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# Test your resolve

## Producing accurate DLS data analysis

**<box one>**

**Definition of terms**

**Aggregation** is defined as the direct mutual attraction between particles or molecules through van der Waals forces or chemical bonding.

**Turbidity**, $\tau$, of a colloidal dispersion is a quantitative measure of the overall loss of energy from a beam of electromagnetic radiation (by whatever mechanism and in whichever direction) which accompanies transmission of the radiation through the dispersion.

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### Introduction

Dynamic Light Scattering (DLS) is an established industry standard technique used extensively and successfully for the analysis of monodisperse and homogenous sample types. It is, however, recognized that accurate analysis of polydisperse samples - which contain a range of particle sizes - is a more complex problem.

Although it is relatively straightforward to gather enormous amounts of data with any sizing technique, it is more challenging to determine the quality and accuracy of that data. Using analytical dynamic light scattering with backscatter detection it is possible to resolve size distributions for concentration of 1 %w/v. While this is much higher than can be measured on conventional DLS instruments, there are still limits to the application of this technique.

This white paper discusses strategies for producing quality data and maintaining analytical accuracy when carrying out DLS measurements of polydisperse samples. By acknowledging the fundamental physical constraints of measurement techniques, researchers can make clearer decisions when selecting application appropriate particle size instrumentation and produce valid data with confidence.

### Characterizing polydispersions

Particle size and size distribution measurements are central to reliable performance and stability in many industrial dispersion products. Depending on the nature of the product and the particle characteristics sought, one or more analytical methodologies are routinely employed in routine quality control. These include electron microscopy, dynamic light scattering, Fraunhofer scattering, single particle detection techniques, optical microscopy, etc.

With so many sizing techniques to choose from, it is essential for researchers to clearly define the questions they need to answer and the type of data that will provide results pertinent to the problem at hand. Is it surface area or proportion of aggregates that is important? How narrow is the size distribution within the sample? Asking questions such as these from the outset guides selection of the appropriate sizing technique.

### Dynamic light scattering (DLS)

Dynamic light scattering (DLS) is a non-invasive technique used for characterizing macromolecules in solution and particles in suspension. The technique measures the time-dependent fluctuations in the intensity of scattered light that occur because the particles are undergoing Brownian motion. The velocity of this Brownian motion is measured and is called the translational diffusion coefficient D. This diffusion coefficient can be converted into a hydrodynamic diameter (DH) using the Stokes- Einstein equation [1]. Results reflect the sample’s polydispersity in the peak width and the number of peaks produced relates to the modality of the dispersion.

The hydrodynamic diameter obtained by the DLS technique is that of a sphere that has the same translational diffusion coefficient as the particle being measured. The translational diffusion coefficient will depend not only on the size of the particle “core”, but also on any surface structure, as well as the concentration and type of ions in the medium. This means that the size can be larger than measured by electron microscopy, for example, where the particle is removed from its native environment. [1]

DLS is highly sensitive to size changes. The intensity of light scattered from a particle is proportional to its size to the sixth power - if a molecule doubles its size its ability to scatter light is increased by a factor of 64. [2] The lower size limit of the DLS technique is dependant upon the relative difference between light scattered by the molecule or particle being studied compared to that scattered by its dispersant medium. This in turn depends on a number of factors such as the refractive indices of the molecule and dispersant, the concentration of the sample, the power and wavelength of the laser used, the sensitivity of the detector and the optical configuration of the instrument. [3]

### Choosing DLS

DLS is the system of choice when determining the presence of aggregates and defining optimum crystallization parameters. [ref?] Additionally, when used with the correct optical arrangement, DLS is one of the few techniques that offer the sensitivity required to characterize particles at the nanoscale. [5]

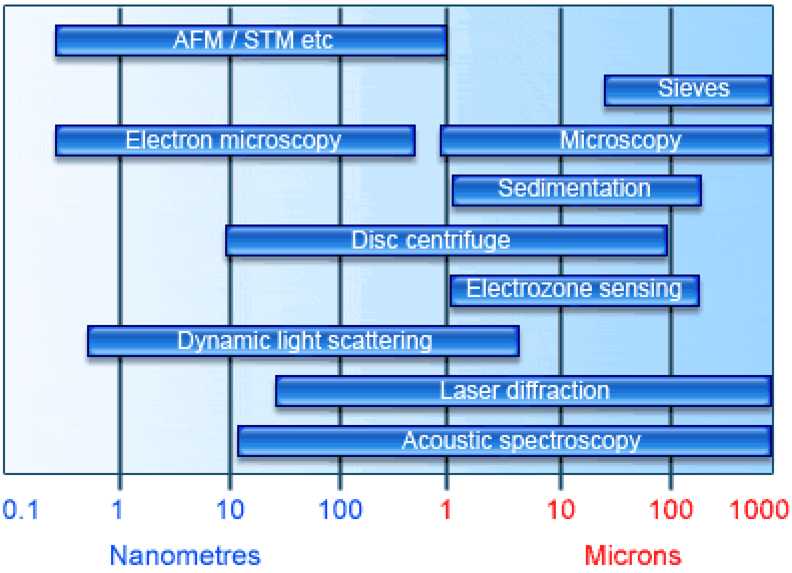

Figure 1: Compared with the number of techniques for measuring the size of a particle greater than 1 micron, there are very few techniques that are able to accurately measure the size of small particles, particularly those less than 10 nanometres.

