

## DATA ANALYSIS

### OVERVIEW

#### SIZE DISTRIBUTION ANALYSIS

The size distribution analysis is performed on the assumption that the refractive index of Si is similar to that of biological particles (e.g., extracellular vesicles, mature viruses).

**Silica Nanosphere Cocktails (S16M-Exo & S17M-MV)** is used as the size standards to create a calibration curve of the particle size and side scattering intensity. Using the calibration curve, the side scattering intensity of every single particle within a sample can be converted into the corresponding particle size.

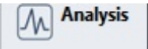
**S16M-Exo:** Range 40-200nm; 4 peaks at 68, 91, 113, 155nm.

**S17M-MV:** Range 150-1000nm; 4 peaks at 155, 298, 535, 850nm.

#### CONCENTRATION ANALYSIS

**0.25µm Fluorescent Silica Microspheres (250nm SiNPs)** of a known concentration are used to quantify the particles of interest within a sample.

### ACCESSING SAVED DATA


Click  and select the folder to import sample files (**NFA Files** only).

- A new folder (named by date) is automatically created in D:\Data (default **File Path**) for each saved sample.
- All saved data and camera images of the day will be saved in this folder.

### SETTING UP STANDARDS



#### CONCENTRATION STANDARD

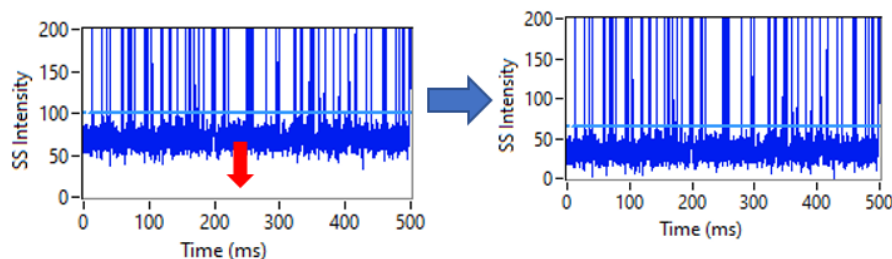
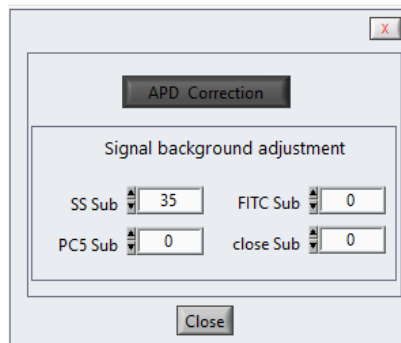
1. Select **recording of QC beads** and select  to automatically set the threshold.


2. Select  to apply standard to all acquired samples.
  - a. If needed, the threshold bar can be moved manually to just above the baseline.

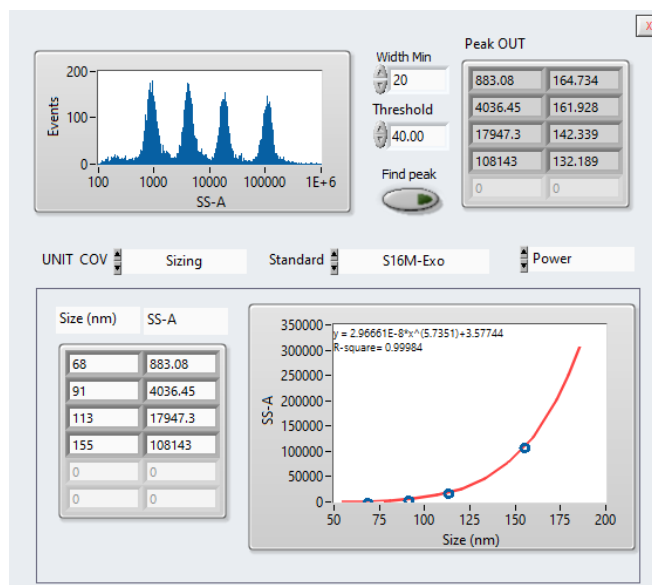
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#### SIZE REFERENCE STANDARD (S16M-EXO)



1. Select **S16M-Exo file** and select  to automatically set the threshold.
2. Select  to open **Signal Processing Control** (as shown below) to manually alter the baseline on the burst trace to reduce background signals for each channel (i.e., side scatter, fluorescence) if required.
  - a. For example, set the side scatter channel to 35 when the blank level is ~70.

