The real-time, simultaneous analysis of nanoparticle size, zeta potential, count, asymmetry and fluorescence

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ABSTRACT

A new nanoparticle sizing and characterization technique is described which allows nanoparticles in a suspension to be individually but simultaneously detected and analysed in real time using a low cost instrument. Nanoparticles of all types and in any solvent can be detected, sized and counted through video-based tracking of their Brownian motion when illuminated by a specially configured laser beam. Depending on particle type, nanoparticles as small as 10nm can be visualized (though not imaged). Advances in the technique are described which allow each particle to be simultaneously analysed not just in terms of its size but also light scattering power (mass/refractive index) and electrophoretic mobility in an applied electric field. Finally, an unprecedented ability to quantify nanoparticle asymmetry in real time, again on a particle-by-particle basis is outlined

Keywords: nanoparticle, analysis, multiparameter, size, zeta

1 INTRODUCTION

The analysis of nanoparticle size is now a central requirement in the development of a wide range of particulate-based materials and substances in which their properties and behaviour are enhanced when produced at nanoscale dimensions. Besides the well established use of electron microscopy and scanning probe microscopy methods, optical ensemble techniques such as Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy (PCS), have long been used routinely in the analysis of nanoparticle dispersions. A number of commercial manufacturers supply instrumentation capable of rapidly and accurately determining particle size from a wide range of sample types.

However, it is well recognised that dynamic light scattering methods become unreliable when presented with samples which contain a wide range of particle sizes, i.e. are polydisperse, and that the basic information obtained, the intensity weighted mean size (the 'z-average'), does not always reflect the sample composition accurately. Furthermore, successful analysis of the correlation function by classical deconvolution algorithms to extract, for instance, bimodal distributions are realistically limited to sample types containing only two or at best three sized particle types, each needing to differ from each other by a size factor of, in practice, >3:1. Finally, DLS is limited in its ability to allow the user to recognise when the sample is unsuitable for analysis by that method and that the data (i.e. the particle size distribution profile) obtained should accordingly be treated with some suspicion.

We report here on a new light scattering technique, Nanoparticle Tracking Analysis (NTA) for determining nanoparticle size through tracking and analysing the trajectories described by individual nanoparticles undergoing Brownian motion in a fluid and which has recently been made commercially available [1-6].

2 THE OPTICAL SYSTEM

The technique is based on laser light scattering in which a finely focussed laser beam (of arbitrary wavelength but which is commercially available at 534nm, 25mW [2]) is passed through a sample of liquid containing a dilute suspension of nanoparticles. The beam is caused to refract at the interface between the liquid sample and the optical element through which it is passed such that it describes a path which is close to parallel to the glass-sample interface. Particles resident in the beam (which is approximately 100µm wide by 25µm deep), are visualised by a conventional optical microscope aligned normally to the beam axis and which collects light scattered from each and every particle in the field of view.

Given NTA is not an imaging technique per se, the total magnification of the system is quite modest (x100 via a 20x 0.4 NA long working distance microscope objective) and for a suitably diluted sample the particles are visualised as light scattering centres moving under Brownian motion in the field of view (approx. 100x80µm, i.e. matched to the beam width) of a camera (640x480 pixels each of 9x9µm) located on a C-mount on the microscope assembly.

The sample chamber is approximately 250µl in volume and 500µm deep and sample introduced by syringe via a Luer port. The sample is allowed to thermally equilibrate for 20 seconds prior to analysis

3 ANALYSIS OF BROWNIAN MOTION.

A video of typically 20-60 seconds duration is taken (30 frames per second) of the moving particles. The video is analysed by a proprietary analysis programme [3] on a

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frame by frame basis, each particle being identified and located automatically and its movement tracked and measured from frame to frame. The thresholds for particle identification can be user adjusted, as can the gain and shutter speed settings of the camera, thus allowing the user to optimise the image for a particular sample type. Particles diffusing into the scattering volume are identified and followed for the duration of the particle presence in the beam or until they diffuse to within a certain distance of an adjacent particle at which point tracking is ceased. This eliminates the possibility of analysing particle trajectories which cross behind each other and which might lead to erroneous results. Particles must be detected by the camera in order to be tracked. The limits to particle sizes which can be analysed by NTA are determined primarily by particle size and refractive index.

