Interpreting the Autocorrelation Functions in DYNAMICS

Summary

The autocorrelation function (ACF) is the primary data stream from DLS measurements (Figure 1). Consequently, it is critical to verify the quality and fit of the ACF before any size information can be interpreted. This technical note will provide information about what a good ACF looks like and provide examples of various ACFs and what they might indicate for respective experiments. Suggestions for improving data quality are also provided.

Related Technical Notes and References

M1406 DYNAMICS User's Guide

M3001 Möbiuζ User's Guide

M3100 DynaPro Plate Reader Plus User's Guide

M3101 DynaPro Plate Reader II User's Guide

M3103 DynaPro Plate Reader III User's Guide

M3300 DynaPro NanoStar User's Guide

TN2005 Cumulants versus Regularization Analysis

TN7002 Screening for Compound Aggregates

TN9000 Quartz Cuvette Cleaning Protocol

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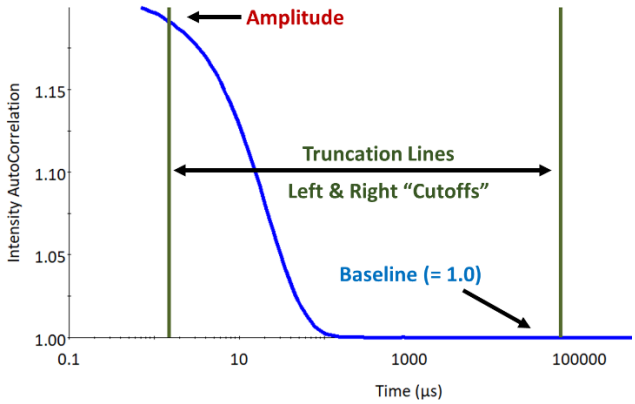


Figure 1: An example of an autocorrelation function (ACF) that is fit to determine the decay rate, which subsequently is used to relate the diffusion coefficient to a hydrodynamic radius. An ACF consists of an amplitude, a baseline, and two cutoffs for data fitting.

Introduction to Autocorrelation Functions (ACFs) in DYNAMICS

Through dynamic light scattering (DLS), also referred to as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS), it is possible to measure the fast (microsecond) fluctuations of scattered light intensity from solute in solution to determine an effective hydrodynamic radius (R_h). It is important to note that DLS does not measure radius directly but instead relates the translational diffusion coefficient (D_t) to the radius of a theoretical sphere that is used to describe the measured sample. Figure 2 summarizes how the hydrodynamic radius is determined from DLS. First, the superposition of scattered waves from particles leads to constructive or destructive interference at the detector. Consequently, Brownian motion results in light intensities that fluctuate with time. The timescale of these fluctuations, related to the decay rate of the ACF, is a measure of the diffusion coefficient. Subsequently, the hydrodynamic radius, R_h, of a particle can be related to the diffusion coefficient by the Stokes-Einstein Relation.

