations.

- Statistical values on the histogram include only the range within the red lines.

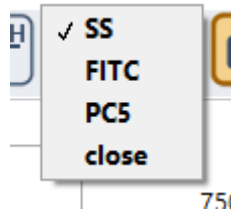
6.6 Histogram Axis Units



The x-axis of the plot area histograms can be set to height (H) or area (A). For accurate sizing, do not change this once the size standard has been set.

- H = signal height.
- A = signal area.

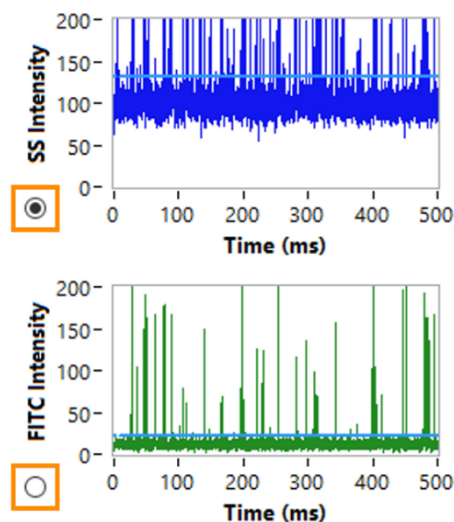
6.7 Trigger Channel Selection



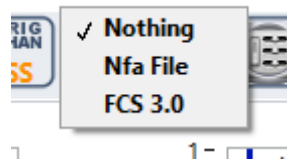
For an event to be recorded by the software, it must exceed threshold in the trigger channel.

- SS, FITC, or PC5 can be selected as the trigger channel.
 - These names change if the channel name is changed.
- The default trigger channel is side scatter (SS).

In newer versions of the software, this button is absent, replaced by selection buttons next to the event burst traces in the plot area.



6.8 Save Data



After each acquisition, the user is automatically prompted to save as an NFA file.

- **NFA File** – save a NFA file after changing sample information or re-save to another location.
- **FCS 3.0** – save the data as an FCS file (for analysis in other software). Only data surpassing the threshold is saved as an FCS, setting the threshold appropriately is therefore essential.

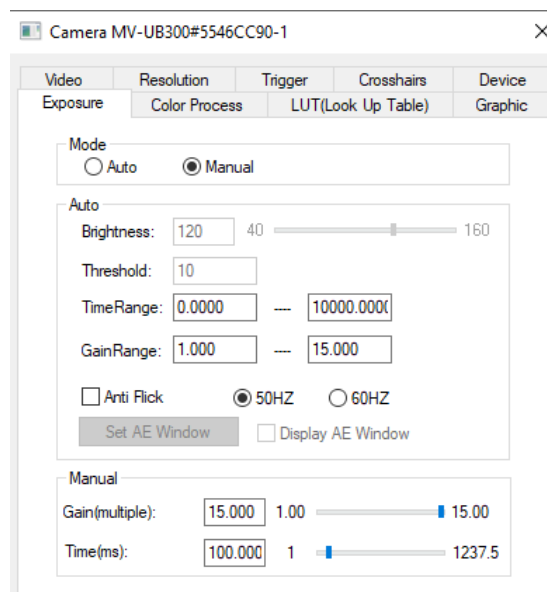
6.9 CCD Camera Power Control



Power control for the CCD camera.

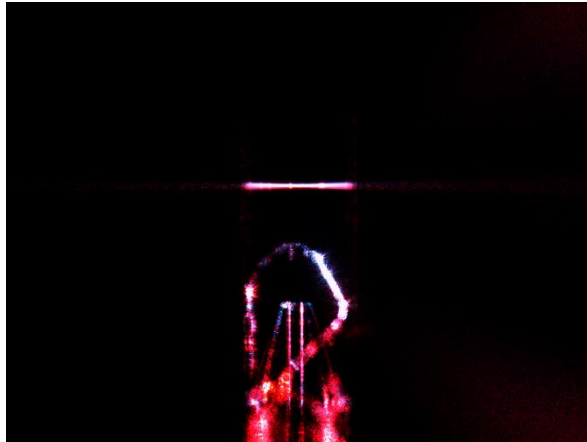
- CCD camera is on by default at system startup.