Providing rapid results with straightforward sample preparation, DLS is the ideal tool for performance and stability testing of colloids. Many industrial products depend on the ability to manufacture dispersions to fine tolerances, without the presence of contaminants or aggregates.

The advantages offered by the DLS technique are also particularly relevant to the production of nanoparticles (e.g. for drug delivery, speciality coatings, etc.). However, at the nanoscale the propensity for particles to aggregate is dramatically increased as solid diffusion results in bridges being formed between particles within picoseconds. [refs – MC to provide] To measure true particle size using DLS at these scales, it is therefore necessary to produce and maintain nanopowders in a dispersion medium at all stages of manufacture. The DLS technique is thus unsuitable for nanomaterials produced by top down methods, such as milling.

### Testing the limits of DLS

Although there are several limitations that must be recognized and controlled to ensure the accurate application of DLS, advances in instrumentation continue to push these boundaries.

Concentration

The measurement of samples using conventional DLS instrumentation is limited to low concentrations due to the onset of a phenomenon called multiple scattering – when light scattered by one particle undergoes scattering by another.
Additionally, a high concentration of particles in solution hinders their rate of diffusion. This in turn affects their apparent size with a marked jump displayed in results when measured against increasing concentration. While comparative results are still highly useful as an indicator of product consistency, for example, this affect places an application dependant testing limit on the system. This testing limit can be predetermined for individual applications. [6]

**Figure 2:** Zetasizer Nano

The problems of multiple scattering have been addressed in advanced light scattering instruments, such as Malvern's Zetasizer Nano (Figure 2), which incorporates non-invasive backscatter optics (NIBS). The scattered light is detected at an angle of 173° and this novel optics arrangement maximizes the detection of scattered light while maintaining signal quality [7]. This provides the sensitivity required to measure turbid samples and nanoparticles, such as surfactant micelles, at low concentrations. [8]

With the advent of NIBS it is now possible to measure at higher particle concentrations (up to 40% w/v) [8]. Though it is often necessary to dilute the sample to lower concentrations in order to understand any effect that any particle-particle interactions are having on the reported size.

Resolution

DLS is inherently a low-resolution technique with limited capability of resolving different sized particles in a distribution. Polydisperse samples can produce unreliable results due to the huge difference between the specific turbidities of the very small and very large particles - scattered light from the smaller mode is 'drowned out' by that from the larger. Although NIBS optics has extended the limits of viable measurements, in practice, for routine analysis and quality control purposes DLS measurements can be unreliable for systems containing particles with relative sizes that differ by a factor of less than two or three? in most circumstances. [ref]

For samples with a limited number of modes distribution algorithms, such as Non-Negative Least Squares (NNLS) and CONTIN, are used to distinguish between the different size populations. Measurements of multimodal distributions are however realistically limited to sample types containing only two or three, monodisperse particle sizes. Real materials with mixed size distributions (particularly emulsions) produce measurement peaks so wide they can become indistinguishable from one another. This situation causes failure in the distribution algorithms. One of the greatest challenges in the application of DLS therefore is the ability to recognize when the process is generating erroneous data.

The resolving ability of distribution algorithms can be tested by performing a series of measurements using mixtures of monosized latex standards prepared at different, relative concentrations. This methodology is detailed in '*How to resolve particle size distributions in concentrated, polydisperse samples using Dynamic Light Scattering (DLS)*' on page <?>.

### Data quality

Acknowledging the inherent difficulty associated with user recognition of erroneous DLS data, Malvern Instruments has developed instrumentation that incorporates robust data quality assessment capabilities. This unique software makes results evaluation accessible to even the most inexperienced user.

The Zetasizer range includes software that quality checks a total of 11 parameters for every piece of data. This information is stored within the system to enable subsequent examination within data groups enabling assessment of comparative size and intensity results. Results are returned in a report. A report of unstable data is accompanied by a recommendation for potential improvements in results – such as further filtration of the solution for example. However, persistent bad quality reporting may suggest that the samples under examination are fundamentally unsuitable for the DLS measurement technique.

