Dynamic Light Scattering: An Introduction in 30 Minutes

Introduction

Dynamic **L**ight **S**cattering (sometimes referred to as **P**hoton **C**orrelation **S**pectroscopy or **Q**uasi-Elastic **L**ight **S**cattering) is a technique for measuring the size of particles typically in the sub micron region.

Brownian Motion

DLS measures Brownian motion and relates this to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. Normally DLS is concerned with measurement of particles suspended within a liquid.

The larger the particle, the slower the Brownian motion will be. Smaller particles are "kicked" further by the solvent molecules and move more rapidly. An accurately known temperature is necessary for DLS because knowledge of the viscosity is required (because the viscosity of a liquid is related to its temperature). The temperature also needs to be stable, otherwise convection currents in the sample will cause non-random movements that will ruin the correct interpretation of size.

The velocity of the Brownian motion is defined by a property known as the translational diffusion coefficient (usually given the symbol, D).

The Hydrodynamic Diameter

The size of a particle is calculated from the translational diffusion coefficient by using the Stokes-Einstein equation;

$$d(H) = \frac{kT}{3\pi\eta D}$$

where:-

$d(H)$ = hydrodynamic diameter

D = translational diffusion coefficient

k = Boltzmann's constant

T = absolute temperature

η = viscosity

Note that the diameter that is measured in DLS is a value that refers to how a particle diffuses within a fluid so it is referred to as a hydrodynamic diameter. The diameter that is obtained by this technique is the diameter of a sphere that has the same translational diffusion coefficient as the particle.

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. Factors that affect the diffusion speed of particles are discussed in the following sections.

Ionic Strength of Medium

The ions in the medium and the total ionic concentration can affect the particle diffusion speed by changing the thickness of the electric double layer called the Debye length (K^{-1}). Thus a low conductivity medium will produce an extended double layer of ions around the particle, reducing the diffusion speed and resulting in a larger, apparent hydrodynamic diameter. Conversely, higher conductivity media will suppress the electrical double layer and the measured hydrodynamic diameter.

The performance of a DLS instrument is normally verified by measurement of a suitable polystyrene latex standard. If the standard needs to be diluted prior to measurement, then dilution in an appropriate medium is important. The International Standard on DLS (ISO13321 Part 8 1996) says that dilution of any polystyrene standard should be made in 10mM NaCl. This concentration of salt will suppress the electrical double layer and ensure that the hydrodynamic diameter reported will be the same as the hydrodynamic diameter on the certificate or the expected diameter.

Surface Structure

Any change to the surface of a particle that affects the diffusion speed will correspondingly change the apparent size of the particle. An adsorbed polymer layer projecting out into the medium will reduce the diffusion speed more than if the polymer is lying flat on the surface. The nature of the surface and the polymer, as well as the ionic concentration of the medium can affect the polymer conformation, which in turn can change the apparent size by several nanometres.

Non-Spherical Particles

All particle-sizing techniques have an inherent problem in describing the size of non-spherical particles. The sphere is the only object whose size
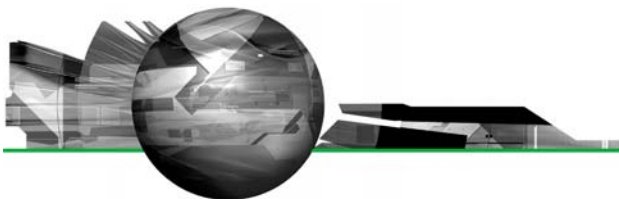
can be unambiguously described by a single figure.

Different techniques are sensitive to different properties of the particle, e.g. projected area, density, scattering intensity, and in general will produce different mean sizes and size distributions for any given sample. Even the size in a microscope image will depend on parameters set such as edge contrast etc. It is important to understand that none of these results are inherently "correct".

The hydrodynamic diameter of a non-spherical particle is the diameter of a sphere that has the same translational diffusion speed as the particle.

If the shape of a particle changes in a way that affects the diffusion speed, then the hydrodynamic size will change. For example, small changes in the length of a rod-shaped particle will directly affect the size, whereas changes in the rod's diameter, which will hardly affect the diffusion speed, will be difficult to detect.

The conformation of proteins and macromolecules are usually dependent on the exact nature of the dispersing medium. As conformational changes will usually affect the diffusion speed, DLS is a very sensitive technique for detecting these changes.