6.10 CCD Camera Settings



Allows the user to change the settings of the CCD camera (not recommended).

6.11 Save CCD Camera Image



Select to save the current frame of the CCD camera as an image in today's data folder.

- The image can give information about the instrument's status (e.g. the image above shows a fibre in the flow cell, which must be removed before further sample acquisition).
 - Refer to the Cleaning Guide for more information.

6.12 Gating Options



Various options for gating sample populations and sub-populations on the dot plot:

- **Line** – Separate two populations.
- **Cross** – Separate four populations.
- **Square** – Isolate one population.
- **Circle** – Isolate one population.
- **Polygon** – Isolate one population.

By default, only one gate can be set at a time, but this can be changed (see Section 4.3 [Dot Plot Settings](#)).

6.13 Set Concentration Standard

Please Check the Concentration and the Event of Standard beads.

STD Concentration Particles/mL

Dilution Factor

Particle Events

Note: The counting result deviations for standard sample obtained at different times do not exceed 15%.

Close

Enter information on the QC concentration standards to calculate the concentration of other samples.

- Refer to section 3.4 Setting the Concentration and Size Standards in the NanoAnalyzer User Guide for more information.

6.14 Set Size Standard

UNIT Convert Sizing Standard S16M-Exo Fit Type Power

CV=18.0% 21.3% 19.1% 16.3%

Events

SS-H

Find peak

Peak out

355.93	134.82
1196.17	110.39
4816.06	130.12
23495.47	126.87

Width Min 10 Threshold 15.1

Size (nm) SS-H

68	355.928
91	1196.17
113	4816.06
155	23495.5

H-SS

Size (nm)

$y = 1.88399E-7 * x^{5.0628} + 0$
R-square = 0.9992

The data Used here is SS-H or SS-A Histogram £-Please make sure threshold level is correct.

Close

Set either the S16M-Exo or S17M-MV size standards to calculate the size of particles in other samples.

- Refer to section 3.4 Setting the Concentration and Size Standards in the NanoAnalyzer User Guide for more information.

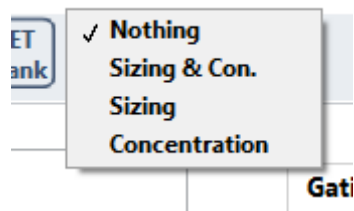
6.15 Set Blank Control



Allows particles in the buffer blank to be subtracted from sample measurements.

- Refer to Section 6.6 Data Analysis Summary in the NanoAnalyzer User Guide for more information.

6.16 Analysis Report (PDF Generation)



PDFs detailing particle size, concentration, or both, can be generated.

- Refer to Section 6.4 Exporting Data in the NanoAnalyzer User Guide for more information.




7 CHANNEL INFORMATION

1	2	3	4	5	6	7	8	9	10
Label	Data Chan.	Filter	Detector	Voltages	Blank Sub	Threshold	Blank Level	Blank SD	S/N
■ SS	0	488/10	SPCM	39526	0	126.7	95.62	10.37	50
■ FITC	1	525/40	SPCM	39527	0	27.1	15.47	3.89	50.81
■ PC5	2	670/30	SPCM	39503	0	18.8	9.16	3.22	2.78
■ close	3	close	Close	Close	0	1	0	0.00	NaN

Information on the instrument parameters for each channel. In each column, left to right:

1. **Label** – Channel name and colour.
 - a. To change: right click table > Label & Color. Change switch from RAW to customer, then enter names and colours to the right.
2. **Data Chan.** – Channel number.
3. **Filter** – Installed bandpass filter.
4. **Detector** – Detector type (SPCM = single photon counting module).
5. **Voltages** – Detector voltages. Cannot be changed.
6. **Blank Sub** – Event burst trace subtraction (see Section 6.2 [Baseline Adjustment](#)).
7. **Threshold** – See Section 6.3 [Threshold Adjustment](#).
8. **Blank level** – Mean intensity of background.
9. **Blank SD** – SD of background intensity.
10. **S/N** – Signal to noise ratio. Calculated from blank level and blank SD.

