Spectradyne nCS1

Operation Manual

Contents

The nCS1 Instrument: Overview & Specifications

The nCS1 is a powerful tool for obtaining detailed size and concentration information about particle distributions in liquids. The core technology utilized in the nCS1 is called Resistive Pulse Sensing (RPS), also referred to as the Coulter Principle, and is a truly orthogonal method to both light scattering methods such as dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA) as well as to resonant mass measurement (RMM) techniques. A clean disposable cartridge targets a specific size range of particles for each

Figure 1. The nCS1 instrument and analysis cartridges.

analysis, eliminating concerns of contamination between samples.

Specifications

- Dimensions: 12" x 12" x 15" (30.5cm x 30.5cm x 38.1cm)
- Weight: 30 lbs (13.6 kg)
- Power: North America: 110V, 5A, 60 Hz;
                        Europe: 230V, 16 A, 50 Hz
- Nitrogen or CDA: 15 to 30 psi regulated supply
- Min. analyte volume: 3 µl
- Min. ionic strength of analyte: 1-10 mM
- Concentration range: $5x10^5 – 5x10^{11}$ particles per ml, cartridge dependent
- Running buffer: PBS buffer + 1% polysorbate 20 filtered to 0.2 µm

A computer is required to interface with the nCS1 through a standard USB link.

Instrument Setup and Sample Prep

Setting up the nCS1

Unpack: Remove all packaging from the instrument and position it on the benchtop.

Power supply: Use the power cable provided with the instrument to connect the nCS1 to a source of electrical power (North America: 110V, 5A, 60 Hz; Europe: 230V, 16 A, 50 Hz). The power input connection is located on the back panel. The fuse drawer also provides a means to set the instrument to run on 110V power (North America) or 230V (Europe). Align the arrows for the desired AC voltage on the fuse drawer and the power entry module.

Pressure supply: Connect the instrument to a source of clean inert gas (e.g., filtered nitrogen or air). The acceptable range of input pressures is 15-30 PSI. The pressure input is located on the back panel.

Load running buffer: Running buffer is used to fill auxiliary portions of the analysis cartridges during operation—it does not contact the sample to be analyzed until after measurement. Recommended running buffer is PBS + 1% polysorbate 20 filtered at 0.2 μm. Add approximately 50 mL to each of the two bottles labeled "Buffer" on the right side of the instrument.

USB connection: Use the USB cable provided with the instrument to connect the nCS1 to a computer with Spectradyne software installed. The USB port is located on the back panel.

Power on: Turn on the instrument with the switch located on the rear panel *before running the nCS1 software installed on the attached computer*.

Software: Run the Spectradyne nCS1 software on the computer. Note that if the instrument is powered off, the software will need to be restarted before next use. Make sure the cartridge calibration file is up-to-date, please contact support@spectradynellc.com for the latest version.

Cleaning cartridge

After setting up the nCS1 for the first time, or after any significant period of inactivity, the cleaning cartridge routine should be performed using the reusable cleaning cartridge supplied with the instrument. Refer to page 13 of this guide for instructions.

Calibration Sample and First Run

A calibration sample is included with the nCS1. The calibration sample should be the first sample run after unpacking the instrument, and can be used at any time by users wishing to familiarize themselves with instrument operation. The calibration sample contains polystyrene nanoparticles in filtered PBS + 1% Tween 20. Guidelines for preparing your own sample for measurement can be found in the Maintenance and Sample Preparation Guidelines section on page 16 of this guide.

Loading the Cartridge

A video demonstrating cartridge loading is available on Spectradyne's website: www.nanoparticleanalyzer.com.

Load the analyte into the cartridge:

➢ Pipette 3-5 microliters of the sample to be measured into the analyte reservoir on the analysis cartridge (Figure 3).
   ❖ IMPORTANT:
      ▪ Ensure that any air bubbles in the sample are acceptably positioned in the reservoir (see Figure 2).
      ▪ Gently tap the cartridge on a flat surface to shift/remove any bubbles if necessary.
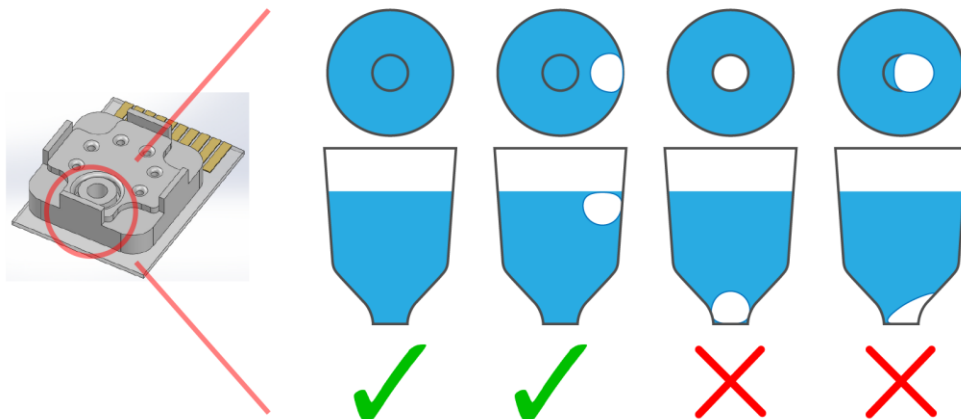
Figure 2. Any air bubbles in the analyte must not occlude the bottom of the reservoir.

Load the cartridge into the nCS1

➢ Load the cartridge into the socket in the front of the nCS1.
   ❖ IMPORTANT:
      ▪ The outer glass edge of the cartridge MUST be flush with the edge of the shelf on which it sits
      ▪ The center indicator light (blue) and the cartridge engage button on the front panel of the instrument must be illuminated before proceeding.
➢ Ensure the nCS1 control software is running on the attached computer.
➢ Press the cartridge engage button on the front of the nCS1 to raise the cartridge into the instrument. The top indicator light (white) will illuminate to indicate that the cartridge is ready for priming.
   ❖ IMPORTANT:
      ▪ If the white light does not illuminate when the stage is raised to the top of its travel, immediately depress the cartridge engage button to lower the stage.