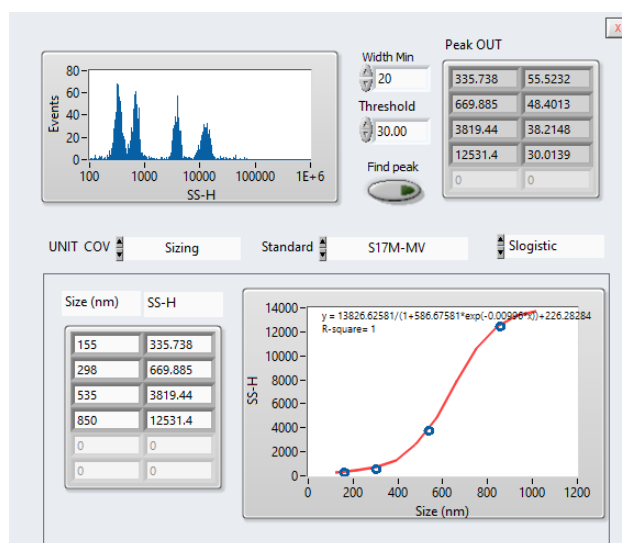


3. Select  to open the calibration panel (see below).
  - a. **Ignore the red text reminder at the bottom of the window.**
4. Select **Sizing** in **UNIT COV** drop down and set **S16M-EXO** as the **Standard**.
5. Click **Find Peak** to generate a calibration curve of particle size and side scattering intensity.





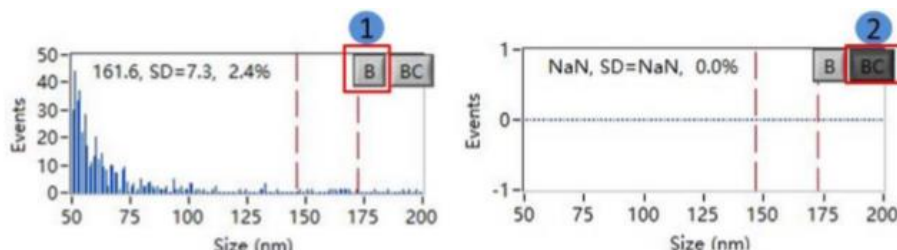
#### SIZE REFERENCE STANDARD (S17M-MV)

1. Select the **S17M-MV** file and select  to open threshold settings. Select **Large Signal** mode from drop down to automatically set the threshold.
2. Select  to open the calibration panel (see below).
3. Select **Sizing** in **UNIT COV** drop down and set **S17M-MV** as the **Standard**.
  - a. Select **Slogistic**.
4. Click **Find Peak** to generate a calibration curve of particle size and side scattering intensity.



## SETTING UP BLANK CONTROL

1. Select blank control data (e.g. PBS) and select  to set the threshold.
  - a. Ensure that the blank has been acquired with the same instrument settings and threshold as your sample (e.g. S16M-Exo or S17M-MV setting).
  - b. Note – The threshold should be set according to your sample (e.g. you may want to increase the threshold to only look at particles >150nm). You may choose to use a set blank for multiple files or match the blank threshold level for each sample individually.
2. Select  and click **B (1)** in the size distribution window to set blank control as the background.
3. Select sample file and click **BC (2)** in the size distribution window to subtract the background from samples.
  - a. Think of “**B**” as selecting the **blank**, and “**BC**” as ‘**Blank correction.**’