3.1 Visibility of particles and detection limits

The amount of light scattered by a particle in any given direction is a function of many variables including incident illumination power, wavelength, angle and polarisation; particle size, refractive index (real and imaginary) and shape, as well as solvent refractive index. Similarly, the amount of light falling on a detector and strength of the resultant signal is dependent, of course, on the efficiency of the collection optics (e.g. Numerical Aperture) and the spectral response and sensitivity of the camera.

The theory of light scattering is well established and the formula for Rayleigh scattering of small particles of radius *a,* refractive index n_1 in a liquid of refractive index n_2 is given below.

$$\frac{I}{I_{in}} = \frac{16\pi^4 a^6}{r^2 \lambda^4}\left(\frac{n^2-1}{n^2+2}\right)\sin^2\psi \qquad (1)$$

Where λ is the wavelength of the incident light beam, *n* relative ref index *(n_2/n_1)*, I_{in} is incident power per unit area, *I* the scattered power per unit area a distance *r* from the scattering region and ψ is the angle between the input polarisation and the scattering direction. The total scattering into an aperture of collection angle θ (numerical aperture NA = sin θ) is then:

$$P_{scat} = \frac{64\pi^4 a^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)\eta I_{in}$$

$$\eta = \frac{(1-\cos\theta)}{4} + \frac{(1-\cos^3\theta)}{12} \qquad (2)$$

For a single mode 25mW laser diode at λ = 534nm and using the camera available on the commercially available instrument discussed in this paper (Marlin, Allied Vision Ltd.), the limits of detection depend primarily on the size and refractive index (R_i) of the nanoparticle. In practice, high R_i materials such as colloidal silver can be visualised down to approx 10nm diameter. For nanoparticles of slightly lower Ri, such as metal oxides (e.g. TiO_2), detection limits may be only >20nm. For weakly scattering materials (e.g. polymer, biological, liposome), the smallest particle visible might only be >50-75nm in diameter.

3.2 Diffusion of nanoparticles under Brownian motion..

Nanoparticles in a liquid are under continuous solvent bombardment and move randomly over length scales related to their size. For spherical particles, the diffusion coefficient (Dt), is related to particle diameter by the Stokes-Einstein relationship

$$Dt = \frac{K_B T}{6\pi\eta r_h} \qquad (3)$$

where K_B is the Boltzmann constant, T temperature, η is viscosity and r_h the hydrodynamic radius.

For non-spherical particles, Dt is a complex function of particle shape (e.g. prolate ellipsoid, oblate spheroid, etc..) [3] but in the technique described here, Dt is expressed only as a sphere equivalent, hydrodynamic radius. For the majority of nanoparticle samples, particle shape is sufficiently spherical (aspect ratios of <2) for the Stokes-Einstein sphere assumption to sufficiently valid to generate useful information about particle size and size distribution.

4 RESULTS.

4.1 Determination of particle diameter through analysis of Brownian motion

The following results show the ability of the technique to resolve different particles sizes at resolutions that exceed those achievable by DLS [10-13]. Fig 1 shows a well resolved bimodal from a mixture of 100 and 300nm polystyrene calibration microspheres. It is important to note that the plot shows number of particles as a function of size, allowing particle concentrations (numbers of particles/ml) for each (user selectable) size class.

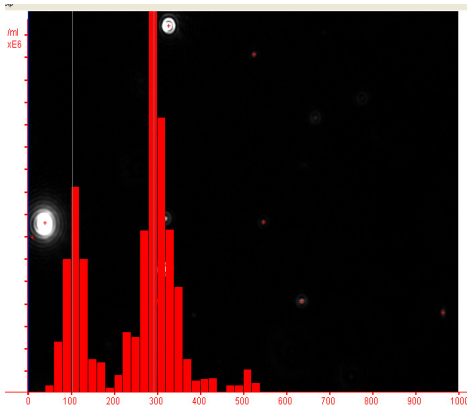

Fig 1. A mixture of 100 and 300nm polystyrene particles as analysed for 166 seconds.