- Call your customer service contact before continuing, or email support@spectradynellc.com for assistance.
- After this point, the nCS1 and data acquisition process are controlled entirely through the Spectradyne Acquisition software on the computer.
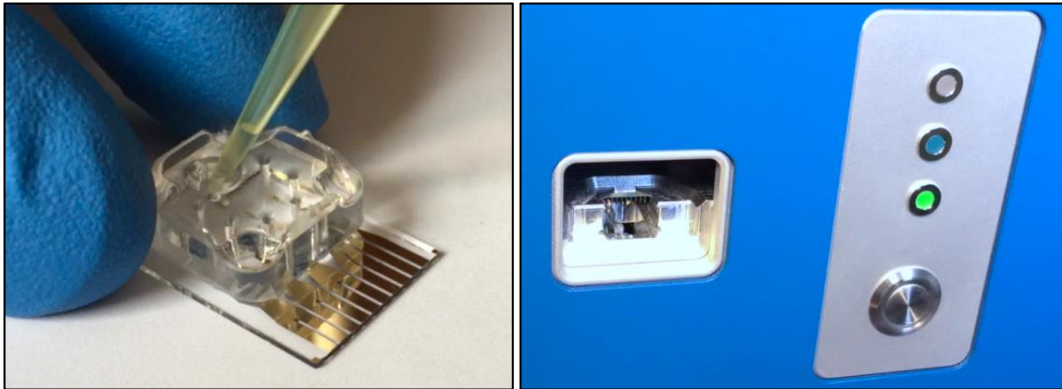
Figure 3. Left panel shows the sample pipetted into the analysis cartridge. Right panel shows the front panel of the Spectradyne nCS1: On the left side is the cartridge insertion slot, and on the right are the cartridge engage button and indicator lights.

nCS1 Control and Data Acquisition Software

Enter Sample Details

Description of fields (see Figure 4):

- **Description:** Enter a sample description, this will be stored with the data file.
- **Calibration: Size (nm)**: Enter the diameter (in nm) of calibration particles mixed in with the sample. If the sample contains no calibration particles, the entry may be left blank.
- **Conc. (1/mL)**: Enter the concentration of calibration particles in particles/mL (e.g., '2E9'). This entry may be left blank.

Figure 4. Sample Details frame.

Initializing the Cartridge

Single-click to acquisition:

NEW TIME-SAVING FEATURE! Prime and acquire data with a single click of the "GO!" button.

➢ Enter the cartridge type in the "Cartridge Mold ID" entry (e.g., P15.2.3, or E16.1.2).
➢ Press "GO!" to prime the cartridge and **start data acquisition automatically** (see Figure 5).
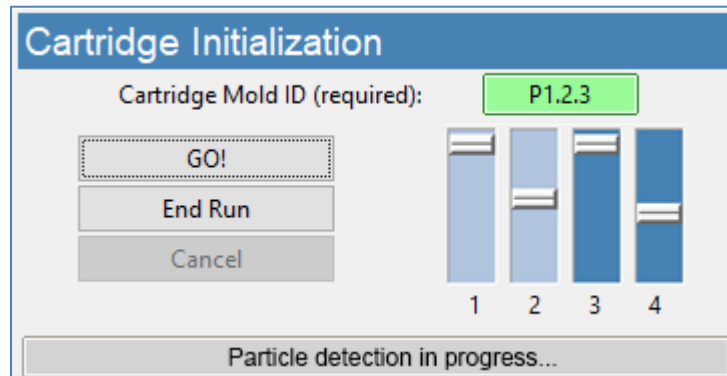
Figure 5. Cartridge Initialization frame and fill monitors after successful

Fill Monitors:

➢ The fill monitors are vertical sliders in the Cartridge Initialization frame that indicate the fill status in various parts of the cartridge (see Figure 5).
➢ The priming routine typically requires 1-2 minutes to complete. During the process, it is normal for fill monitors to vary between all levels and show different colors.
➢ After normal priming, the fill monitors should have approximately the pattern shown in Figure 5.
➢ When the routine is complete, the software beeps and **data acquisition begins automatically**.

Troubleshooting Tip:

➢ If the priming routine encounters an issue, one or more of the fill monitors will remain in a red color and the priming routine will not complete.

➢ After 5 minutes, a red fill monitor indicates that a portion of the cartridge was not successfully primed.

➢ If Fill Monitor 1 or 3 is red:
  ❖ An issue with the running buffer is likely.
  ❖ Check that sufficient running buffer remains in the reservoirs
  ❖ Stop the priming by clicking "Cancel" and then "End Run" to eject the cartridge.
    ▪ IMPORTANT: Before attempting to disconnect buffer bottles, run cleaning cartridge.
  ❖ Replace the running buffer and use the cleaning cartridge to flush the system with the new running buffer.

➢ If Fill Monitor 2 or 4 is red:
  ❖ There may be an issue with the sample:
    ▪ Is there insufficient surfactant in the sample?
    ▪ Is the conductivity of the sample too low?
    ▪ Are the particles in the sample too highly concentrated?
  ❖ There may be an issue with the cartridge:
    ▪ Try measuring the sample in a new cartridge.

Acquisition Control

NEW FEATURE! After priming of the cartridge is complete, continuous data acquisition will begin automatically using bias values that are optimized for the cartridge type.
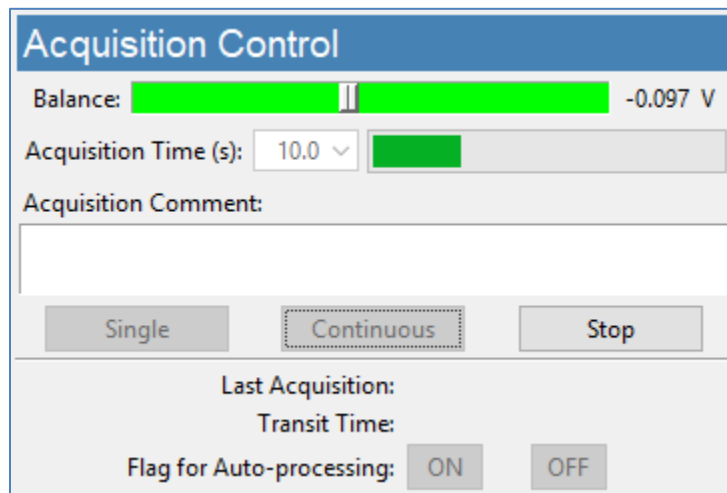
Figure 6. Acquisition Control frame.

Continuous-mode Data Acquisition:

➢ Continuous-mode acquisition may be manually started by pressing the "Continuous" button (Figure 6).
➢ Repeat acquisitions are collected until the user presses the "Stop" button.
➢ The length of each acquisition is specified using the "Acquisition Time" dropdown (10 s default).
➢ In continuous mode, each acquisition is automatically flagged for processing unless a significant number of defects are detected in the data (see page 14).
➢ Interrupt continuous-mode acquisition by pressing "Stop".

> **Data Saving Tips:**
> ➢ Raw data files are saved by date within the Spectradyne data directory, C:\Spectradyne\Data\. For example, if data is acquired on April 17 2017, files will be saved in the directory: C:\Spectradyne\Data\2017_04_17 by default.
> ➢ Acquisitions are saved with a date and an incremented extension number starting at 0. For example, a raw data file acquired on April 17 2017 might be named 20170417_003.h5.
> ➢ Record significant acquisition numbers in your notes for future reference.