Light Scattering Theories

Rayleigh Scattering

If the particles are small compared to the wavelength of the laser used (typically less than $d = \lambda/10$ or around 60nm for a He-Ne laser), then the scattering from a particle illuminated by a vertically polarised laser will be essentially isotropic, i.e. equal in all directions.

The Rayleigh approximation tells us that $I \propto d^6$ and also that $I \propto 1/\lambda^4$, where I = intensity of light scattered, d = particle diameter and λ = laser

wavelength. The d^6 term tells us that a 50nm particle will scatter 10^6 or one million times as much light as a 5nm particle. Hence there is a danger that the light from the larger particles will swamp the scattered light from the smaller ones. This d^6 factor also means it is difficult with DLS to measure, say, a mixture of 1000nm and 10nm particles because the contribution to the total light scattered by the small particles will be extremely small. The inverse relationship to λ^4 means that a higher scattering intensity is obtained as the wavelength of the laser used decreases.

Mie Theory

When the size of the particles becomes roughly equivalent to the wavelength of the illuminating light, then a complex function of maxima and minima with respect to angle is

observed.

Figure 1 shows the theoretical plot of the log of the relative scattering intensity versus particle size at angles of 173° (the detection angle of the Zetasizer Nano S and Nano ZS in aqueous media) and 90° (the detection angle of the Nano S90 and Nano ZS90) assuming a laser wavelength of 633nm, real refractive index of 1.59 and an imaginary refractive index of 0.001. Mie theory is the only theory that explains correctly the maxima and minima in the plot of intensity with angle and will give the correct answer over all wavelengths, sizes and angles. Mie theory is used in the Nano software for conversion of the intensity distribution into volume.
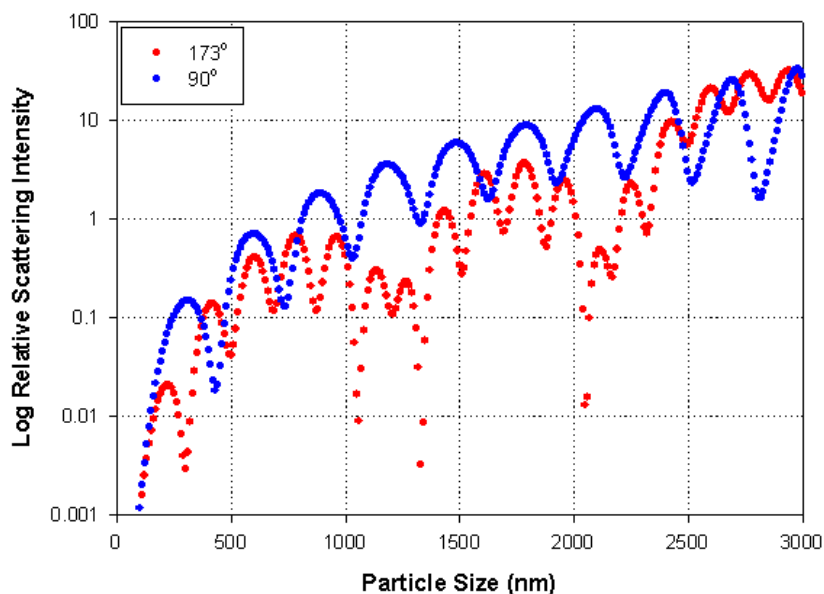
Figure 1: Theoretical plot of the log of the relative intensity of scattering versus particle size at angles of 173° (the detection angle of the Nano S, and Nano ZS in aqueous media) and 90° (the detection angle of the Nano S90 and Nano ZS90) assuming a laser beam at a wavelength of 633nm, real refractive index of 1.59 and an imaginary refractive index of 0.001

How DLS Works

In dynamic light scattering, the speed at which the particles are diffusing due to Brownian motion is measured. This is done by measuring the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement. How do these fluctuations in the intensity of scattered light arise?

Imagine if a cuvette, containing particles which are stationary, is illuminated by a laser and a frosted glass screen is used to view the sample cell. A classical speckle pattern would be seen (figure 2). The speckle pattern will be stationary both in speckle size and position because the whole system is stationary. The dark spaces are where the phase additions of the scattered light are mutually destructive and cancel each other out (figure 3A). The bright blobs of light in the speckle pattern are where the light scattered from the particles arrives with the same phase and interfere constructively to form a bright patch (figure 3B).