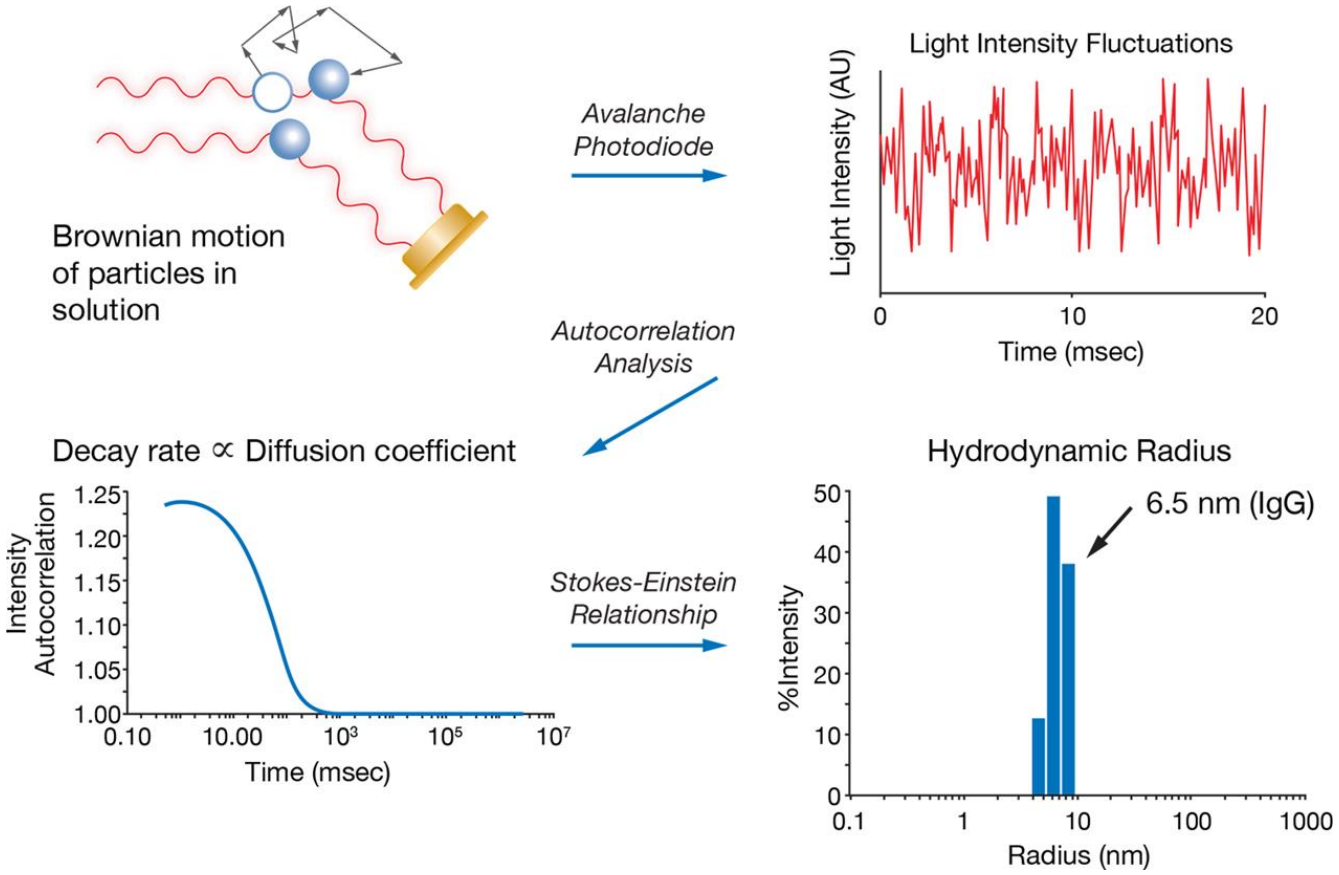


Figure 2. Process through which light intensity fluctuations due to Brownian motion from a sample in solution are utilized to determine how quickly light intensity changes with time. How the intensity fluctuations change can be related via an autocorrelation analysis to determine the decay rate. Smaller particles, which diffuse more quickly and fluctuate faster, lead to shorter decay times than larger particles, which can be related to hydrodynamic radius.

Once data has been collected from a DLS measurement, DYNAMICS proceeds to fit the ACF and provides data in the **Datalog Grid** and **Regularization Graph**. Before this data can be interpreted correctly, however, the ACF should be inspected to ensure its quality. Provided in Figure 3 below, the ACF window in DYNAMICS is outlined by selecting the appropriate button in the analysis ribbon. On the left side of the window, the average of the

unmarked acquisitions is provided for each measurement while individual ACFs for each acquisition can also be viewed. On the right side of the window, the control panel enables the display of the Cumulant and Regularization Fit, which when enabled will display a residuals graph below the ACF as a measure of the error of the fit over time.

Note: Use the DYNAMICS data filter to remove ACFs that contain artifacts due to dust or aggregates. More information can be found in **TN2009 – Data Filtering in DYNAMICS.**