8 GATE INFORMATION

	1	2	3	4	5	6	7	8	9
Population	ROI Events	% of All	SS-H Median	SS-H CV%	FITC-H Median	FITC-H CV%	Size (nm) Median	Size (nm) CV%	
 Total	5953	100.0	310.1	330.4	36.6	134.3	68.8	13.7	
 GFP	4193	70.4	304.3	351.2	44.9	130.8	68.6	13.8	
 GFP	1760	29.6	326.0	265.2	23.3	25.2	69.5	13.2	

Information on the whole sample and gated populations within the sample (see Section 7.12 [Gating Options](#)). In each column, left to right:

1. **Population** – Population name and colour.
 - a. To change: right click table > Name & Color. Enter names and colours in the pop-up to the right of the table.
 - b. To reset names: right click the table > Initialisation Name.
 - c. To reset colours: right click the table > Initialisation Color.
2. **ROI Events** – Number of events in each region of interest (population).
3. **% of All** – % of total particles in each population.

In columns 4-9..., statistical data about each population is displayed:

- To change the displayed channels (below, left), right click the table > Channel. Select the channels whose statistics will be displayed.
- To change the statistics displayed (below, right), right click the table > Statistical Info. Select the statistics which will be displayed for each channel.

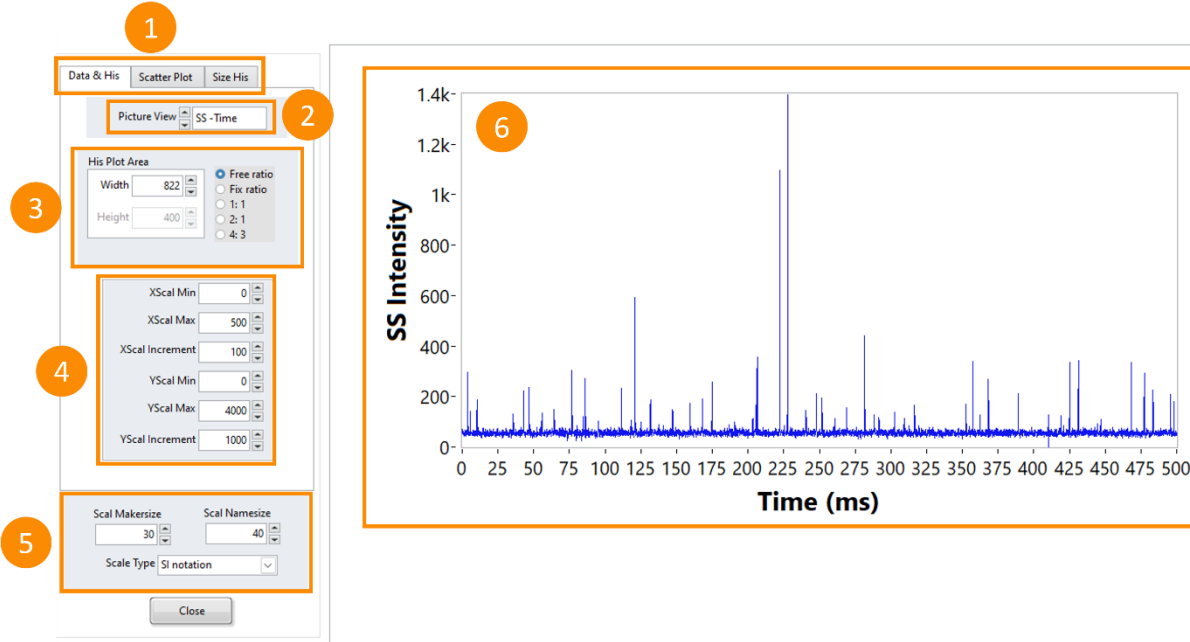
9 IMAGE AMPLIFICATION WINDOW

The image amplification window is used to export high resolution images of the event burst traces, peak histograms, dot plots, and gated size distributions. It is accessed by right clicking the event burst trace, peak histogram, or dot plot and selecting Image Amplification.