For a system of particles undergoing Brownian motion, a speckle pattern is observed where the position of each speckle is seen to be in constant motion. This is because the phase addition from the moving particles is constantly evolving and forming new patterns. The rate at which these intensity fluctuations occur will depend on the size of the particles. Figure 4 schematically illustrates typical intensity fluctuations arising from a dispersion of large particles and a dispersion of small particles. The small particles cause the intensity to fluctuate more rapidly than the large ones.
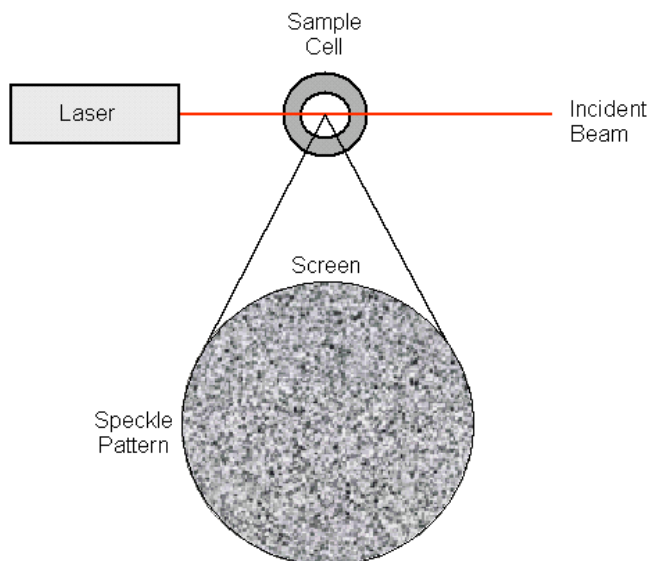
Figure 2: Schematic representation of a speckle pattern
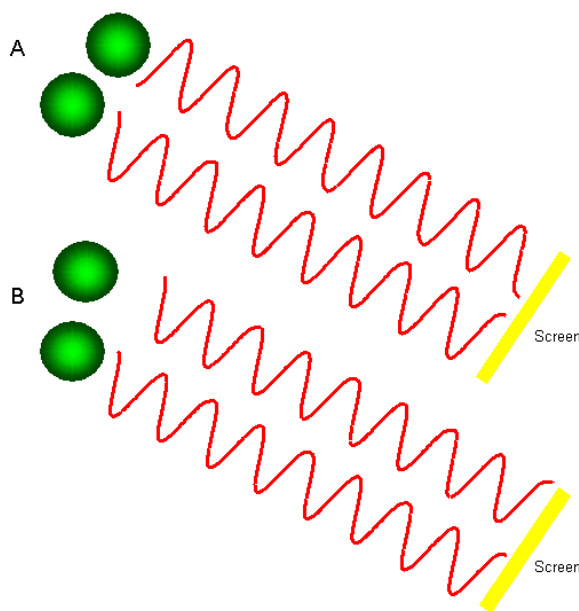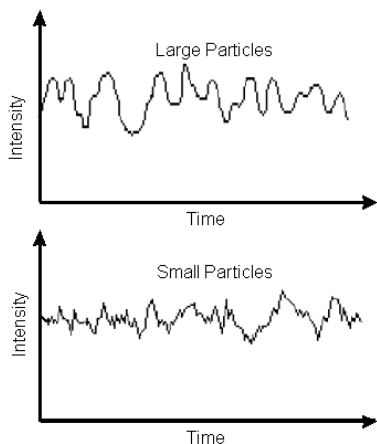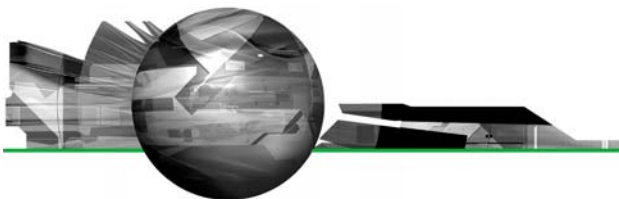
Figure 3: The observed signal depends on the phase addition of the scattered light falling on the detector. In example A, two beams interfere and 'cancel each other out' resulting in a decreased intensity detected. In example B, two beams interfere and 'enhance each other' resulting in an increased intensity detected.