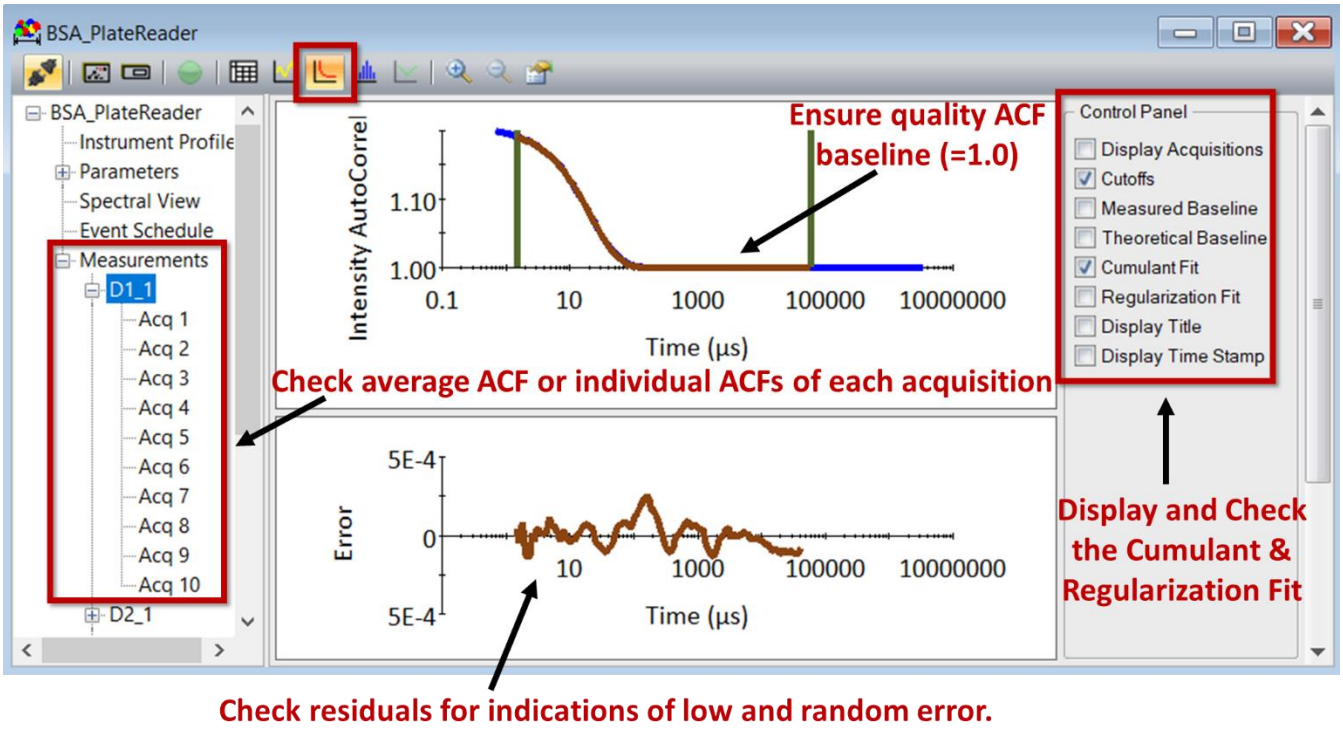


Figure 3. The experiment window demonstrating the correlation graph button (top ribbon), the measurements (left side), the control panel (right side), the autocorrelation function (top middle), and the residual graph (bottom middle), which displays the difference between the fitted and measured autocorrelation curve.

Recommendation: Always observe the shape and quality of the ACF before interpreting particle sizes from Cumulants or Regularization analyses.

This technical note will detail various behaviors that can occur in ACFs and how to interpret them. Once a quality ACF has been identified, the resulting Datalog Grid and Regularization Graphs can be more confidently trusted to be representative of the sample and not due to other factors such as contamination or noise.

Interpreting ACFs in DYNAMICS & Guidelines for Improving their Quality

An ACF can be described by several parameters and is typically fit within limits to ensure only the highest quality data is used for fitting. These are outlined below in Figure 4, which include the amplitude of the ACF, the determined baseline after decay, and the region of fitting—determined by the truncation lines, also referred to as the left and right "cutoffs." Ultimately, the quality of the fit will determine the reliability of the data but understanding the appropriate behavior of the ACF will increase confidence in the data correctly describing the sample of interest instead of contamination or other causes of intensity fluctuations.