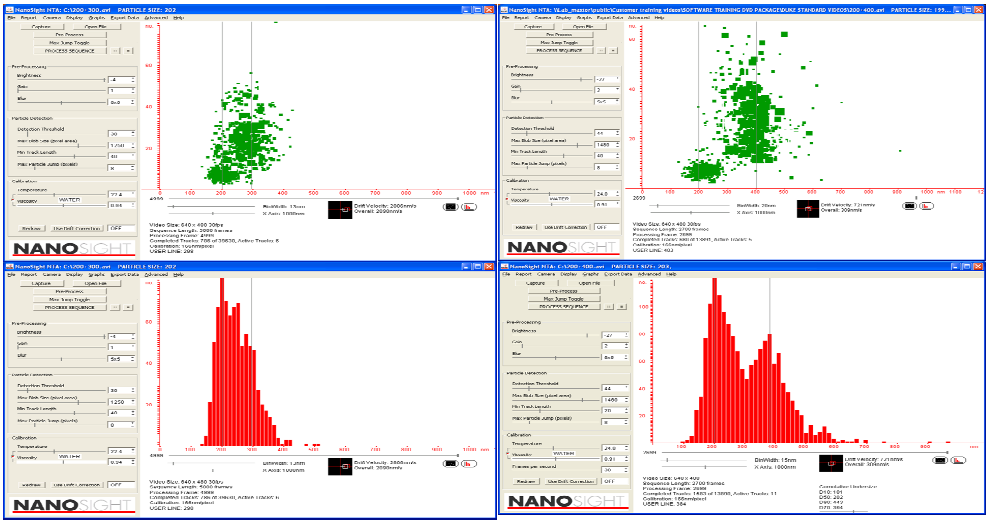

Fig 2. Size plots (a; histograms) and two dimensional plots of size v. intensity (b; scatter plots) for mixtures of 200 and 300nm particles (left) and 200 and 400nm particles (right).

4.2 Simultaneous analysis of particle size and intensity

The analysis program is capable of measuring the intensity of light scattered by the particle at the same time as tracking its Brownian motion from which its size is calculated. This offers the ability to generate two dimensional plots of intensity v. size. As can be seen from Fig 2, the 2D plots of a mixture of titanium dioxide nanoparticles of 200, 300 and 400nm diameter can more easily be resolved by 2D plotting even though the samples of each were not particularly monodisperse. Fig 2a shows plots from a mixture of 200 and 300nm particles, Fig2b is for a mixture of 200 and 400nm particles.

4.3 Analysis of changes in particle refractive index with simultaneous size determination.

In the following example, suspensions of a virus particle were incubated over a 2 hour period in a metal salt solution which resulted in the formation of highly scattering metallic coating on many of the virus particles. While there was, except for the formation of a few aggregates, no significant increase in particle diameter through the formation of a molecularly thin metallic film, and hence no significant change in particle size, the increase in particle refractive index was clearly seen in the 2D, intensity v. size plot Fig. 3