Figure 4: Typical intensity fluctuations for large and small particles

It is possible to directly measure the spectrum of frequencies contained in the intensity fluctuations arising from the Brownian motion of particles, but it is inefficient to do so. The best way is to use a device called a digital auto correlator.

How a Correlator Works

A correlator is basically a signal comparator. It is designed to measure the degree of similarity between two signals, or one signal with itself at varying time intervals.

If the intensity of a signal is compared with itself at a particular point in time and a time much later, then for a randomly fluctuating signal it is obvious that the intensities are not going to be related in any way, i.e. there will be no correlation between the two signals (figure 5). Knowledge of the initial signal intensity will not allow the signal intensity at time t = infinity to be predicted. This will be true of any random process such as diffusion.

However, if the intensity of signal at time = t is compared to the intensity a very small time later (t+δt), there will be a strong relationship or correlation

between the intensities of two signals. The two signals are strongly or well correlated.

If the signal, derived from a random process such as Brownian motion, at t is compared to the signal at t+2δt, there will still be a reasonable comparison or correlation between the two signals, but it will not be as good as the comparison at t and t+δt. The correlation is reducing with time. The period of time δt is usually very small, maybe nanoseconds or microseconds and is called the sample time of the correlator. t = ∞ maybe of the order of a millisecond or tens of milliseconds.

If the signal intensity at t is compared with itself then there is perfect correlation as the signals are identical. Perfect correlation is indicated by unity (1.00) and no correlation is indicated by zero (0.00).

If the signals at t+2δt, t+3δt, t+4δt etc. are compared with the signal at t, the correlation of a signal arriving from a random source will decrease with time until at some time, effectively t = ∞, there will be no correlation.

If the particles are large the signal will be changing slowly and the correlation will persist for a long time (figure 6). If the particles are small and moving rapidly then correlation will reduce more quickly (figure 7).
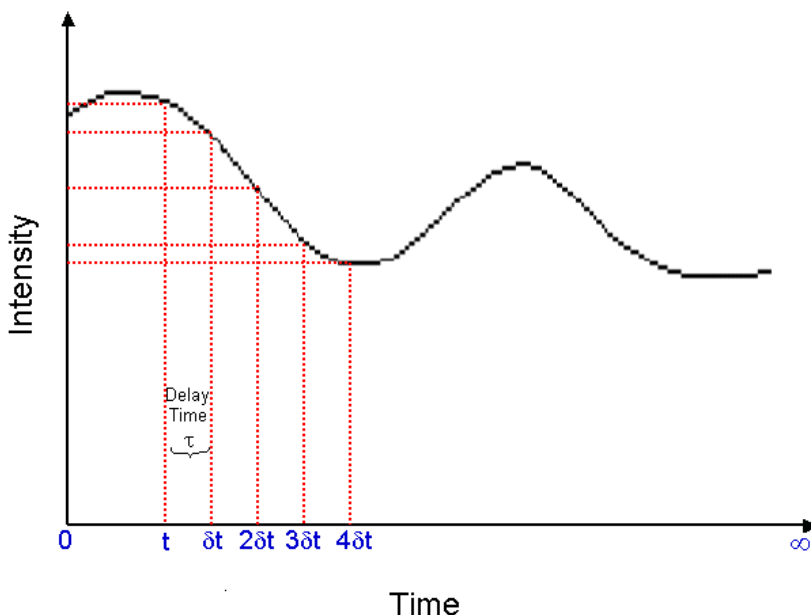
Figure 5: Schematic showing the fluctuation in the intensity of scattered light as a function of time
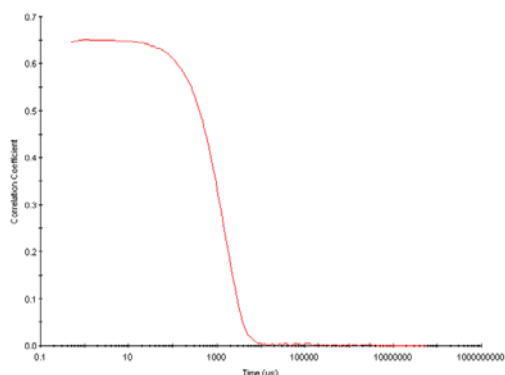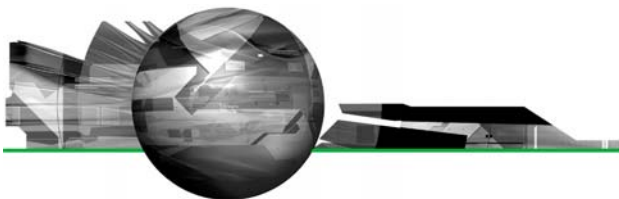
Figure 6: Typical correlogram from a sample containing large particles in which the correlation of the signal takes a long time to decay
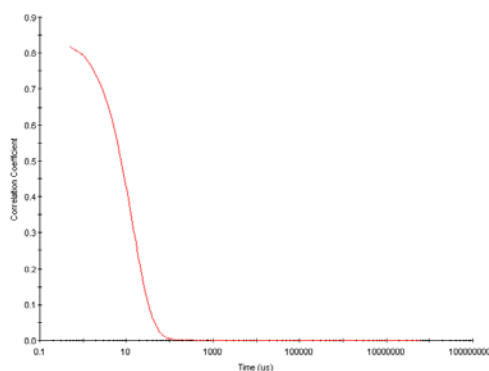
Figure 7: Typical correlogram from a sample containing small particles in which the correlation of the signal decays more rapidly