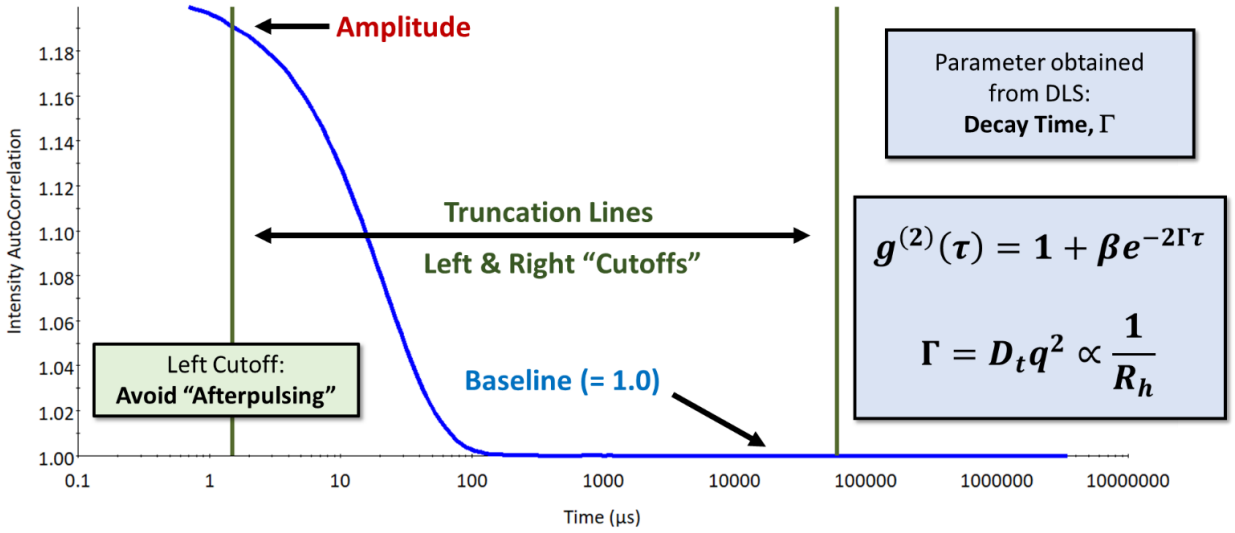


Figure 4. The components of an ACF that are provided in the correlation graph analysis, which include the amplitude, the baseline, and the cutoffs. DLS as a measurement technique provides the decay time of a sample's autocorrelation function by fitting the measured ACF, which is subsequently related to the diffusion coefficient and then the hydrodynamic radius, R_h.

The signal amplitude (sometimes also expressed as intercept) can range between 0 and 1 and is treated as a variable parameter when fitting the correlation function. The amplitude is not related to any specific property of the sample per se as it is primarily determined by the size of the detection (scattering) volume and optical characteristics such a stray light. For low concentration samples, stray light dominates the signal and ACF amplitudes are typically low and show a correlation to sample concentration. However, is not possible to quantify concentration by measuring the amplitude. For comparing concentrations, it is most appropriate to use the *Normalized Intensity* data available in the Datalog Grid. As the light scattering signal is proportional to molar mass and the concentration, doubling the concentration for a given sample will result in twice the light scattering intensity, which is expressed as *Normalized Intensity* in DYNAMICS.

Typically, a few data points at the beginning and the end of the ACF are excluded using the cutoffs or truncation lines to minimize noise and any potential artefacts. In most cases, the default cutoffs of 1.5 µs and 6.0 x 10^{-4} µs provided in DYNAMICS are appropriate.

The remainder of this technical note will explore examples of ACFs and strategies for interpreting or improving them for data analysis.

Example ACFs of Good Quality Monomodal Populations

ACFs shown below in Figure 5 are typical for monomodal size distributions and are two examples of good quality decays. Provided that the experimental conditions are similar (i.e. solvent and temperature), large particles can qualitatively be distinguished from small particles by the longer decay of the ACF.

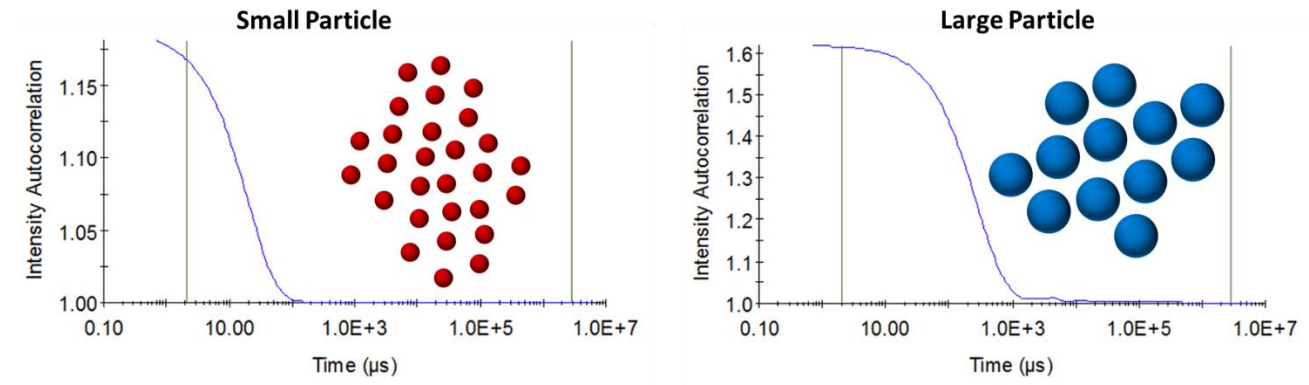


Figure 5. ACFs for typical monomodal size distributions.