Balance Monitor:

➢ When the bias is ON, the Balance monitor indicates the electrical balance of the system.
➢ The balance should remain approximately centered on the scale, within the range of -0.800 V to +0.800 V, and the color of the balance indicator should be green.

➤ If the scale moves outside the normal range, run the "Clear Constriction" routine (page 13).

Single-mode Data Acquisition

➤ Press "Single" to acquire one acquisition of length specified by the "Acquisition Time" dropdown (10 s default).
➤ Interrupt the current acquisition with the "Stop" button. Partial acquisitions are not saved.

Assessing data quality
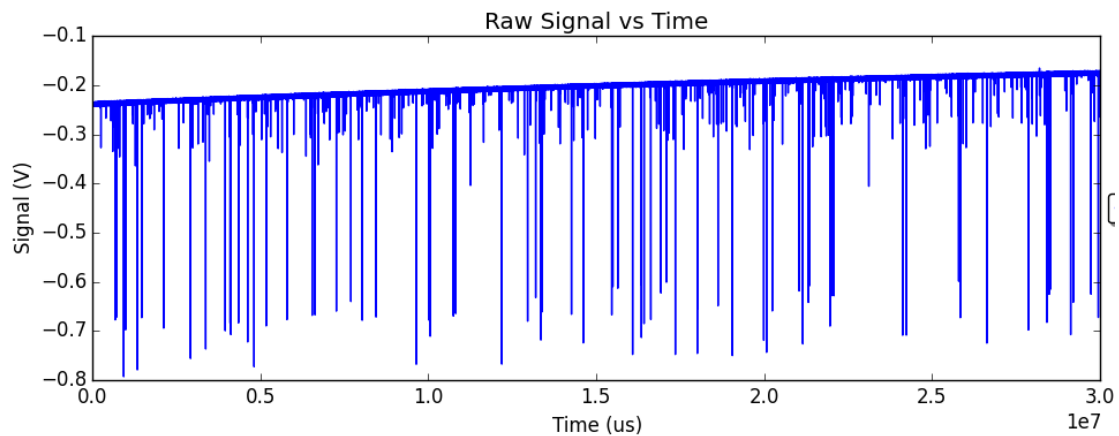
Figure 7. Exemplary raw data: Smooth baseline with particle detection events appearing as downward spikes.

➤ Raw data should have the form shown in **Figure 7**—it has a smooth baseline with particle detection events appearing as downward spikes from the baseline.
➤ Drifting of the baseline is acceptable.
➤ In some situations, there may be occasional steps in the baseline. These steps may be acceptable, depending on their severity.
➤ If the raw data shows too many steps, or poor display of calibration particles, click on the "Clear Constriction" button (page 13), wait for completion, then reattempt acquisition.
➤ In single acquisition mode, the user may manually flag the most recent acquisition for automatic processing by pressing the "ON" button in the Acquisition Control frame.
➤ Flagged acquisitions are highlighted blue in the Viewer software for rapid identification (refer to the Data Analysis and Viewer Software section on page 18 of this guide).

> **How much data is enough?**
> ➢ The 1-σ error reported for a concentration measurement in a given size range is derived from statistical error in counting particle detection events.
> ➢ Particle detection events are Poisson-distributed in time; therefore, the error scales as $1/N^{1/2}$ (with small corrections for N < 5).
> ➢ Example: N = 100 particles must be counted in a given size range to obtain a concentration measurement on that range having standard error of 10 %.
> ➢ The particle concentration (with error) measured over any user-defined size range can be obtained in real time using the Live CSD feature of the Auto-analysis engine.

Utilities
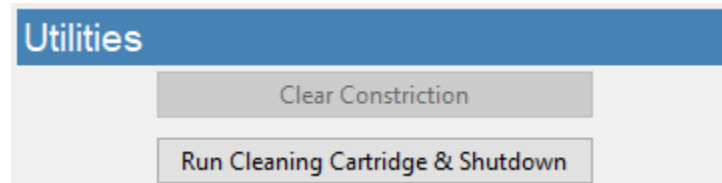
Figure 8. Utilities frame.

Clear Constriction:

This automatic sequence is used during a measurement run to clear the sensing constriction of the cartridge in the event of a blockage.

➢ Press the "Clear Constriction" button (Figure 8) and wait for the routine to complete.
➢ Continue acquisitions as normal.

Run Cleaning Cartridge & Shutdown:

This automatic sequence flushes the internal fluid lines of the nCS1 and requires a reusable cleaning cartridge. **This routine must be run before the nCS1 is shut down.**

➢ Insert the reusable cleaning cartridge provided with the nCS1 and raise it into the instrument.
➢ Press the "Run Cleaning Cartridge & Shutdown" button (Figure 8) to begin the automated process.
➢ Wait for the first stage of the process to complete.
➢ Follow the prompts:
   o Remove the cleaning cartridge and store it in a safe place for re-use.
   o Click OK to complete the routine.

The Auto-analysis Engine
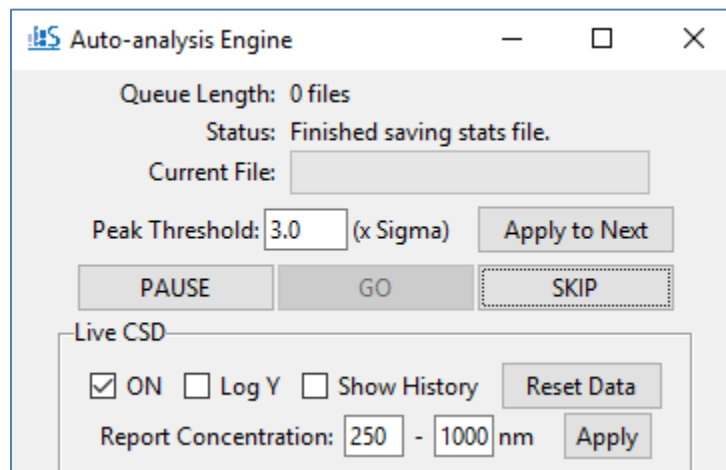
Figure 9. Auto-analysis Engine interface.

Automatic Processing of Raw Data:

➢ Flagged acquisitions are placed in the Auto-analysis Engine queue for automatic processing into Spectradyne Stats files (Figure 9).
➢ Raw data is processed using standard parameters (refer to page 19 for details):
  - o Peak Detection Threshold: 3.0 x the standard deviation of the baseline noise.
    - ▪ The threshold can be changed in the Auto-analysis engine interface.
  - o Fit Width: 4.0 x the mean transit time of high signal-to-noise particle events.
➢ Control the auto-processing engine using the "Pause", "Go" and "Skip" buttons.