Viewing the correlogram from a measurement can give a lot of information about the sample. The time at which the correlation starts to significantly decay is an indication of the mean size of the sample. The steeper the line, the more monodisperse the sample is. Conversely, the more extended the decay becomes, the greater the sample polydispersity.

The Correlation Function

It has been seen that particles in a dispersion are in a constant, random Brownian motion and that this causes the intensity of scattered light to fluctuate as a function of time. The correlator used in a PCS instrument will construct the correlation function $G(\tau)$ of the scattered intensity:

$$G(\tau) = <I(t).I(t+\tau)>$$

Where τ = the time difference (the sample time) of the correlator.

For a large number of monodisperse particles in Brownian motion, the correlation function (given the symbol [G]) is an exponential decaying

function of the correlator time delay τ:

$$G(\tau) = A[1 + B\ exp(-2\Gamma\tau)]$$

where A = the baseline of the correlation function, B = intercept of the correlation function.

$\Gamma = Dq^2$

where D = translational diffusion coefficient

$q = (4\ \pi\ n\ /\ \lambda_o)\ sin\ (\theta/2)$

where n = refractive index of dispersant, λ_o = wavelength of the laser, θ = scattering angle.

For polydisperse samples, the equation can be written as:

$$G(\tau) = A[1 + B\ g_1(\tau)^2]$$

where $g_1(\tau)$ = is the sum of all the exponential decays contained in the correlation function.

## Obtaining Size Information From the Correlation Function

Size is obtained from the correlation function by using various algorithms. There are two approaches that can be taken (1) fit a single exponential to the correlation function to obtain the

mean size (z-average diameter) and an estimate of the width of the distribution (polydispersity index) (this is called the Cumulants analysis and is defined in ISO13321 Part 8), or (2) fit a multiple exponential to the correlation function to obtain the distribution of particle sizes (such as Non-negative least squares (NNLS) or CONTIN.

The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and is therefore known as an intensity size distribution.