The navigation bar (top left) consists of three options:

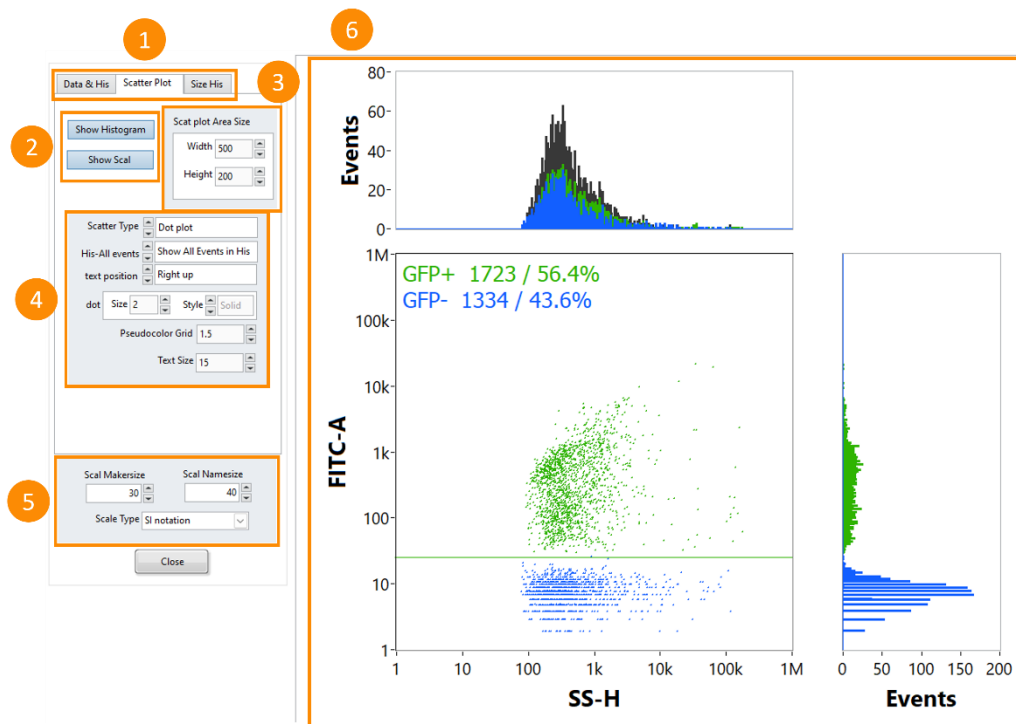
- **Data & His** – Section 9.1. High resolution images of the event burst trace or peak histograms.
- **Scatter Plot** – Section 9.2. High resolution image of the dot plot.
- **Size His** – Section 9.3. High resolution image of the size distributions.