Example ACFs of Control Solvent or Weak Signal Samples

ACFs shown below on the left-side of Figure 6 are typically expected for pure buffer. Measuring buffer under the same filtration procedure as your sample can be useful to confirm the absence of aggregates or contamination in the buffer itself. Some buffers that contain larger molecules or micelles, for example sucrose, PEG, Tween, or SDS, may exhibit a noisy decay or may even appear as a small particle. For example, the weak signal ACF in Figure 6 is filtered buffer containing sucrose. If your sample, however, exhibits this weak signal, it may be due to insufficient analyte. In this case, it may be necessary to:

- Collect more acquisitions
- Extend acquisition time (provided samples are clean)
- Increase laser power (if manually set)

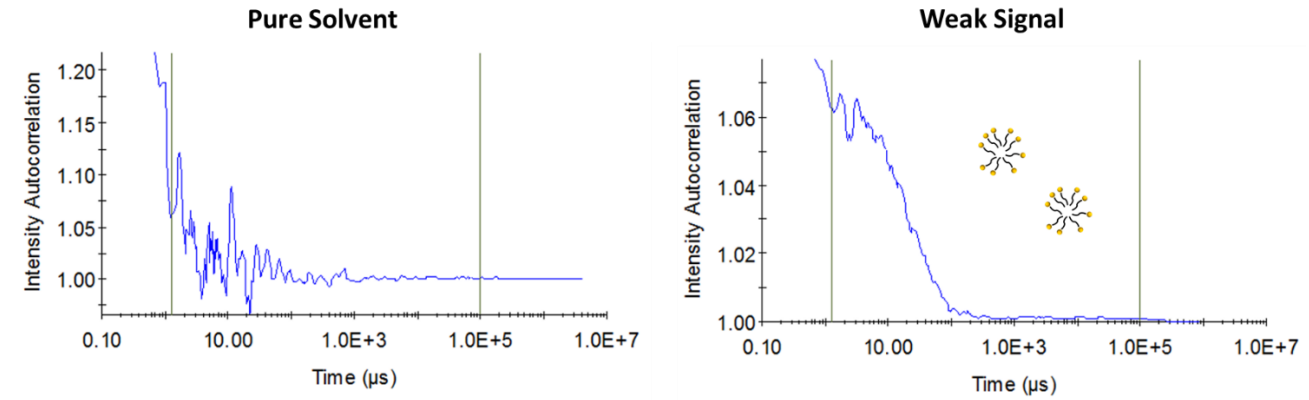


Figure 6. ACFs for pure solvent or weak signals.

Example ACFs of Multimodal Populations or Large Aggregates

ACFs shown below in Figure 7 demonstrate two situations where attention to the baseline is critical. On the left side, this multimodal population decay has a well-defined baseline and can therefore be readily analyzed; however, the large aggregate decay on the right side does not decay to baseline within the acquisition time. For cases of multimodal populations, these are two decays representing a smaller particle and a larger particle. For cases of premature termination, the following considerations apply:

- Error is likely for size distribution analysis if not baseline resolved.
- Acquisition time is too short relative to decay time (for the large molecules); however, longer acquisition times are generally unsuccessful because of the presence of these aggregates.
- The best strategy is to centrifuge or filter samples to remove the very large aggregates to increase reproducibility and reliability of the results.