Live CSD – Quantitative Real-time Data Analysis:

NEW FEATURE! A live CSD plot enables quantitative evaluation of the current run in real-time.

➢ The Live CSD feature displays a combined size distribution from the current cartridge run.
➢ The Live CSD is updated each time a new stats file is generated by the auto-analysis engine.
➢ Display Options (see Figure 9):
  - o "ON" – Control display of the Live CSD.
  - o "Log Y" – Plot the y-axis in log scale to view concentrations over multiple orders of magnitude in a single sample.
  - o "Show History" – Display all acquisitions that are included in the combined distribution.
  - o "Reset Data" – Clear the history of acquisitions displayed in the Live CSD.
➢ Concentration Measurements on the Live CSD:

- o Specify an integration range using the entries provided and click "Apply".
  - o The measured concentration for a specific size range (with statistical error) is displayed.
- ➢ Which data is included in the Live CSD?
  - o All data from the current cartridge run and since the 'Reset Data' button was last pressed.
  - o <u>No peak filtering is applied</u>—*false-positive detection events may be included*. For more in-depth data analysis use the Data Viewer (page 18).

After Data Acquisition

End measurement run:

NEW FEATURE! Flushing the cartridge is no longer required after measurement, and ending a measurement run now only requires approximately 30 s.

- ➢ After acquiring sufficient data, press "End Run" (Cartridge Initialization frame, see Figure 5).
- ➢ Wait for the process to complete.
- ➢ Follow the instructions in the prompts at the end of the routine.
- ➢ **NOTE: The cleaning cartridge routine must be performed before shutting down the nCS1.**

Prepare the next cartridge for analysis (if continuing with measurements):

- ➢ Prepare the sample as necessary.
- ➢ Load the new sample into a new analysis cartridge.

Remove the used cartridge:

- ➢ After completion of the End Run routine, lower the stage by pressing the button on the front panel of the instrument and remove the cartridge.
- ➢ The nCS1 is now ready for the next measurement run—load a new cartridge for analysis.

Insert the next analysis cartridge (if continuing with measurements):

- ➢ Return to the section entitled, 'Initializing the cartridge' on page 7.

nCS1 Shutdown Procedure

Run the Cleaning Cartridge & Shutdown routine:

- ➢ **This routine must be run before instrument shutdown.**
- ➢ Refer to Run Cleaning Cartridge & Shutdown: page 13 for detailed instructions.
- ➢ Wait for the procedure to complete and follow all prompts.

➢ Reminder: <u>store the reusable cleaning cartridge in a safe place for next use.</u>

Remove the running buffer bottles and refrigerate

➢ The running buffer bottles should be refrigerated during periods of inactivity (e.g., overnight).
➢ Detach the buffer bottles from the supply lines using the quick-connect tube fittings and store in the refrigerator until next use.
➢ Running buffer should be replaced with fresh filtered buffer approximately every two weeks.

Turn off power to the instrument

➢ Power off the instrument during long periods of inactivity (e.g., overnight).
➢ Use the power switch at the back of the instrument to power down.
➢ The power indicator light (green) on the front of the instrument may take a few seconds to turn off.

Maintenance and Sample Preparation Guidelines

Buffer Maintenance

Refrigerate the running buffer bottles

➢ The running buffer should be refrigerated during long periods of inactivity (e.g., overnight) to minimize algae growth.
➢ Running buffer should be replaced with fresh filtered buffer approximately every two weeks.

Preparing for long periods of inactivity

Before long periods of inactivity (e.g., longer than about 1 week), the cleaning cartridge routine should be performed using **deionized water in place of the normal running buffer.**

➢ Drain fluid lines using the "Instrument Shutdown" procedure described above.
➢ Disconnect the running buffer bottles from the fluid lines using the quick-connect tube fittings.
➢ Empty the running buffer from the bottles (store refrigerated if intended for future use).
➢ Add approximately 25-50 mL of deionized water to each of the two buffer bottles, and replace in the instrument.

➢ Follow the steps of the nCS1 Shutdown Procedure described on page 15, using DI water in place of running buffer.

Aborting a measurement run

End the measurement run using the steps described in "After Data Acquisition" on page 15.

Guidelines for Sample Preparation

Some optimization of sample preparation may be required when analyzing a sample for the first time. Once the best sample prep for the analyte in question is determined, that procedure can be used for future analyses of that analyte and its variations. The parameters below are suggested targets for first-run samples.

Optimal sample parameters:

Concentration:	10^9 particles/ml below 300 nm, 10^8 particles/mL above 300 nm
Calibration beads:	Add calibration beads to final concentration ~10^9/ml below 300 nm, ~10^8/ml above 300 nm. Recommended size ~30% below the cartridge max size.
Ionic strength:	~ 1 S/m (e.g., ~100 mM NaCl)
Surfactant:	1% polysorbate 20 or equivalent

Notes:
➢ Sample diluent and calibration particles:
  ❖ If ionic strength or surfactant in the diluent is a concern, then lower values can be used.
  ❖ For first-run analyses, Spectradyne highly recommends mixing calibration particles of a known size (typically NIST-traceable polystyrene beads) with the analyte.
  ❖ Keep in mind that the sample diluent may be a source of nanoparticles!

➢ Running buffer:
  ❖ **Important:** Running buffer does not contact the analyte sample until after its particle distribution has been analyzed. The user should not be concerned that ionic strength or surfactant content of the running buffer will affect the measurements.
  ❖ Running buffer should be refrigerated during periods of inactivity (e.g., overnight)
  ❖ Running buffer should be replaced approximately every 2 weeks in order to avoid contamination of the fluid lines from algae, etc.

Data Analysis and Viewer Software
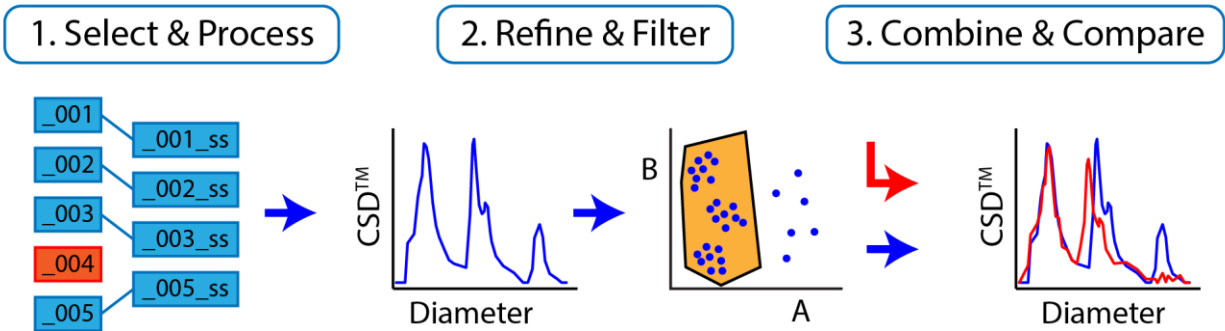
Workflow Overview:

Figure 10. Overview of data processing workflow.