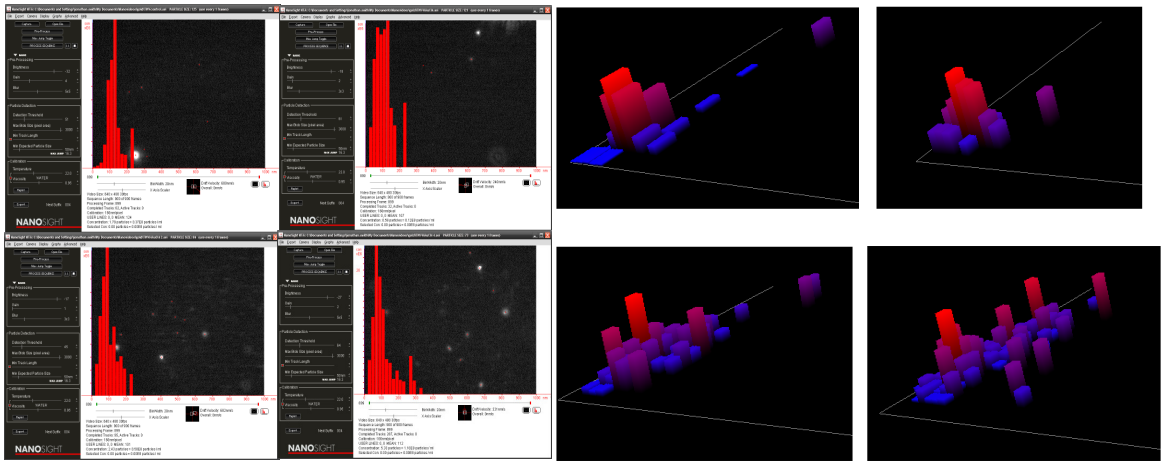

Fig 3. Size profiles (left) obtained for virus particles over a 4 hour incubation period in the presence of a metal salt solution which resulted in the reduction of the metal onto the virus particles causing their refractive index to increase without significant increase in virus size (right). Note that the aggregates can be clearly differentiated by virtue of the increase in both size and scattered intensity

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4.4 Simultaneous measurement of particle size and electrophoretic mobility.

By the introduction of electrodes into the sample cell, it is possible to apply an electric field across the liquid region through which the laser beam passes [8,9]. Applying an electric field causes charged particles to move in this field. We have shown, Fig 4, that electrophoretic mobility is linearly dependent on applied voltage

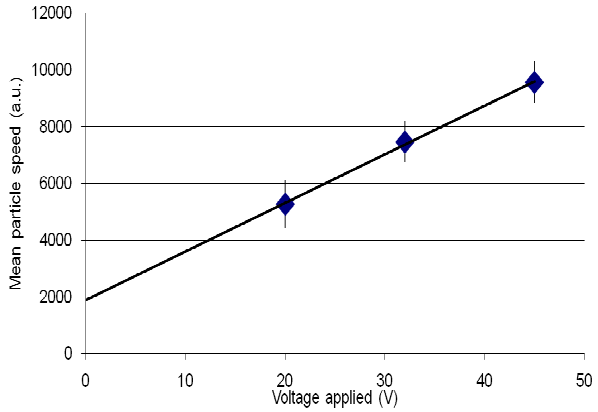

Fig 4. Electrophoretic mobility as a function of applied voltage

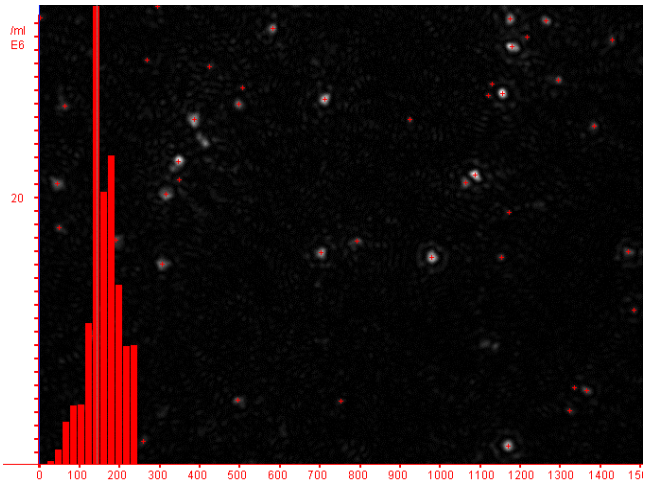

Fig 5. Particle size distribution profile of carboxylated particles with no applied electric field.

It is possible to determine the size of a negatively charged 150nm particle (carboxylated), through analyzing its Brownian motion prior to applying an electric field, Fig 5

On the application of an electric field (30V), the particles are seen to move towards the anode and their electrophoretic tracks can be visualised and analysed by the analysis program (Fig 6). The Brownian motion from which the size is obtained can be simultaneously extracted from the overall electrophoretic motion.

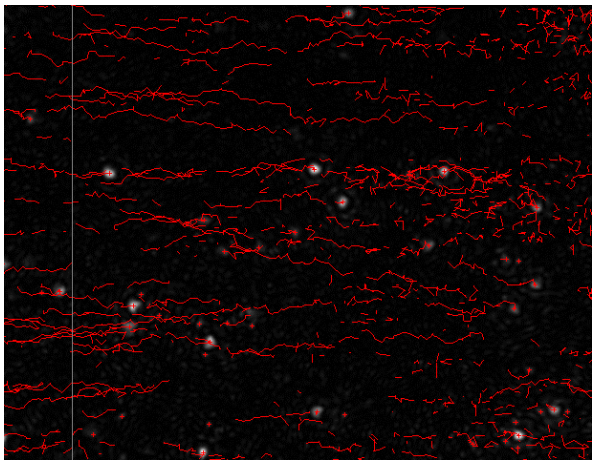

Fig 6. Tracks described by particles moving under an applied electric field.