If the distribution by intensity is a single fairly smooth peak, then there is little point in doing the conversion to a volume distribution using the Mie theory. If the optical parameters are correct, this will just provide a slightly different shaped peak. However, if the plot shows a substantial tail, or more than one peak, then Mie theory can make use of the input parameter of sample refractive index to convert the intensity distribution to a volume distribution. This will then give a more realistic view of the importance of the tail or second peak present. In general terms it will be seen that:-
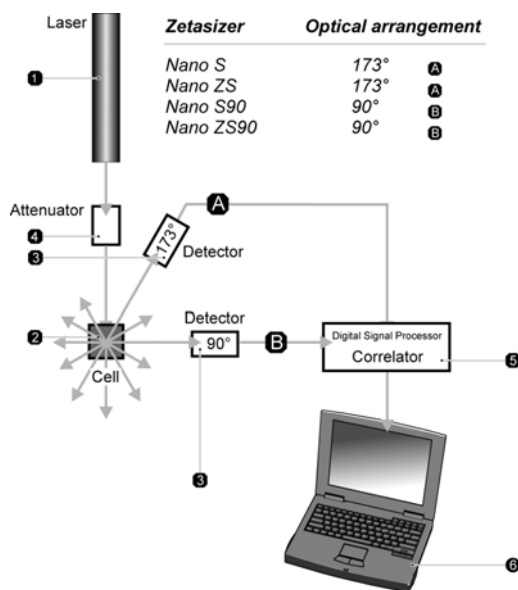
d(intensity) > d(volume) > d(number)
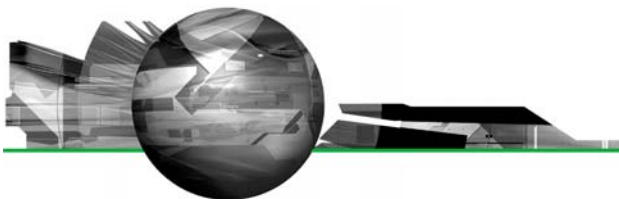
A very simple way of describing the difference between intensity, volume and number distributions is to consider 2 populations of spherical particles of diameter 5nm and 50nm present in equal numbers (figure 8). If a number distribution of these 2 particle populations is plotted, a plot consisting of 2 peaks (positioned at 5 and 50nm) of a 1 to 1 ratio would be obtained. If this number distribution was converted into volume, then the 2 peaks would change to a 1:1000 ratio (because the volume of a sphere is equal to $4/3\pi(d/2)^3$). If this was further converted into an intensity distribution, a 1:1000000 ratio between the 2 peaks would be obtained (because the intensity of scattering is proportional to d^6 (from Rayleighs approximation)). Remember that in DLS, the distribution obtained from a measurement is based on intensity.
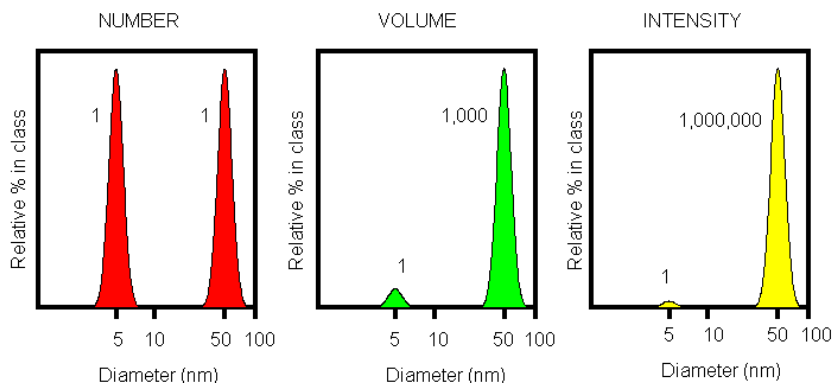
Figure 8: Number, volume and intensity distributions of a bimodal mixture of 5 and 50nm lattices present in equal numbers

Optical Configuration of a Dynamic Light Scattering Instrument

A typical dynamic light scattering system comprises of six main components. Firstly, a laser ① provides a light source to illuminate the sample contained in a cell ②. For dilute concentrations, most of the laser beam passes through the sample, but some is scattered by the particles within the sample at all angles. A detector ③ is used to measure the scattered light. In the Zetasizer Nano series, the detector position will be at either 173° or 90°, depending upon the particular model.

The intensity of scattered light must be within a specific range for the detector to successfully measure it. If too much light is detected, then the detector will become saturated. To overcome this, an attenuator ④ is used to reduce the intensity of the laser source and hence reduce the intensity of scattering. For samples that do not scatter much light,

Figure 9: Optical configurations of the Zetasizer Nano series for dynamic light scattering measurements

such as very small particles or samples of low concentration, the amount of scattered light must be increased. In this situation, the attenuator will allow more laser light through to the sample.

For samples that scatter more light, such as large particles or samples at higher concentration, the intensity of scattered light must be decreased. The appropriate attenuator position is automatically determined by the Nano software and covers a transmission range of 100% to 0.0003%.

The scattering intensity signal from the detector is passed to a digital processing board called a correlator ⑤. The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. This correlator information is then passed to a computer ⑥, where the Nano software will analyze the data and derive size information.

