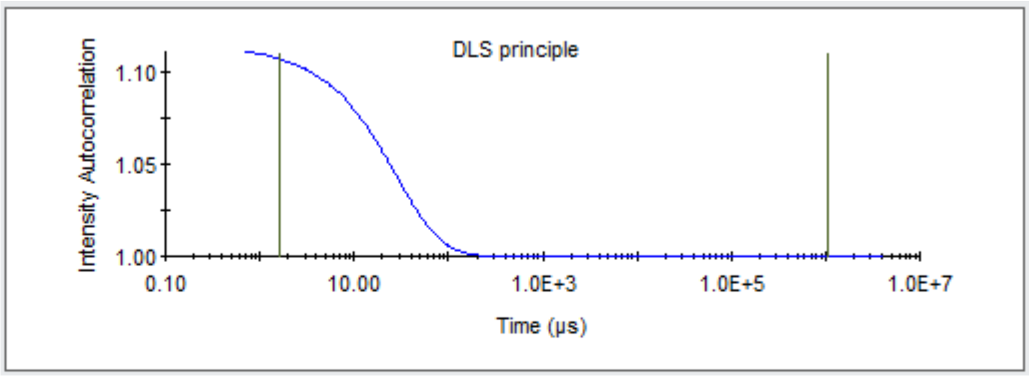
From DYNAMICS User's Guide (M1406 Rev. F)

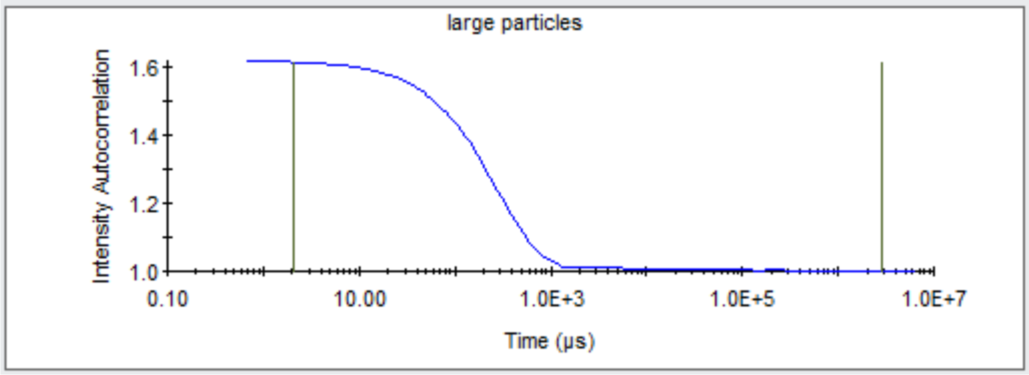
Evaluating Correlation Functions

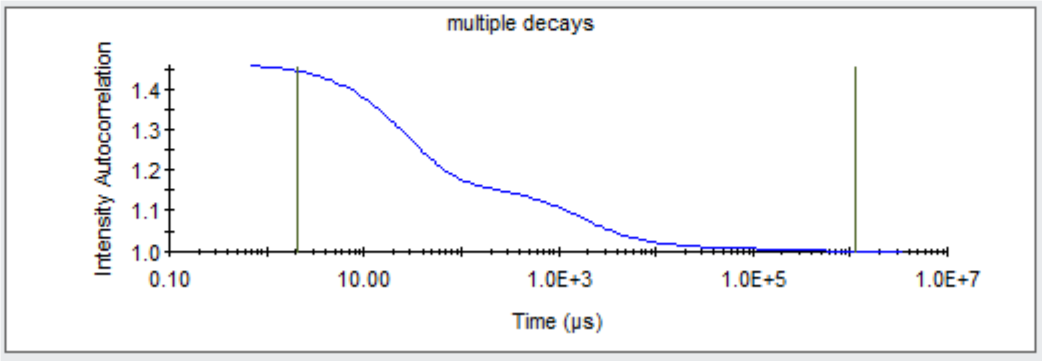
The following figures show examples of when you should continue with data interpretation, when you should try to improve the quality of the data before proceeding, or when you should stop.

