

NANOCOMPOSIX'S GUIDE TO DYNAMIC LIGHT SCATTERING MEASUREMENT AND ANALYSIS

FEBRUARY 2015, v 1.4



4878 RONSON CT STE K
SAN DIEGO, CA 92111
858 - 565 - 4227
NANOCOMPOSIX.COM

Note to the Reader: *We at nanoComposix have published this document for public use in order to educate and encourage best practices within the nanomaterials community. The content is based on our experience with the topics addressed herein, and is accurate to the best of our knowledge. We eagerly welcome any feedback the reader may have so that we can improve the content in future versions.*

Please contact us at info@nanocomposix.com or 858-565-4227 with any questions or suggestions.

GUIDELINES FOR DYNAMIC LIGHT SCATTERING MEASUREMENTS

This document is designed to help our customer maximize the value of DLS measurement data. After analyzing thousands of nanoparticle samples we've assembled a set of guidelines that can be used to determine how to maximize the quality of your DLS data and, more importantly, how to interpret your results. We hope that you find this information useful and if you have any questions or comments, please feel free to contact us at info@nanocomposix.com or (858) 565-4227 x 2.

CONTENTS

Introduction to Dynamic Light Scattering.....	3
What to Expect from your DLS Data	3
High Confidence Samples	3
More Challenging Samples	3
Sample Preparation Advice.....	4
How Much Material to Submit	4
Sample Preparation Techniques for High Quality Data	5
Interpreting Your Results	5
Data Package Description	5
Size Distributions by Intensity, Volume and Number	6
Intensity Distribution	6
Volume Distribution.....	6
Number Distribution	7
Cumulants/Correlogram	7
Malvern Tech Note Bibliography:	8

INTRODUCTION TO DYNAMIC LIGHT SCATTERING

Dynamic Light Scattering (DLS) is an important tool for characterizing the size of nanoparticles in solution. DLS measures the light scattered from a laser that passes through a colloidal solution and by analyzing the modulation of the scattered light intensity as a function of time, the hydrodynamic size of particles and particle agglomerates can be determined. Larger particles will diffuse slower than smaller particles and the DLS instrument measures the time dependence of the scattered light to generate a correlation function that can be mathematically linked to a particle size.

DLS is a valuable tool for determining and measuring the agglomeration state of nanoparticles as a function of time or suspending solution. When DLS sizing data is compared to Transmission Electron Microscopy images, the aggregation state of the particles can be determined. In an unagglomerated suspension, the DLS measured diameter will be similar or slightly larger than the TEM size. If the particles are agglomerated, the DLS measurement is often much larger than the TEM size and can have a high polydispersity index (large variability in the particle size).

WHAT TO EXPECT FROM YOUR DLS DATA

At nanoComposix, DLS is an important tool for us but we are also very aware of the instruments strengths and weaknesses which allows us to interpret the data accordingly. Here is our ladder of confidence for DLS measurements:

HIGH CONFIDENCE SAMPLES

- 1) For monodisperse (Coefficient of Variation (CV) < 20%) suspensions of unaggregated nanoparticles that have diameters > 20 nm, the DLS produces highly reproducible and reliable measurements. The hydrodynamic diameter is typically larger than the TEM determined diameter by an offset that is a function of capping agent.
- 2) Polydisperse nanoparticle solutions or stable solutions of aggregated nanoparticles (no visible particulates and no particle settling). This class of materials includes dried nanoparticles dispersed with sonication. Typically, the DLS measured diameters will be in the 100 – 300 nm range with a polydispersity index of 0.3 or below. If your sample is in this range it is important that you understand how to interpret intensity, volume and number normalized diameters.

MORE CHALLENGING SAMPLES

- 1) *Any solution that contains large aggregates (visible floating in solution or particles that settle with time).* The presence of the larger particles will dominate the light scattering signal and

mask the presence of the smaller particles. We interpret readings of sizes above 500 nm with PI >0.5 as “big and agglomerated” without giving much weight to the actual numbers.

- 2) *Bimodal solutions.* Using DLS to identify the presence of multiple narrow peaks (say the mixture of 20 nm and 100 nm monodisperse nanoparticles) is possible but highly dependent on the ratio of the particles in solution, their relative size, and the mathematical algorithm used to analyze the correlation function. Use Centrifugal Particle Sizing (CPS) analysis instead!
- 3) *Very dilute samples, especially if the particles are small.* On the instrument report, there is a field that shows the attenuation factor. When the attenuation factor is 11, the sample will be highly sensitive to any dust or other impurities in the solution and the results may be less repeatable than when run at higher concentrations.
- 4) *When measuring very small (<20 nm) nanoparticles.* At this size, the nanoparticles are mostly absorbing and will scatter very little light. In order to get an accurate measurement, much higher mass concentrations of particles will be required compared to the measurement of larger samples. If the attenuation factor is 11, the results may be questionable.
- 5) *Heterogeneous samples.* If you are trying to measure the size of nanoparticles in a solution that has other components, this may affect the result. DLS of nanoparticles in biological media, from an unpurified powder, or in a sample with very broad size distributions can be problematic.
- 6) *Unknown solution properties.* If the temperature dependent viscosity and refractive index of the solvent are not known this will affect the calculated DLS diameter. If there is a high concentration of dissolved solids in solution (PEG, sugars, polymers, etc.) this can affect the viscosity and refractive index.
- 7) *Unknown nanoparticle properties.* The density and refractive index of the nanoparticle is not needed to determine a z-average (cumulants determined hydrodynamic size). However, volume and number calculated densities require a Mie Scattering calculation in order to correctly estimate a diameter. If this is known then it should be provided with a sample so this information can be included in the analysis.