Unique Features of the Zetasizer Nano

Non-Invasive Backscatter Detection (NIBS)

The Nano S and Nano ZS instruments detect the scattering information at 173°. This is known as backscatter detection. In addition, the optics are not in contact with the sample and hence the detection optics are said to be non-invasive. There are several advantages in using non-invasive backscatter detection:

- The laser does not have to travel through the entire sample. This reduces an effect called multiple scattering, where light from one particle is itself scattered by other particles. As the light passes through a shorter path length of the sample, then higher concentrations of sample can be measured.

- Contaminants such as dust particles within the dispersant are typically large compared to the sample size. Large particles mainly scatter in the forward direction. Therefore, by using backscatter detection, the effects of dust are greatly reduced.

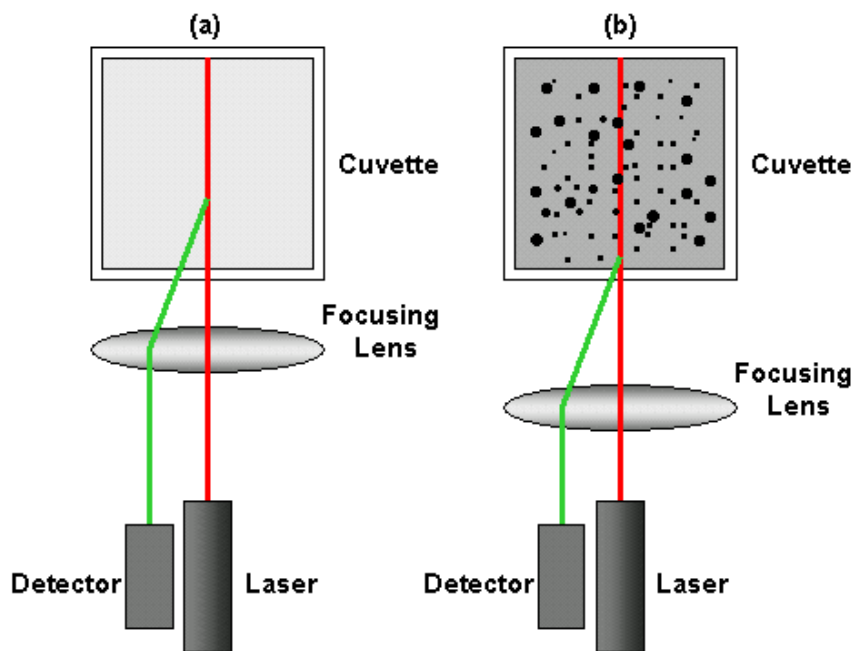### Variable Measurement Position For Sizing

The measurement position within the cuvette of the Nano S and Nano ZS can be changed. This measurement position is changed by moving the focusing lens and is determined automatically by the Nano software (figure 10).

For small particles, or samples at low concentrations, it is beneficial to maximise the amount of scattering from the sample. As the laser passes through the wall of the cuvette and into the dispersant, the laser will cause "flare".

This flare may swamp the signal from the scattering particles. Moving the measurement point away from the cuvette wall towards the centre of the cuvette will remove this effect (figure 10a).

Large particles or samples at high concentrations scatter much more light. In this situation, measuring closer to the cuvette wall will reduce the effect of multiple scattering by minimising the path length over which the scattered light has to pass (figure 10b). The measurement position is determined automatically through a combination of the intercept of the correlation function and the intensity of the light scattered.

Figure 10: Schematic diagram showing the measurement position for (a) small, weakly scattering samples and for (b) concentrated, opaque samples. The change in measurement position is achieved by moving the focusing lens accordingly

Additional Reading

[1] International Standard ISO13321 Methods for Determination of Particle Size Distribution Part 8: Photon Correlation Spectroscopy, International Organisation for Standardisation (ISO) 1996.

[2] Dahneke, B.E. (ed) Measurement of Suspended Particles by Quasi-elastic Light Scattering, Wiley, 1983.

[3] Pecora, R. Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy, Plenum Press, 1985.

[4] Washington, C. Particle Size Analysis In Pharmaceutics And Other Industries: Theory And Practice, Ellis Horwood, England, 1992.

[5] Johnson, C.S. Jr. and Gabriel, D.A. Laser Light Scattering, Dover Publications, Inc., New York 1981

more information at www.malvern.co.uk