9.1 Data & His



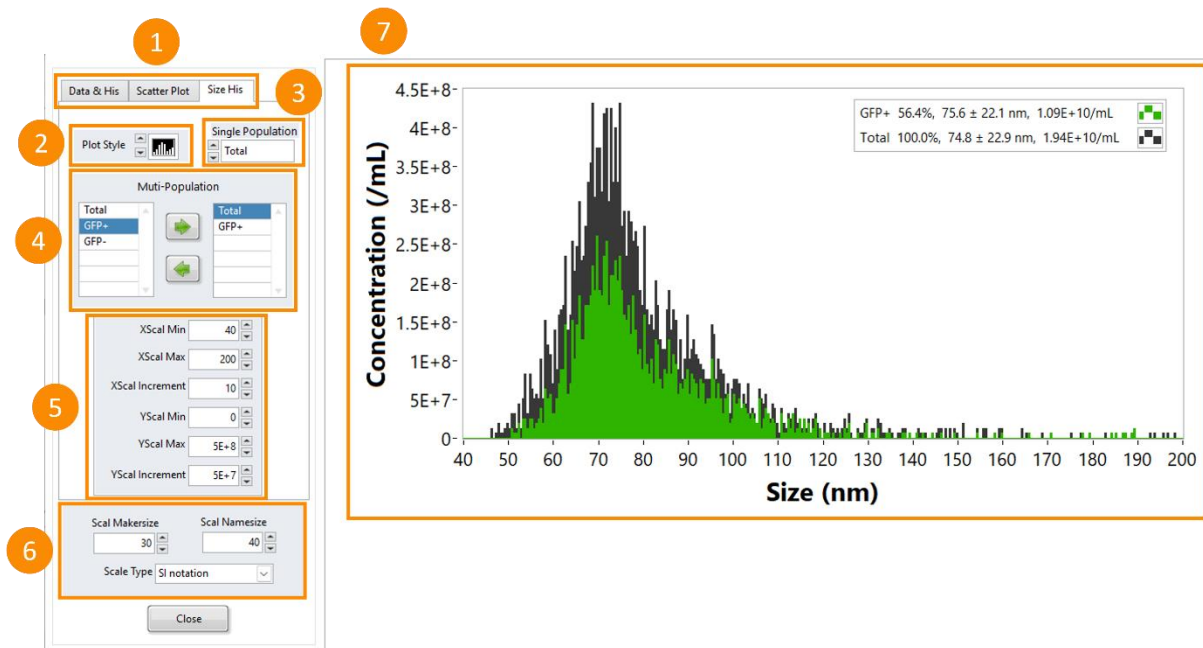
1. **Navigation Bar.**
2. **Picture View** – Select image source (event burst trace or peak histogram).
3. **His Plot Area** – Change the size and aspect ratio of the image.
4. **Axis Settings** – Change the range and increment of the axes.
5. **Label Settings** – Change the size and notation of axis labels.
6. **Image Preview** – Preview of the high resolution image. Right click > Export Image to save.

9.2 Scatter Plot



1. **Navigation Bar.**
2. **Histogram Settings** – Toggles to show/hide the histograms and their scales.
3. **Scat Plot Area Size** – Change the size and aspect ratio of the image.
4. **Dot Plot Settings** – Change the display settings of the dot plot.
 - a. **Scatter Type** – Dot plot or pseudocolor.
 - b. **His-All Events** – Show/hide the total population in the histograms.
 - c. **Text Position** – Change the position of the population text within the dot plot.
 - d. **Dot** – Change the size and shape of dots.
 - e. **Pseudocolor Grid** – Change the sensitivity of the pseudocolor density colouring.
 - f. **Text size** – Change the size of the population text within the dot plot.
5. **Label Settings** – Change the size and notation of axis labels.
6. **Image Preview** – Preview of the high resolution image. Right click > Export Image to save.

9.3 Size His



1. **Navigation Bar.**
2. **Plot Style** – Choose from multiple bar chart or line chart styles.
3. **Single Population Selection** – Select a single population to display its size distribution.
4. **Multiple Population Selection** – Using the green arrows, add (top) or remove (bottom) populations from the image. The left list is all populations, while the right list is populations shown in the image.
5. **Axis Settings** – Change the range and increment of the axes.
6. **Label Settings** – Change the size and notation of axis labels.
7. **Image Preview** – Preview of the high resolution image. Right click > Export Image to save.

SS-H	FITC-H	FITC-H	Size (nm)	FITC-H	FITC-H	Size (nm)
CV%	Median	CV%	Median	Median	CV%	Median
266.4				998.9	421.9	76.4
252.0						79.3
551.0						

Channel	Statistical Info	Name & Color	Initialization Name	Initialization Color	Exit
SS-A					
FITC-A					
PC5-A					
close-A					
SS-H	✓				
FITC-H	✓				
PC5-H					
close-H					
Size (nm)	✓				

Channel	Statistical Info	Name & Color	Initialization Name	Initialization Color	Exit
Median	✓				
Mean					
CV%	✓				
SD					