In Fig 7 can be seen a 2 Dimensional plot of size of particle in a) the absence of an applied electric field and b)_when the filed is switched on. This shows that particle sizing is unaffected by the electrophoretic motion of the particles.

Shown in Fig 8 is a three dimensional plot of Fig 7 in which the vertical axis represents numbers of particles of any given class over the analysis time.

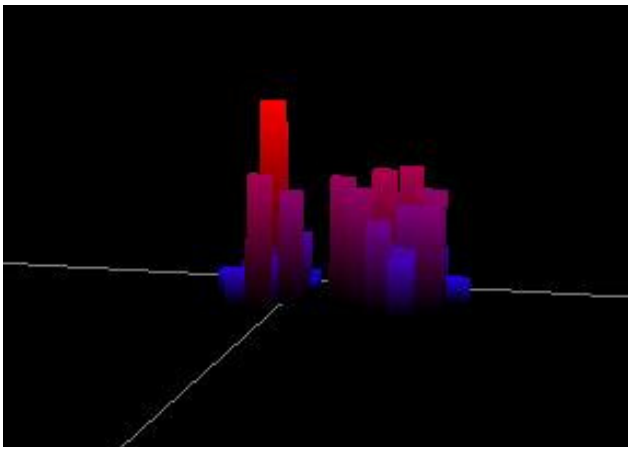

Fig 8 A three dimensional plot of Fig 7 in which the vertical axis represents numbers of particles of any given class over the analysis time.

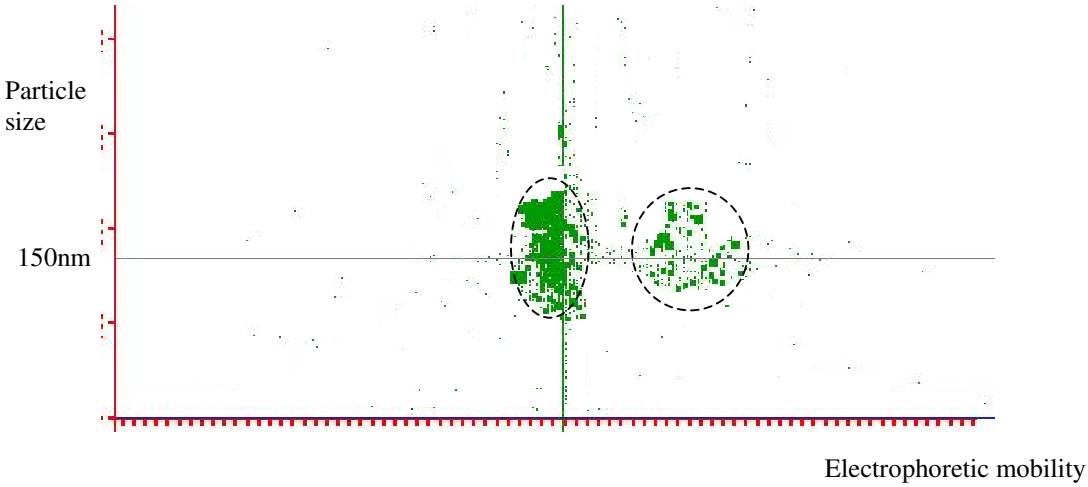

Fig 7 shows a 2 Dimensional plot of size of particle in a) the absence of an applied electric field and b)_when the field is switched on. This shows that particle sizing is unaffected by the electrophoretic motion of the particles.

5 CONCLUSION

The technique outlined above allows for the multi-parameter analysis of nanoparticle suspension on a particle-by-particle basis [10-12]. Particles which are known to be asymmetric with an aspect ratio of >2:1 also exhibit a specific optical signature in which the light scattered by the particle varies in intensity on a timescale related to the major axis length through interference effects associated with the rotation of the particle under Brownian motion.

Accordingly, it is possible that complex nanoparticle suspensions comprising many different particle types can be resolved into different populations through the application of two or more of the above measureands allowing higher resolution analyses than have hitherto been possible.

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