Proceed Category

The following three figures show examples of the correlation function in the "Proceed" category. If the correlation function is in the Proceed category, continue with the data interpretation.





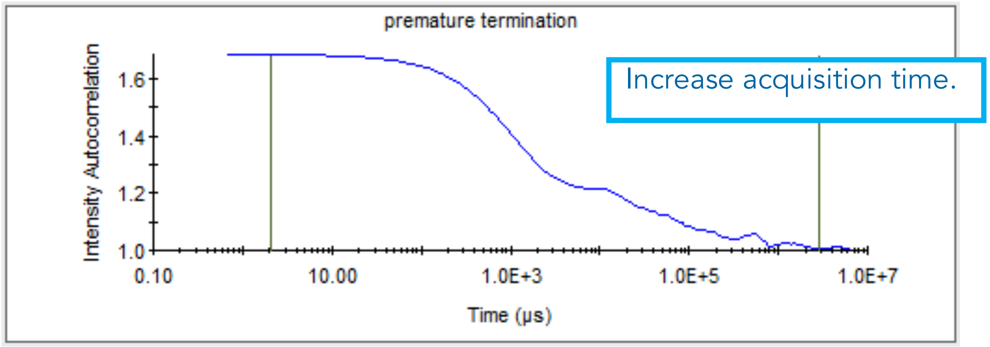


Caution Category

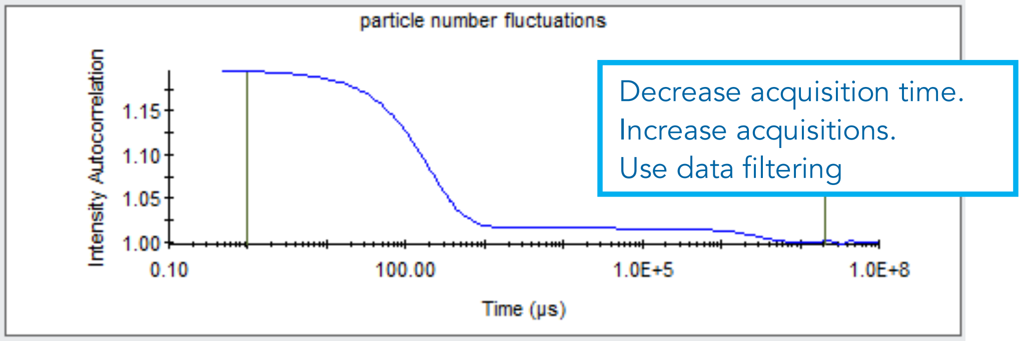
The following three figures show examples of the correlation function in the caution category. Before proceeding, attempt to improve the quality of the data by following all or some of the recommended changes to the experiment.

Leave the sample in the instrument and follow these steps:

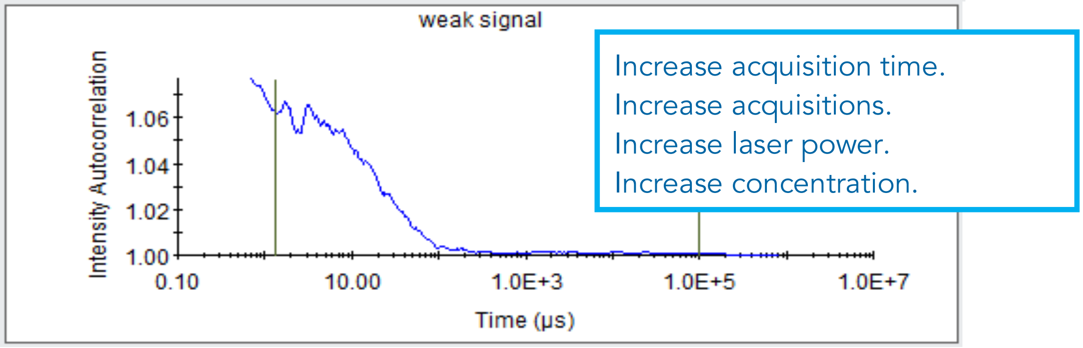
- Change the acquisition time: Increase the time for incomplete decays, or decrease the time if a number of fluctuations are present, and/or increase the number of acquisitions.
- Increase laser power (to a maximum of 100%). Or, change both the acquisition time and laser power as described in the figures below.
- If none of these steps lead to functions shown in the "Proceed" category, it may be necessary to increase the concentration of the analyte. Ultimately, you may accept the imperfect data from this category and continue.