Three workflow steps for data analysis

1. Select & Process:
    - ➤ Raw data acquisitions are selected and processed into Spectradyne Stats files. Likely this step will have been completed by the Auto-analysis Engine at the time of acquisition (described on page 10), however a manual process is available for customizing the choice of raw data and processing parameters.
    - ➤ This step yields a quantitative Concentration Spectral Density (CSD™) distribution.
2. Refine & Filter:
    - ➤ Any necessary refinement to the diameter scaling is performed (e.g., to account for possible buffer effects)
    - ➤ Low-confidence particle measurements are eliminated by data filtering (e.g., false positives near the small particle detection limit)
    - ➤ This step yields the final CSD for the collection of measurements.
3. Combine & Compare:
    - ➤ The collection of refined Spectradyne Stats files is saved as a single combined file.
    - ➤ The CSDs of different samples are quantitatively compared at the combined file level.

Data Viewer Software User Interface:
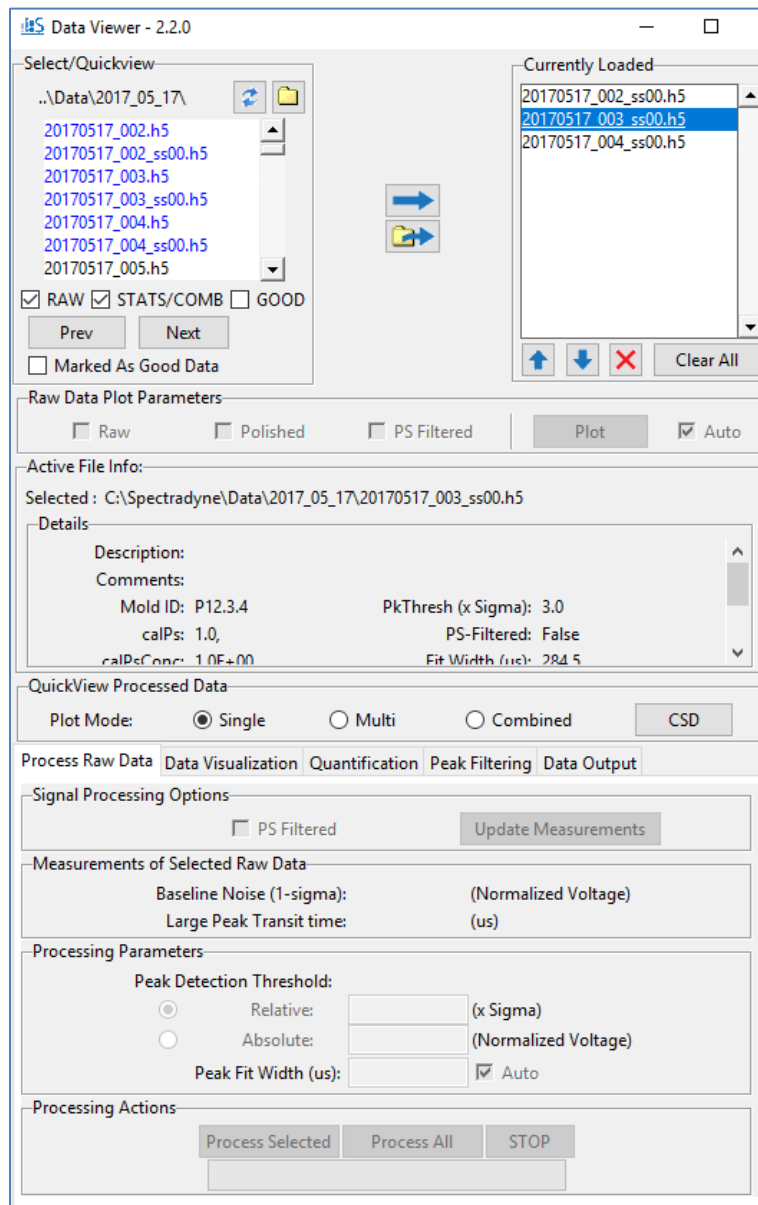
Figure 11. Overview of the Spectradyne Viewer software user interface.

1. Select & Process Raw Data

In a typical workflow, data to be used in analysis will have been flagged at the time of acquisition, and processed into Spectradyne Stats files by the Auto-analysis Engine (see page 14). In such a scenario, the user may skip to the section, "Viewing the Results of Raw Data Processing" on page 21, below. If the raw data has not already been processed, or if the user wishes to employ non-standard processing parameters (i.e., a user-defined peak fit width or

peak threshold), then the manual method for processing raw data described next should be used.

Viewing and Selecting Raw Data for Analysis

Navigate to data location

- ➢ Open the Data Viewer software.
- ➢ In the Select/Quickview frame (Figure 11), use the folder icon to the right of the current path to navigate to the location of the raw data.

Inspect raw data

- ➢ Check one of the plot options in the Raw Data Plot Parameters frame:
  - ❖ "Raw" – display the raw data as it was collected
  - ❖ "Polished" – display the raw data after default signal processing.
  - ❖ "PS Filtered" – display the raw data after PS Filter signal processing.
    - ▪ PS Filter signal processing is a new feature (beta-release) that is intended to improved reductions in baseline noise.
    - ▪ PS Filter signal processing is most effective for large size cartridges (e.g., TS2000)
    - ▪ PS Filter signal processing requires additional computational time.
- ➢ Select a file from current folder in the Select/Quickview list box—the raw data will be displayed.
- ➢ The "Prev" and "Next" buttons navigate through the files in the current directory for rapid inspection of raw data.

> **Note: Select/Quickview Frame**
>
> - ➢ Files flagged for automatic data processing are highlighted with blue text.
> - ➢ The flag can be toggled for selected files using the "Marked as Good Data" checkbox.
> - ➢ The Select/Quickview list can be filtered to show subsets of the folder contents using the checkboxes below the list (e.g., raw files only).
> - ➢ Metadata for any selected file is shown in the Active File Info frame.

Load raw data files for processing

- ➢ After selecting the raw data to be analyzed, load the files into the Currently Loaded file list at right using one of two methods:
  1. Use the load button (➡) to load selected files from the Select/Quickview list.
  2. Use the remote load button (↪) to select files from another folder with a dialog window.
- ➢ The Currently Loaded file list may be modified with the control buttons at the bottom of the frame.