## Combining technology

Enhanced resolution can be achieved by combining DLS with chromatography. Due to its chromatography flow mode, the Zetasizer Nano can be successfully coupled with Size Exclusion Chromatography SEC apparatus, which separates materials based on their hydrodynamic size. This combination confers the ability to perform absolute size exclusion chromatography (ASEC) enabling size measurements in real time, under flow. This has enormous benefit for applications such as protein characterization. [9]

## Conclusion

In common with all particle sizing techniques, accurate DLS analysis comes with inherent constraints. Understanding and accepting these limits is critical towards the selection of both technique and instrumentation as well as, most importantly, the production of valid, quality data.

Advanced instrumentation, such as the Zetasizer Nano, can enhance the possibilities afforded by conventional measurement set-ups. Additionally, using accepted methodologies to verify a sample's suitability for testing and the application of robust data quality assessment can prevent misplaced acceptance of unsound results. Armed with relevant knowledge and the correct tools, researchers can confidently perform both robust routine measurements as well as more exploratory measurements along the outer boundaries of what is currently accepted as standard DLS.

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## How to resolve particle size distributions in concentrated, polydisperse samples using Dynamic Light Scattering (DLS)[10]

As a test of the resolving ability of an algorithm, measurements can be done using mixtures of monosized latex standards prepared at different relative concentrations. A series of samples where the relative concentrations of different size latex standards were varied were measured. Measurements were made on mixtures of latex standards at neat concentration on a Zetasizer Nano S (173°).

### Experimental

All latex standards used for the measurements discussed in this paper were obtained from Duke Scientific, Palo Alto, California and were traceable to NIST, Gaithersburg, Maryland. Nanosphere™ size standards from the 3000 series were used. Specifically, these were the 60nm (catalogue number 3060A), the 220nm (3220A) and 900nm (3900A) standards respectively. All of these standards are supplied at 1% w/v concentration.
The analysis model used in the Zetasizer Nano software was Multiple Narrow Modes.

### Results

The Zetasizer Nano S incorporates non-invasive backscatter optics (NIBS) that allows for the measurement of concentrated samples. Therefore, measurements of mixtures of 60 and 220nm latex standards at different ratios were made at neat concentrations of 1% w/v. Table 1 summarises the results obtained for these measurements and contains the ratios of the 60 and 220nm latex mixtures, the z-average diameters, the polydispersity index values, the peak analysis of the intensity and volume size distributions obtained (peak modes and percentages). Conversions of the measured intensity size distributions into volume were done using Mie theory with particle refractive index values of 1.59 and particle absorption values of 0.01. The corresponding intensity and volume size distributions obtained for the various mixtures are shown in figures **<?>** and **<?>** respectively.
The z-average diameters obtained from the latex mixtures decreases as the concentration of 60nm latex in each mixture is increased. Conversely, the polydispersity index values increase as the percentage of 60nm latex contained in each sample increases.
The measured volume ratios are in good agreement with the actual volume ratios of the latex mixtures.

### Conclusions

Even though dynamic light scattering is a low-resolution technique, the results presented show that, not only can different size particles be resolved, but changes in the relative concentrations of each size population can be monitored.
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## Images

**Figure 1 <from Malvern website http://www.malvern.com/LabEng/industry/nanotechnology/nanoparticle_measurement.htm >**: Compared with the number of techniques for measuring the size of a particle greater than 1 micron, there are very few techniques that are able to accurately measure the size of small particles, particularly those less than 10 nanometres.

**Figure 2: Zetazier Nano**

<box images – from Application note: Resolving Particle Size Mixtures of Concentrated Samples Using Dynamic Light Scattering>

**Figure 1:** Intensity size distributions obtained for (a) 60nm latex standard (b) 220nm latex standard (c) 1:1 v/v mixture of 60:220nm latex (d) 2:1 v/v mixture of 60:220nm latex (e) 4:1 v/v mixture of 60:220nm latex (f) 12:1 v/v mixture of 60:220nm latex and (g) overplot of all the intensity size distributions

**Figure 2:** Volume size distributions obtained for (a) 60nm latex standard (b) 220nm latex standard (c) 1:1 v/v mixture of 60:220nm latex (d) 2:1 v/v mixture of 60:220nm latex (e) 4:1 v/v mixture of 60:220nm latex (f) 12:1 v/v mixture of 60:220nm latex and (g) overplot of all the intensity size distributions

**Table 1:** The results obtained for measurements of mixtures of 60 and 220nm latex standards at different ratios made at neat concentrations of 1% w/v. The table contains the ratios of the 60 and 220nm latex mixtures, the z-average diameters, the polydispersity index values, the peak analysis of the intensity and volume size distributions obtained (peak modes and percentages).