Increase acquisition time.



Decrease acquisition time.
Increase acquisitions.
Use data filtering

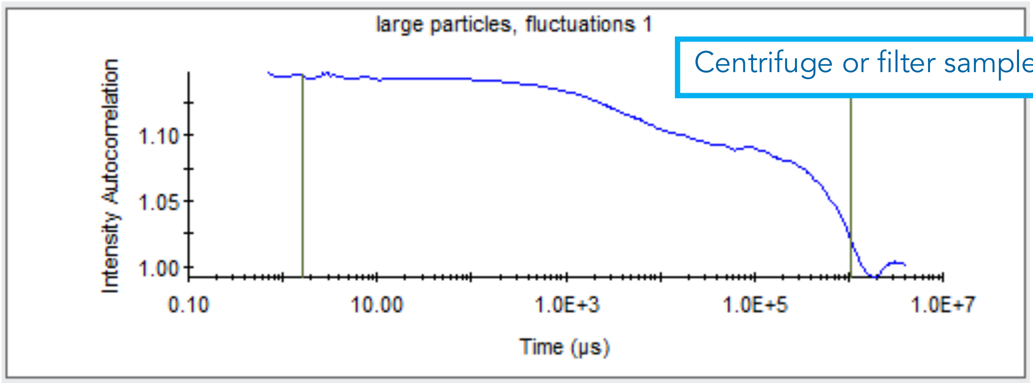


Increase acquisition time.
Increase acquisitions.
Increase laser power.
Increase concentration.

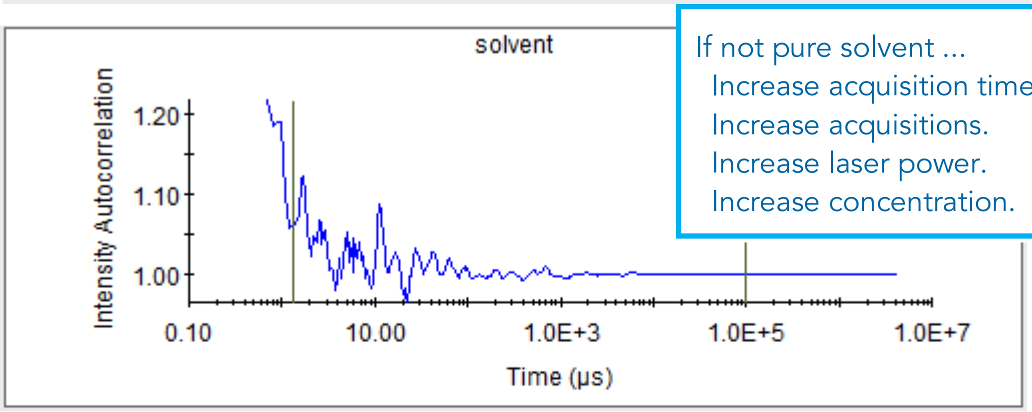
Stop Category

Do not proceed with data interpretation if the correlation function appears as shown in the following two figures.

- If the graph appears as in the first figure, the sample probably contains large particles, and should be centrifuged (6,000 x g for 10-30 min works well to remove micrometer size particles) or filtered using syringe filters (0.1 µm).
- If the graph appears as shown in the second figure, make sure the cuvette or well plate is inserted properly, the lid is closed, and that the sample is not pure solvent.
- If all of these items check out OK, follow the recommendations under the "Caution" category. If these steps fail, contact Wyatt Technology Support.



Centrifuge or filter sample.



If not pure solvent ...
Increase acquisition time.
Increase acquisitions.
Increase laser power.
Increase concentration.