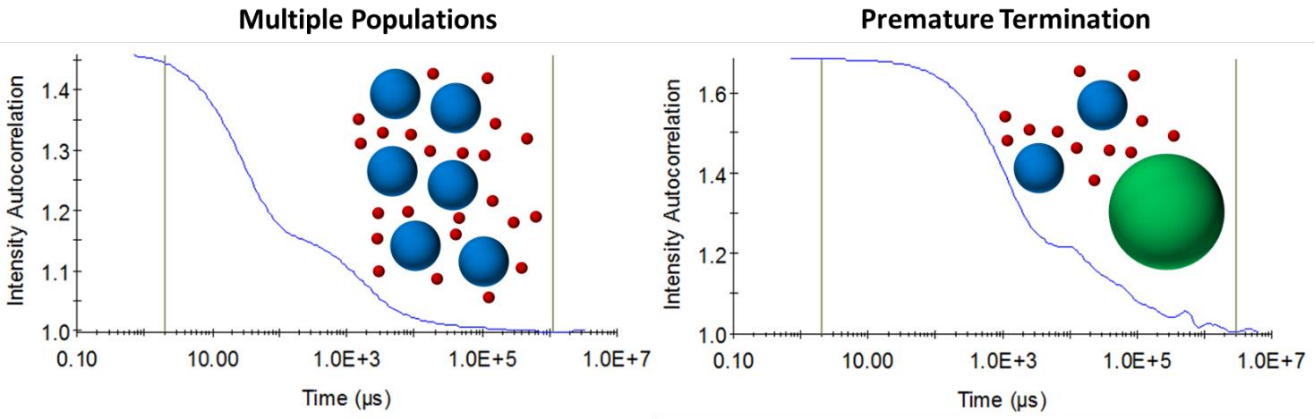


Figure 7. ACFs for multimodal populations or premature termination

Example ACFs of Particle Fluctuations or Large Particulates

ACFs shown below in Figure 8 demonstrate two situations that may suggest the sample needs treatment to remove particulates that are potentially beyond the measurement limits of the instrument or to address rapid or large intensity changes. On the left is an example of an ACF with a "foot" or a plateau that is indicative of particle number fluctuations. The following considerations are applicable:

- It is appropriate to mark out these acquisitions, which result from very low particle concentrations that cause the number of particles in the scattering volume to fluctuate during the acquisition.
- If persistently present, one may need to filter or centrifuge the sample.
- Shorter acquisitions times with a higher number of acquisitions, where acquisitions with a second plateau are marked out, can help to acquire good data.

On the right is an example of an ACF of large particulates. For these types of measurements, the DYNAMICS data filter is often of limited success. Instead, the presence of these large particulates will necessitate filtration or centrifugation (these particles are larger than the measurable size range by DLS anyway).

This shape of the autocorrelation function may also be caused by dirty cuvettes and can be improved by thoroughly cleaning cuvettes as described in *TN9000 Quartz Cuvette Cleaning Protocol*. Typically, these bubbles, aggregates, or large particulates are beyond the maximum size that is compatible with the instrument (radius of several microns). The use of a new well plate is recommended when measuring samples with the Plate Reader. Well plate cleanliness or the presence of bubbles may also be monitored by the built-in camera.

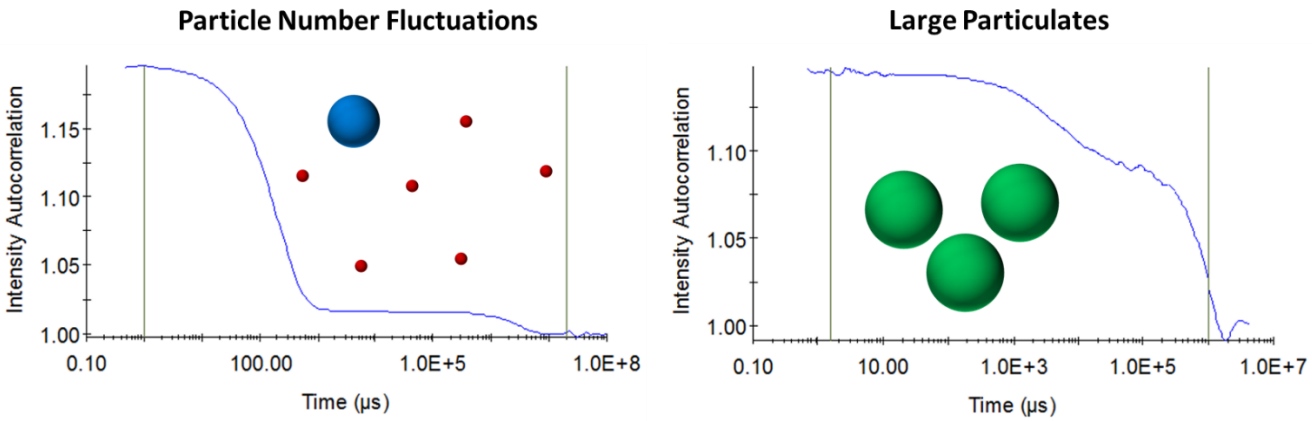


Figure 8. ACFs for particle number fluctuations or large particulates.

Strategies for Data Filtering

DYNAMICS contains a Data Filter that can be used to automatically mark out acquisitions to exclude them from the measurements and results. By right-clicking on the Datalog Grid view, the Data Filter (Ctrl + F) Settings can be opened. An example with typical settings for DLS filter parameters is shown below. Here, a **Baseline Limit**, **Maximum SOS**, and **Intensity Fluctuation (%)** can be assigned a numerical limit that helps to automatically filter measurements. These filters can be toggled on or off and manually marking out data will override these filters, which enables flexibility in analyzing ACFs.