Processing raw data

Each raw data file is analyzed to count and measure particles detected during acquisition. The result of this analysis is a Spectradyne Stats file that contains detailed information about all particles detected during the acquisition as well as the acquisition itself.

Procedure for processing raw data

➢ The Process Raw Data tab of the Data Viewer contains all the controls for processing raw data, as well as relevant measured values for the selected raw data file.
➢ The default processing parameter values are appropriate for use in most cases. These default values are the same as those used by the Auto-analysis Engine during acquisition:
   ❖ Peak Detection Threshold: 3.0 x the standard deviation of the baseline noise.
   ❖ Fit Width: 4.0 x the mean transit time of high signal-to-noise particle events.
➢ The above parameters can be customized for more in-depth analysis using the entries in the Processing Parameters frame.
➢ To analyze the raw data, use the controls in the Processing Actions frame:
   ❖ "Process Selected": Processes only the currently selected raw data file from the Currently Loaded list.
   ❖ "Process All": Processes all raw files in the Currently Loaded files list using the same (adaptive) parameters.
   ❖ "Stop": Interrupts processing of the current raw file with no partial data saved and stops batch processing.

Note: Stats Files

➢ A Stats file shares the same base filename as the raw file from which it was generated, with an additional extension of the form _ssNN.
➢ In the extension, NN is an integer that increments each time the raw file is processed.
➢ Stats files are saved in the same folder location as the raw file from which they are generated.
➢ For full functionality, a Stats file should be kept in the same folder as its parent file.

➢ Processed raw files are replaced by the newly generated Stats file in the Currently Loaded list.

Viewing the Results of Raw Data Processing

Ensure that the Stats files of interest are loaded into the Currently Loaded files list.

Concentration Spectral Density (CSD):

➢ The nCS1 is capable of very high resolution size and concentration measurements of particles in a liquid, and is well suited for characterizing and comparing subpopulations of particles in polydisperse mixtures.
➢ A useful quantity is therefore the concentration of particles in the sample over a given size range, so that different size populations in a mixture can be compared directly.
➢ The Concentration Spectral Density (CSD) is a measure of particle concentration (particles/mL) per unit diameter (nm).
➢ The integral of a CSD over any size range yields the concentration of particles (in units of particles/mL) in that range.

Aggregate Data View: Generating the CSD

➢ Select a plot mode from the QuickView Processed Data frame:
  ❖ "Single" – Display results from only the selected Stats file in the Currently Loaded file list.
  ❖ "Multi" – Display results from all Stats files in the Currently Loaded file list separately on the same plot.
  ❖ "Combined" – Combine results from all Stats files in the Currently Loaded file list as if they derived from a single long acquisition.
➢ Plot the CSD using one of two available "CSD" buttons:
  ❖ In the QuickView Processed Data frame (always visible)
  ❖ In the Aggregate Views frame of the Data Visualization tab.

Single-particle Views: Particle events in raw data

➢ Navigate to the Data Visualization tab in the DataViewer.
➢ Use the "Peaks in Raw Data" button to show all peaks detected in the stats file that is selected in the Currently Loaded list.
  ❖ Use the tools in the plot window to zoom in on individual peaks.
  ❖ Clicking on the peak (at sufficiently high zoom) displays the details of the peak measurement (Figure 12).
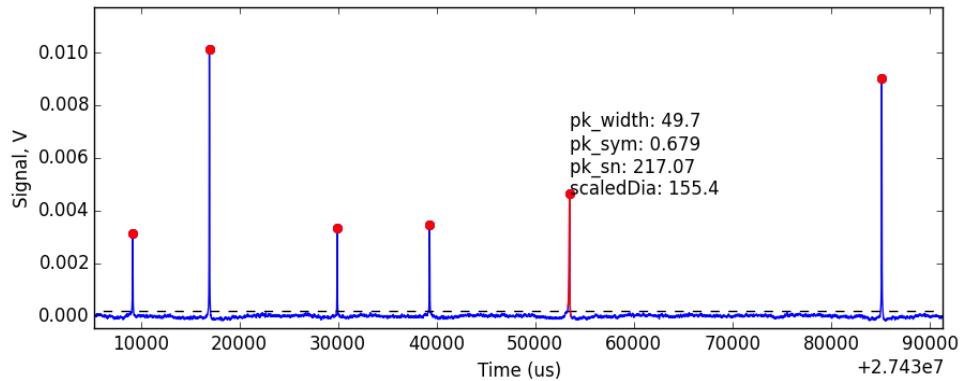 ❖ Ctrl + Click selection allows concurrent display of multiple peak measurements.

Figure 12. Inspecting individual peaks detected in the raw data.

Single-particle Views: Parametric Scatter Plots

➢ The "Scatter" button in the Data Visualization tab generates a scatter plot of particle diameter vs the parameter selected in the dropdown list (e.g., transit time) for each detected particle event.

➢ The plot mode selected in the QuickView Processed Data frame determines whether data from a single Stats file or multiple Stats files will be displayed.

Note: Refine Data Group

Any outlier Stats files should be eliminated from the loaded file list at this point in the analysis workflow.

➢ Generate a CSD plot in multi-mode to show the CSDs for all Stats files in the Currently Loaded list.

➢ CSDs corresponding to measurements of the same sample should be consistent with each other—any outliers should be removed from the Currently Loaded list using the red "X" button below the list.

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2. Refine & Filter

At this point in the data analysis workflow, multiple measurements of the same sample have been processed to generate Stats files in the Currently Loaded files list. These Stats files will contain measurements of many individual particles in the same sample under the same measurement conditions, and can therefore be considered as a single analysis of the sample for downstream processing. The Spectradyne Viewer provides efficient tools for working with multiple Stats files as a group, and after the Refine & Filter workflow step the multiple files are combined into a single entity for comparison with other sample measurements.

> **Note: Treating multiple datasets as one for Refine and Filter**
>
> ➢ In the QuickView Processed Data frame of the Spectradyne Viewer, use the Combined plot mode to display data from all the loaded Stats files as a single dataset.

The two main goals of this step in the workflow are:

1. Refine diameter and concentration scaling as necessary.
2. Filter the particle set to keep only high-confidence detection events and eliminate false-positives.

2.1 Refine the diameter and concentration scaling of the CSD:

Particles are detected and measured in an analysis cartridge that is cast from a master mold. Different cartridges may be cast from different molds, and the analysis software automatically compensates for variation between cartridges using a mold-specific calibration factor. This factor establishes the correct scaling for the diameter axis of the CSD in standard measurement conditions. Certain factors, (e.g., composition of the sample diluent), may cause slight deviations from the default calibration, and the user may wish to refine the diameter scaling to align known features of the CSD (e.g., a peak of calibration particles) with the appropriate diameter or concentration.