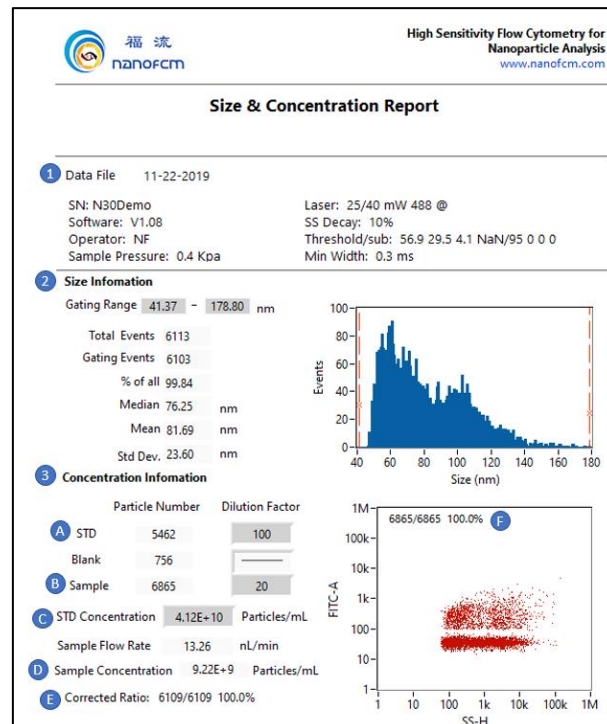
## THRESHOLD SETTINGS

When particle sizes between multiple samples need to be compared, the threshold should be consistent i.e. the highest threshold should be used for particle size analysis of each sample.

Theoretically, when the sample purity is equal the threshold level is similar, and therefore the auto-threshold function can be used. High sample concentration or low sample purity can result in the sample's threshold being higher than that of the blank control – if this is the case, then the sample needs to be re-processed (e.g. diluted).



## EXPORTING DATA

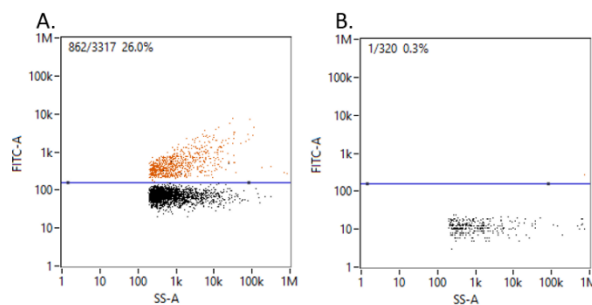
1. Click  and select **Sizing & Con** to save particle size and concentration information as a pdf (see below for a total population report example).
2. Input into the report:
  - a. Concentration standard (aka 250nm QC beads) dilution factor (e.g., 100).
  - b. Sample dilution factor
  - c. Concentration standard (aka 250nm QC beads) concentration




1. **Sample acquisition information** e.g., data file name, laser and pressure settings, threshold.
2. **Size Information** – sizing analysis for sample e.g., range, median, mean, and standard deviation.
3. **Concentration Information** – concentration analysis for total or gated sub-populations.
  - A. **STD** - Input 250nm QC SiNP (concentration standard beads) dilution factor
  - B. **Sample** – Input sample dilution
  - C. **STD Concentration** – Input original 250nm QC SiNP concentration.
  - D. **Sample Concentration** - shows concentration of total or gated sub-population. This is automatically calculated by software.
  - E. **Corrected Ratio** - shows number of gated events out of total events, and its percentage. Takes into account events from the blank control.
  - F. **Uncorrected Ratio (no blank correction)** –shows number of gated events out of total events, and its percentage. Does not take into account events from the blank control.

## FLUORESCENCE ANALYSIS

1. Select sample file and select  to set the threshold.
2. Set the dot-plot axis e.g., SS-A vs. FITC-A (depends on type of fluorescence).
3. Select the tools  to gate population of interest in dot plot. A line gate is shown below.
  - a. Use the same gate on your blank control to show no fluorescent-positive particles are present. Recommended to gate on your blank control (e.g., PBS) and check it against each sample.



*Fluorescent gating of FITC + particles – A) shows gated FITC+SS+ population; B) shows blank control (i.e. PBS diluent) with same gate to take into account any fluorescent false positive arising from diluent.*

4. Click  and select **Concentration** or **Sizing & Con** to generate a report showing the gated sub-population.
  - a. Ensure blank control has been subtracted – can check **Concentration** report to confirm.
  - b. The corrected ratio now takes the blank into account and can be found above the sample dot plot in the concentration report.