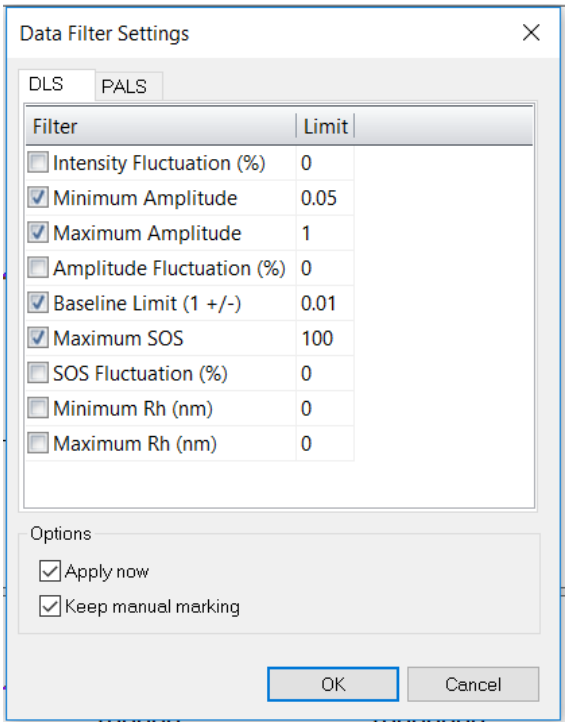


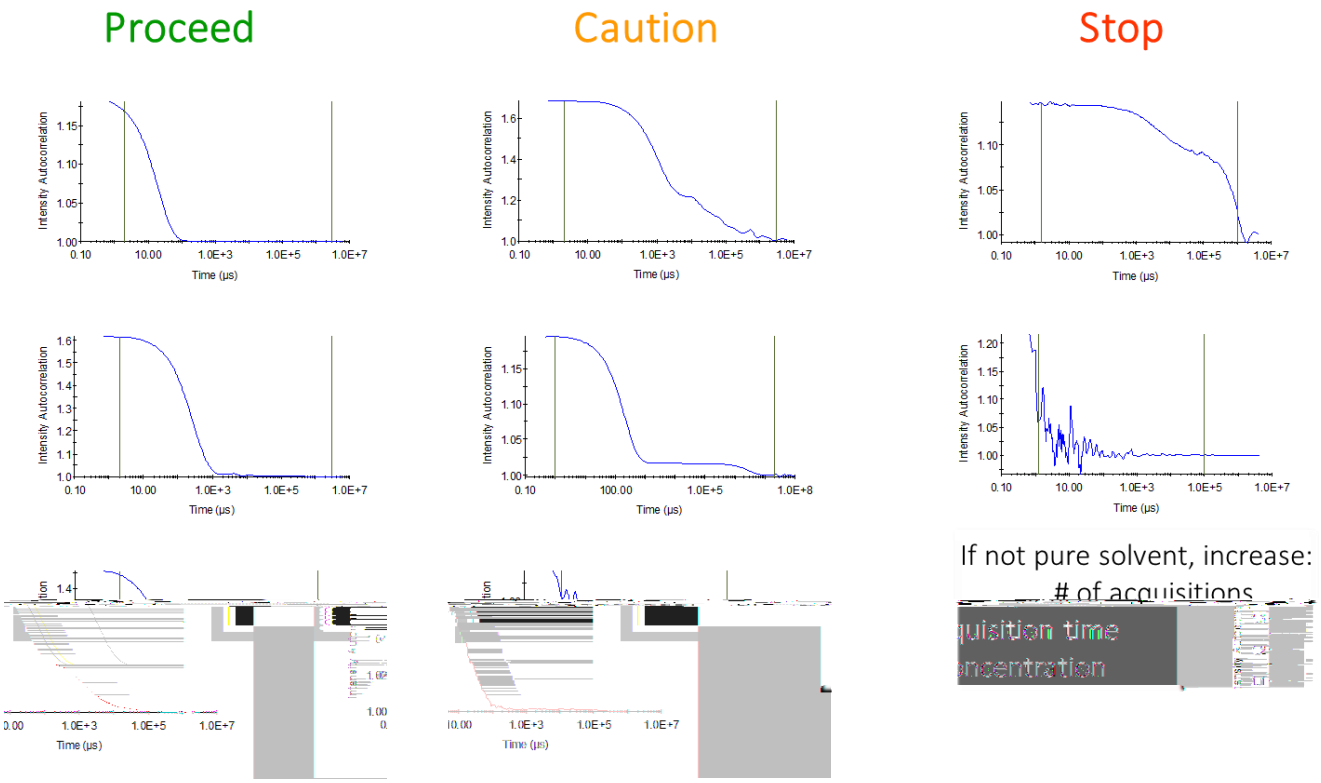
Figure 9. Data Filter Settings by Right-Clicking the Datalog Grid View ( )