SAMPLE PREPARATION ADVICE

HOW MUCH MATERIAL TO SUBMIT

When submitting a colloidal dispersion for DLS analysis, it is important to submit enough material in order to obtain a sufficient signal and have high quality data. An ideal sample submission for DLS analysis has a volume of 1-2mL and is sufficiently concentrated as to have strong colored hues, or opaqueness/turbidity in the case of a white or black sample. Alternatively, 100-200uL of highly concentrated sample can be submitted and diluted to 1mL or analyzed in a low-volume microcuvette.

SAMPLE PREPARATION TECHNIQUES FOR HIGH QUALITY DATA

In order to maximize the accuracy and reproducibility of DLS samples submitted to nanoComposix, here's what we suggest:

- 1) **Minimize Particulate Contaminants:** Any particulate contaminants will affect the DLS measurements. It is common for a single particle contaminant to scatter a million times more light than a suspended nanoparticle which can dramatically affect your data quality. When preparing your particles, make sure that you use ultra high purity water or solvents, extensively rinse any containers and pipettes with clean solvent before use, and keep samples tightly sealed.
- 2) **Filter:** If there are any visible particulates in your sample or if your particles settle out onto the bottom of your container a few minutes after mixing, it is highly unlikely you are going to get a successful DLS measurement. One strategy is to filter your sample through a 0.2 or 0.45 micron filter before sending it to us for analysis.
- 3) **Concentrate:** If your sample is very dilute spin to concentrate before sending it to nanoComposix. Typically, if the particles are >20 nm and there is a visible color to the solution, the sample is sufficiently concentrated. Otherwise you may have to wait to get an initial analysis back to determine if concentration is a limiting factor for the accuracy of your measurements.
- 4) **Avoid probe sonication:** In some cases, probe sonication can eject particulates from the tip that can interfere with DLS measurements. Bath sonication is better.

INTERPRETING YOUR RESULTS

DATA PACKAGE DESCRIPTION

After your sample is analyzed, you will receive a report that has a lot of important information that needs to be interpreted. Here's what you should look for:

- 1) Your sample will have been run multiple times. We make 6 separate DLS measurements with each measurement consisting of 5 sub-runs with a 10 second duration. A representative run is selected and provided as a print out in your data package. The remainder of the data is summarized in the attached spreadsheet with each run.
- 2) Look at the Expert System Opinion: Malvern has a built in expert system that examines a number of different parameters to determine the confidence in the reliability of the data. If the Result Quality field is "good" then typically there is a good fit to the cumulant data and the data quality is high.

If this is the case, then you will typically quote the Z-Average value as the mean value of the hydrodynamic diameter of your particle. A second important number is the polydispersity index which

is a measure of the width of the particle size distribution. Polydispersity indices less than 0.1 are typically referred to as "monodisperse". The formula for Polydispersity is

$$Pdl = \left(\frac{\sigma}{\bar{d}}\right)^2$$

where the polydispersity (Pdl) = the square of the standard deviation / mean diameter. For example, a particle with a Z-Average value of 100 nm and a Pdl of 0.1 would have a standard deviation of 31.6 nm.

If the polydispersity index is > 0.1, if the cumulants fit error is > 0.001, or if the Result quality is Poor, then the data is more suspect and further analysis should be done to determine if valid data has been generated.

SIZE DISTRIBUTIONS BY INTENSITY, VOLUME AND NUMBER

Your result package will contain a .pdf report, with sizing distributions by intensity, volume and number. When interpreting DLS results, it is important to remember that the measurement is not based upon particle counting, but upon intensity fluctuations of the pattern of light that is scattered by the particles when dispersed. The Intensity distribution, and resulting Z-Ave, is what is actually measured. The other distributions, volume and number, are very useful but somewhat less accurate distributions that are calculated from the measured intensity data, and the optical properties of the material, which aren't often known. Additionally, there are more assumptions used to generate the volume and number graphs.

INTENSITY DISTRIBUTION

The Intensity Distribution describes how much light is scattered by the particles in different size bins. This distribution is the most accurate and reliable distribution, and is closely related to the raw measurements that the instrument takes. When interpreting these data, it is important to keep in mind that there is a very strong dependence of the intensity of light scattered, with respect to particle diameter. These two values have a sixth-power relationship, which means, for example, that a 100 nm particle will scatter one million times as much light as a 10nm particle! The larger portion of a particle distribution *by number* will dominate the signal at the detector of a DLS instrument. It is important to keep this in mind.