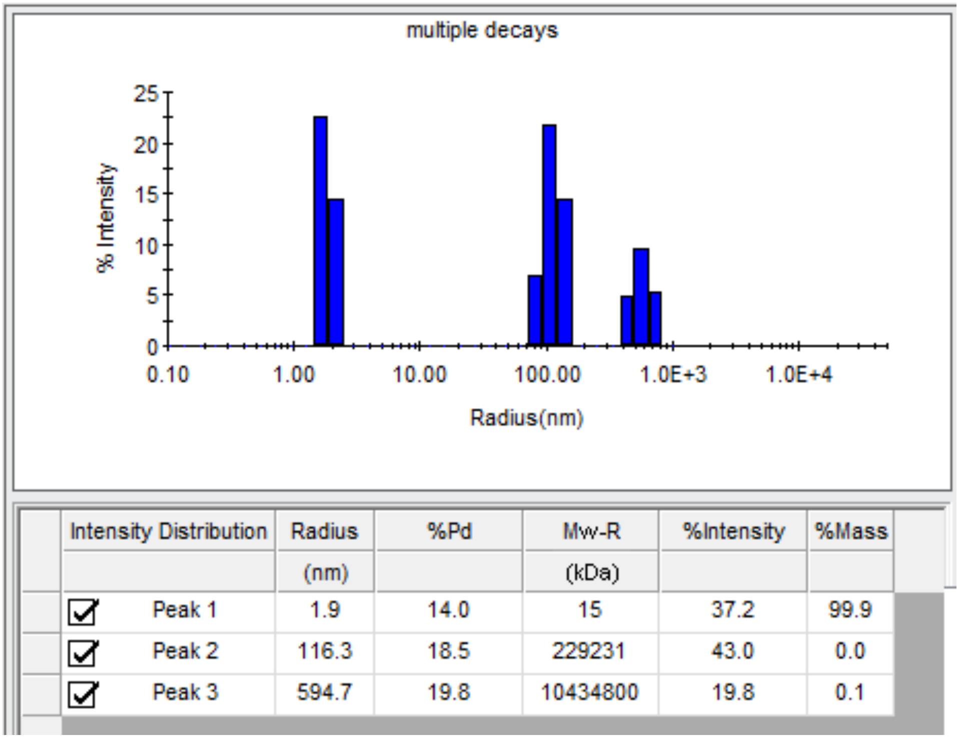
Size Distribution Results

You view the size distribution results in the Regularization Graph.

- Click the Regularization Graph button in the toolbar to display a Regularization Graph.

The Regularization Graph shows the calculated size distribution for the autocorrelation curve associated with a measurement or acquisition selected in the experiment tree.

The Results Summary table located below the size distribution histogram describes the number of peaks and their mean size (Radius), % polydispersity (%Pd), molar mass estimated from the measured radius (Mw-R), relative intensity scattered by each population (%Intensity), and estimated relative amount of mass (%Mass) or number of particles (%Number) in each peak or species.



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☑ Peak 1	1.9	14.0	15	37.2	99.9
☑ Peak 2	116.3	18.5	229231	43.0	0.0
☑ Peak 3	594.7	19.8	10434800	19.8	0.1

Monomodal Size Distribution

The following histogram has one peak, so it is called a monomodal size distribution. The peak is defined by the mean radius and polydispersity. The width of the peak is the standard deviation of the weighted bin values, also known as the Polydispersity. The mean radius of the peak is defined by a weighted average of the number of bins comprising the histogram, in this case three. The bins by themselves do not represent real, distinct, physical particles; however, their mean and standard deviation do.

Multimodal Size Distribution

Histograms with more than one peak are multimodal size distributions. The presence of different and resolvable species in the sample cause modes in the size distribution. To be resolved as a separate peak, a species must have a size (radius) larger than another species by a factor of five or more, and be detectable (produce sufficient scattered light for detection). When the sizes of the species are below these requirements, a separate peak will not be resolved for each species.

By definition, a multimodal size distribution is heterogeneous—the sample contains distinct populations of particles that are not the same size. The instruments can resolve up to four or five modes in a size distribution. For each mode, DYNAMICS estimates the relative amount of light scattered and the relative amount of mass based upon one of several possible particle scattering properties. Often, the relative amount of mass of a peak is quite small, for example, less than 0.1%, and is considered to be negligible.