Some common DLS filters that are recommended are:

- **Baseline Limit (1 ±)**: A typical value of 0.01 can exclude ACFs that suffer from particle contamination, number fluctuations, premature termination, or other sources of noise.
- **Maximum SOS**: A typical value of 100 can exclude multimodal data and data with no ACF, such as solvent. This parameter is intended to filter out outliers and is most often varied.
- **Minimum Amplitude**: A typical value of 0.05 can exclude data from low concentration samples.
- **Maximum Amplitude**: A typical value of 1 is considered the maximum value for a good ACF.

Summary

As the primary data stream in DLS measurements, visually inspecting the autocorrelation function (ACF) is invaluable prior to interpreting the results provided by DYNAMICS. This can be optimized with the built-in data filter. Below is an overall summary of the most common type of observed ACFs:



Appendix.

Sample Fluorescence

DLS analysis may be hampered by sample fluorescence, as the fluorescence background may eventually dominate over the usable signal, i.e., intensity fluctuations due to the Brownian motion of the sample itself. Generally, some sample fluorescence can be tolerated as the DLS analysis does not depend on the absolute light scattering intensity. When fluorescence is present, a very high count rate (fluorescence background) will be observed while the ACF will exhibit a low amplitude and might appear noisy. Note that the correct particle size will still be measured. If the excitation wavelength is very close to the laser wavelength of the DLS instrument, the ACF will resemble that of a pure solvent or buffer and no usable signal can be obtained. It is always recommended to measure absorption and fluorescence spectra of a sample when fluorescence is suspected.

The DynaPro NanoStar is available with a standard 660-nm or custom 785-nm laser, with the near-IR laser less prone to excite fluorescence. The DynaPro Plate Reader uses an 830-nm laser, where fewer fluorescent labels or polymers will emit; however, these higher wavelengths come at the cost of less sensitivity for a given laser power. The Mobius is available with a standard 532-nm laser but has the benefit of being compatible with an inline narrow passband fluorescence-blocking filter on the APD.

Below in Figure 10 is an example of the effects of fluorescence on a measurement of a gold nanoparticle with an absorption maximum near 532 nm. For this sample, the measurement with the NanoStar at 660 nm wavelength resulted in a good ACF, whereas due to the fluorescence emission maxima for the sample occurring near the 532-nm laser wavelength of the Mobius, the signal was obscured by strong fluorescence and provided no useable autocorrelation function.

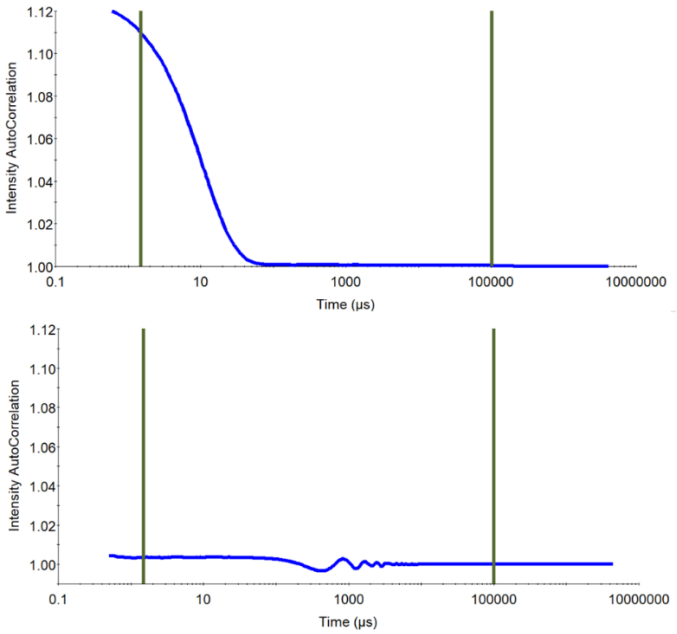


Figure 10. Two ACFs of a gold nanoparticle in chloroform measured via the NanoStar (top) and Mobius (bottom), where the fluorescence emission maxima for the sample is at the laser wavelength of the Mobius. Because of the extreme fluorescence at the Mobius wavelength, the signal is overwhelmed and provided an exceptional high count rate but a low amplitude with no useable signal.