VOLUME DISTRIBUTION

The Volume Distribution shows the total volume of particles in the different size bins. This distribution is computed from the intensity distribution, the optical properties of the material (specifically, Refractive Index and Absorption at 632 nm), and the formula for the volume of a sphere: $V = (4/3)\pi r^3$. Note in the formula, that particle diameter (or more specifically, radius) has a third power relationship with volume. This means that, similar to the intensity distribution, the volume distribution will be more heavily weighted towards the larger, more voluminous particles in a distribution.

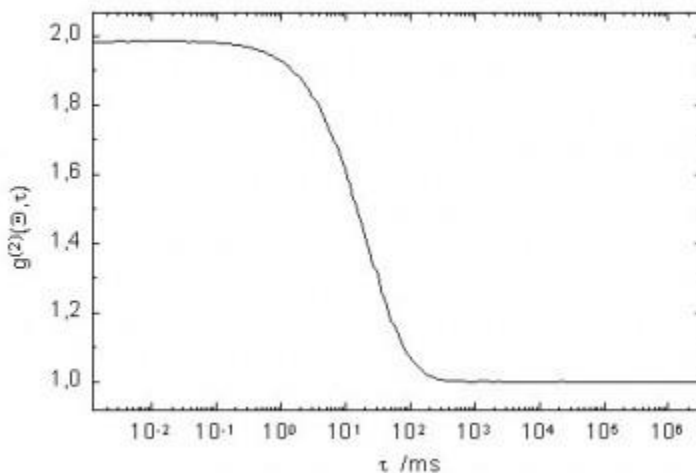
NUMBER DISTRIBUTION

The number distribution, quite simply, shows the number of particles in different size bins. This distribution is meant to depict a distribution that would be obtained from a particle-count technique, like TEM or CPS. In this distribution, there is a simple first-power relationship between particle size and contribution to the distribution. Like the Volume Distribution, this distribution is computed from the intensity distribution and the optical properties of the material. Typical, high-quality DLS results usually see a decrease in diameter when going from Intensity Mean, to Volume Mean, to Number Mean values.

In Summary, it is very important to keep your interpretation of the results in context. The obtained Z-Ave and Intensity values should always be larger than a diameter obtained from TEM – the reasons for this are twofold. Firstly, the results are based upon light scattering, and due to the previously mentioned sixth power relationship between light scattering intensity and particle diameter, the larger particles dominate the signal. Secondly, DLS measures the hydrodynamic diameter, which is the diameter of the particle, plus and ligands, ions or molecules that are associated with the surface and travel with the particle in solution. These ligands, ions or other associated molecules make the particle appear larger to the instrument in comparison to TEM. The difference of hydrodynamic to TEM diameter is dependent on the concentration and molecular weight of the ligands or molecules.

CUMULANTS/CORRELOGRAM

Step #1: Look at the cumulants fit function. Typical correlation data from a DLS measurement is shown below and this function is fit with different mathematical routines to extract a measurement value.



The cumulants fit error should be < 0.005 . If this is not the case then the sample is polydisperse and contains a range of different sizes. Since each size has a different correlation function, the broader the

distribution, the worse the cumulants fit will be. Typically if the polydispersity is > 0.3 , then the Z-Average cumulants fit number cannot be used.

At this point, you have to determine if the data is usable or not. Look at the run to run repeatability of the data in the excel chart. If the Multimodal mean Number Distribution data is consistent run to run, and $>90\%$ of the "% By Number" is in Peak 1, then we would recommend that you report the Number Distribution mean as the DLS result. For this to be meaningful, you must have supplied us with the refractive index of the particle (or told us what it was so that we could use a value that we have validated in house). When you report this result, it is very important to state that this is a Number Distribution mean that was calculated using a Non-Negative Least Squares (NNLS) algorithm and the particle refractive index used in the calculation.

If the multimodal distributions are showing multiple peaks, this is a further sign that the sample is either polydisperse or there are particulate contaminants mixed in with your sample. If the excel data is repeatable, you can use any of the reported metrics as a qualitative assessment of the aggregation state of the particle but the actual number will not be accurate. Even though the DLS software is designed to identify multiple peaks this is almost always wrong and misleading - polydisperse samples are much more common than true bimodal particle size distributions. CPS is a much better technique for measuring samples that have a larger particle size distribution.

If the sample quality is poor, if the attenuator value is 11, if the polydispersity is unexpectedly high, or if there is large variance in the individual runs in the excel sheet, you should follow the steps in Sample Preparation Advice to see if the material can be re-prepared to get a more consistent result.

Malvern has done an excellent job preparing varying technical reports that provide even more information on DLS interpretation. Some of the best articles are linked below:

MALVERN TECH NOTE BIBLIOGRAPHY:

- [Dynamic Light Scattering – An Introduction in 30 Minutes](#)
- [Size Quality Report for the Zetasizer Nano](#)
- [Characterization of Colloidal Gold Using Dynamic Light Scattering](#)