Polydispersity

Polydispersity refers to the level of homogeneity of the sizes of the particles. When the level of homogeneity is high, the particles can be considered to be virtually identical in their size, or monodisperse. **The level of homogeneity is considered high when the percent polydispersity is less than 15%**.
When the level of homogeneity is low (percent polydispersity greater than 30%), the particle population can be considered to contain significantly different sizes, or to be polydisperse. If the sample is polydisperse, the Datalog Grid contains "Multimodal" in the %Pd column.

Polydispersity is caused by the presence of different species that cannot be resolved by the technique of dynamic light scattering (species with sizes differing by a factor of less than five cannot be resolved). A peak containing 100% monomer will have a smaller polydispersity than peak containing a mixture of monomer:octamer. The peaks shown here all have % Polydispersity greater than 30%.

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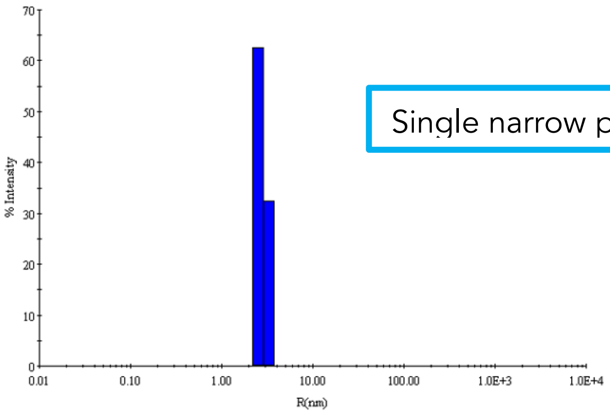
Size Distribution Interpretations

Monomodal Monodisperse

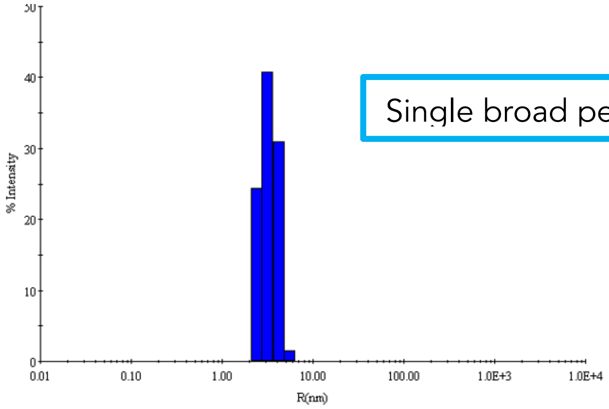
BLGA, 4 mg/ml, PBS, T = 25 °C
Peaks: 1
Mean Radius: 2.8 nm
% Pd: 13.8%
Majority monomer



Single narrow peak

Monomodal Polydisperse

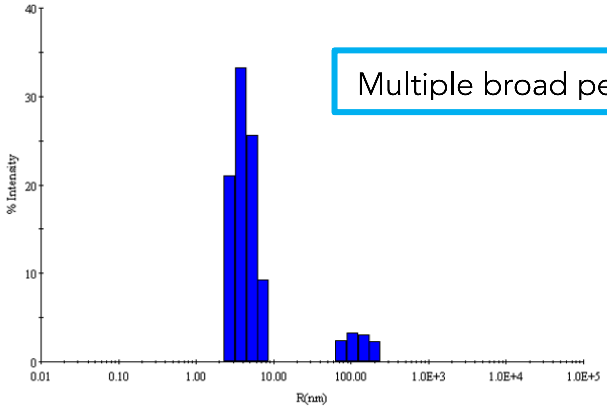
BLGA, 4 mg/ml, PBS, T = 5 °C
Peaks: 1
Mean Radius: 3.4 nm
% Pd: 22.%
Increasing amounts of Dimer



Single broad peak

Multimodal Polydisperse

BSA, 2 mg/ml, PBS, T = 25 °C
Peaks: 2
Peak 1:
Mean Radius: 4.3 nm
% Pd: 32.1%
Monomer, Dimer, Trimer
Peak 2:
Mean Radius: 130.9
% Pd: 34.5%
Various non-specific aggregates



Multiple broad peaks