Procedure for refining scaling

➢ Select the Quantification tab in the Spectradyne Viewer
➢ In the Refine Scaling frame, use the Manual Scaling Refine entries to scale the diameter and concentration. The value of the entry multiplies the CSD; for example, a Diameter refine factor value of 1.02 would 'stretch' the diameter scale by a factor of 1.02, and a population of particles originally centered at 100 nm would shift to a new center of 102 nm.
➢ Occasionally, more challenging samples may require adjustment of the concentration scaling in an analogous way.

> **Note: Refining the diameter scaling using a known feature in the CSD**
>
> The diameter scaling of a CSD can quickly be refined using a known feature in the CSD (e.g., a population of calibration particles added to the sample that exhibit a clear peak in the particle size distribution):
>
> ➢ Fit a peak in the CSD to a Gaussian curve using the steps described in the section, "Combine and Compare" on page 28 of this guide.
> ➢ The "Rescale Last Fit To:" button will automatically refine the diameter scaling so that the mean of the Gaussian fit coincides with the diameter specified by the user in the entry adjacent to the button.

2.2 Filter the Set of Detected Particles to Exclude False-positives

Particle detection events manifest as peaks in the raw data. The algorithm used to detect and characterize these peaks employs a thresholding method: Any excursion of the normalized output signal crossing above the peak detection threshold is considered to be a possible peak and measured (see Figure 13). The metrics for each peak found in this way include quality of fit parameters (i.e., signal-to-noise), as well as physical parameters of the transit event such as transit time through the sensing constriction and symmetry of the peak shape.

As the peak detection threshold approaches the baseline noise, some false positives (i.e., peaks in the raw data that do not likely represent real particles) naturally are included in the Stats file data set (see Figure 13). The goal of this step in the workflow is to eliminate false positives from the data by excluding particle detection events whose measured peak parameters fall outside the normal range for true particles. The signal-to-noise, peak symmetry, and transit time parameters are all available for use as filter parameters.
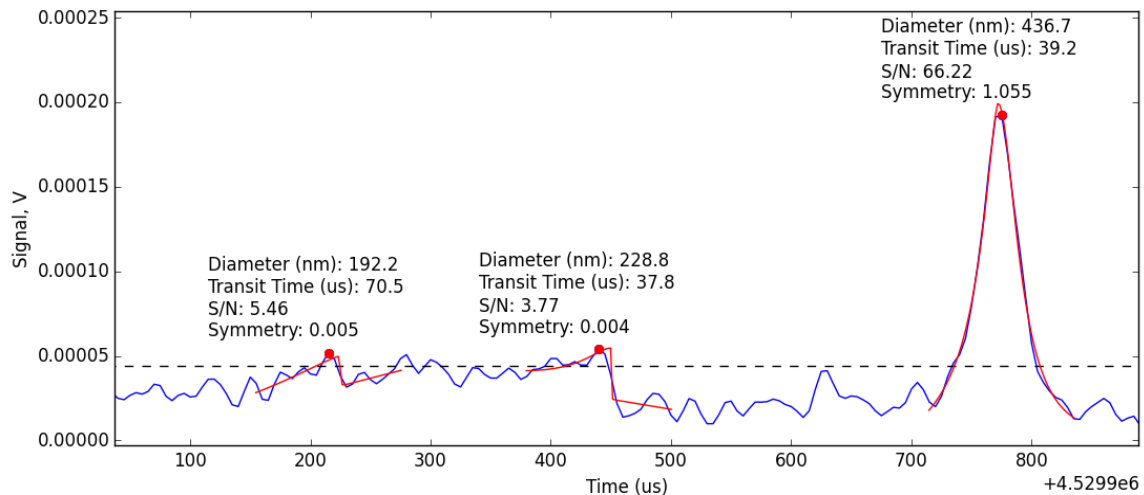

Figure 13. The peak detection threshold indicated by the dashed line is close enough to the baseline noise that two false positives are detected near 200 us and 450 us in the local time window; a real particle event is clearly detected at 750 us. A red point highlights the peak value, and the red line shows the fit for each peak. Peak-filtering tools are available in the Viewer for identifying and removing false-positives in the data.

Identifying False-Positives Among the Measured Peaks

False-positives generally are characterized as follows (example shown in Figure 14):

> ➢ Diameter:
>> o Diameter near or below the small size limit of detection of the cartridge.
> ➢ Transit time:
>> o A group of small diameter particle events having a broad distribution of transit times.
> ➢ Symmetry:

- o Symmetry outside the range of 0.2 – 4.0 (approx.)
- ➢ Signal-to-noise (S/N):
  - o S/N < 15 (cartridge dependent).
  - o A general sense for an appropriate S/N cutoff can also be obtained by inspecting peaks in the raw data (Figure 13 and "Viewing the Results of Raw Data Processing," page 21).
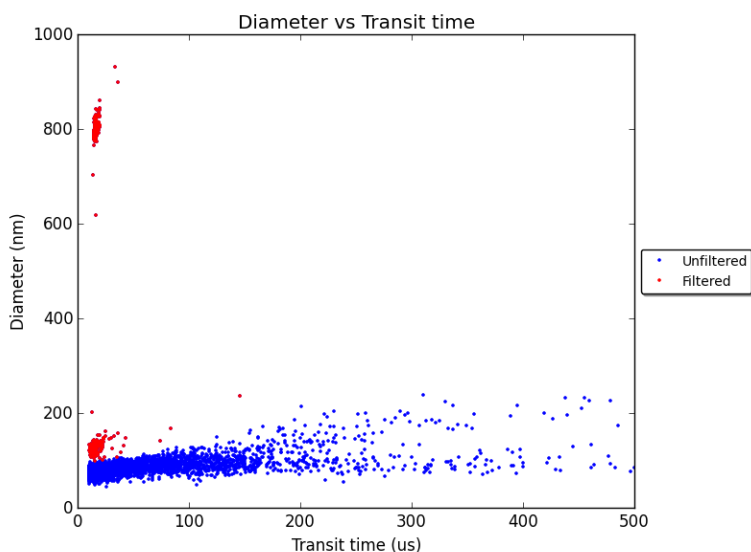

Figure 14. Representative Scatter plot of Diameter vs. Transit Time showing two distinct populations of true particle detection events (red) and false-positives excluded from the data set (blue). False positive detection events are characterized in part by having diameter close to the minimum detectable size for the cartridge (here approx. 130 nm) and a broad distribution of transit times.

Procedure for removing false-positives from the set of detected peaks

- ➢ Load the desired Stats files into the Currently Loaded files list
- ➢ Navigate to the Peak Filtering tab of the Spectradyne Viewer
- ➢ Use the appropriate action buttons in the Filters frame of the Peak Filtering tab to apply and remove existing filters as desired from files in the Currently Loaded file list.
- ➢ To create a new filter, use the "+" button at the bottom of the available filters list
- ➢ In the New Filter dialog box:
  - o Choose one of two options for type of filter:
    - ▪ Linear – Filter bounds have constant value of the non-diameter parameter and include an infinite range of diameters
    - ▪ Polygon – Filter has 2D, user-defined polygonal boundaries similar to a gate commonly used in analysis of flow cytometry results.
  - o Select the filter parameter of choice from the dropdown (e.g., Transit Time)
  - o Specify a descriptive name for the filter
  - o Specify the limits of the filter in one of two ways:

- Enter min and max values for the non-diameter parameter in the entries provided (linear filters only)
- Specify the bounds of the filter directly on a scatter plot using the mouse
  - Click "Create" to add the filter to the list of available filters.
- The effects of peak filtering will be reflected in any of the viewing methods available (e.g., CSD plots, parametric scatter plots). In Single and Combined viewing mode, both the raw and filtered datasets are displayed in the scatter plots.

The refined and filtered Stats files can now be saved individually or combined into a single Combined file format as described in workflow step 3, below. To save one or more Stats files with the applied filters and scaling, navigate to the Data Output tab of the Viewer and use the controls in the Save frame.

- Saving a Stats file creates a new file with an incremented extension number, and does not overwrite existing Stats files present in the current working folder.

3. Combine and Compare

At this point in the workflow, the Currently Loaded file list typically contains multiple Stats files that together represent a polished analysis of the particle content of a single physical sample. The goals of this workflow step are to:

1. Combine the multiple Stats files into a single entity for simpler manipulation.
2. Use the Viewer's quantitative analysis tools to compare the measurement to others.

3.1 Combine multiple Stats files into a single Combined file
Combine Procedure

- Load the desired Stats files into the Currently Loaded file list.
- Navigate to the Data Output tab of the Spectradyne Viewer.
- In the Combine Stats frame, use the "All Loaded" button to combine all loaded Stats files into a single Combined file.
- A new file will be generated in the current working directory with file name of the form, "YYYYMMDD_ccNN.h5", where NN is an incrementing number unique for each day.

3.2 Compare Multiple Analyses

- Load the analyses of interest into the Currently Loaded file list.
- In the QuickView Processed Data frame of the Spectradyne Viewer, select Plot Mode Multi.
- Use the tools in the Data Visualization and Quantification tabs of the Viewer to make quantitative comparisons of the analyses.
- Scatter and CSD plots will display separate results from each loaded analysis on the same axes, and quantitation tools will report results for each analysis separately.

Quantifying Particle Size Distributions – Concentration in the CSD

➢ The concentration of particles over a certain size range is readily obtained after plotting the CSD (Figure 15):

❖ In the Quantification tab of the Viewer, click the "Select Range" button.
❖ Click and drag the mouse cursor across the CSD over the size range of interest.
❖ Click "Measure Concentration" to report the concentration over the selected size range.
❖ The concentration measurement is displayed on the plot.

Quantifying Particle Size Distributions – Fitting Gaussian Peaks in the CSD

➢ Peaks in the CSD can be fit to a normal distribution to obtain the mean and width of the peak (Figure 15):

❖ In the Quantification tab of the Viewer, click the "Select Range" button.
❖ Click and drag the mouse cursor across the CSD over the size range of interest.
❖ Click "Fit to Gaussian" to fit the selected region to a normal distribution.
❖ Fit results and the concentration of particles over the fit range are displayed on the plot

The above quantification methods perform the same functions when plotting multiple CSDs (multi-mode). The CSD corresponding to the file selected in the Currently Loaded Files dropdown is the one that is measured.
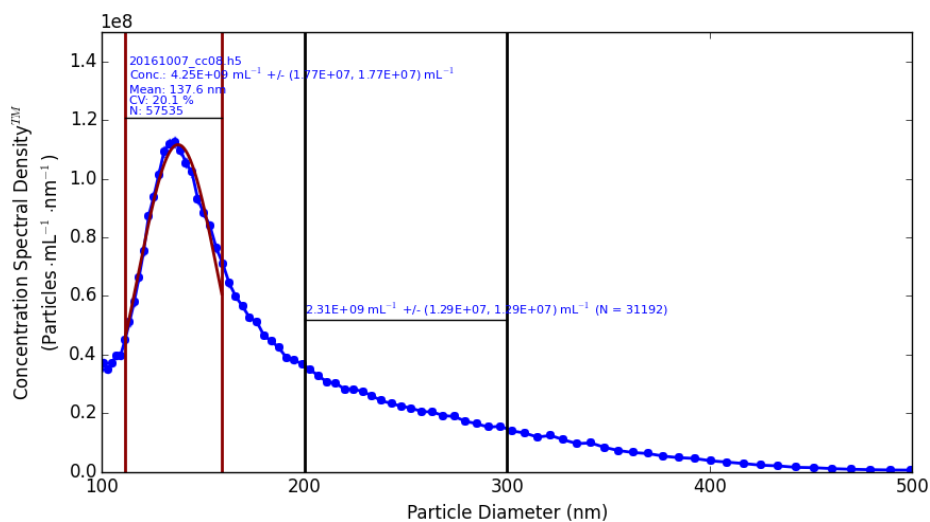

Figure 15. Quantification of a CSD plot. The peak is fit to a Gaussian distribution and the concentration of particles in the range of 200-300 nm is measured directly.

Note: Exporting Stats data to other file formats

Navigate to the Export frame in the Data Output tab of the Viewer.

- ➢ Two formats are available for export:
  - o Microsoft Excel format (.xlsx)
  - o MS DOS CSV format (.csv)
- ➢ When exporting to Excel format ("Excel" button), the file selected in the Currently Loaded files list is exported to two sheets in a single file named <original stats filename>.xlsx:
  - o Sheet 1 contains the metadata associated with the file (e.g., acquisition parameters, processing parameters, etc.).
  - o Sheet 2 contains the CSD data and a chart showing the CSD.
- ➢ When exporting to CSV format ("CSV" button), the selected file is exported to a single sheet in a file named <original stats filename>.csv. CSD data is placed 'below' the metadata in the sheet.
- ➢ The "All…" button saves all the stats files in the Currently Loaded files list to a single file.
  - o A dialog window permits the user to choose the export format and the filename.

More Information and Technical Support

More detailed information on the operation of the nCS1 and its software can be obtained by sending email to support@spectradynellc.com.

At Spectradyne we are eager to help with your particle analysis. Please contact us for help with specific measurement challenges and for help with the analysis of specific particle populations.

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