Size and Count of Nanoparticles by Scattering and Fluorescence Nanoparticle Tracking Analysis (NTA)

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ABSTRACT

A novel addition to a technique for the analysis of nanoparticles in a suspension is described. The Nanoparticle Tracking Analysis (NTA) technique sizes individual nanoparticles, based on their Brownian motion. NTA allows nanoparticles to be sized on a particle-by-particle basis, resulting in a higher resolution analysis and therefore a better understanding of polydispersity than ensemble methods (such as dynamic light scattering, DLS) and it also yields directly a count/concentration measurement. Analysis of scattering intensity is a recent development allowing sub-populations of nanoparticles with varying scattering characteristics to be resolved in a complex mixture.

Now this technique has been extended to the analysis and differentiation of fluorescently labeled nanoparticles. With the appropriate wavelength lasers and optical filters, the technique has been shown to be able to differentiate between sub-populations in a heterogeneous mixture.

Keywords: fluorescence, aggregate, nanoparticle, characterization, sizing.

1 INTRODUCTION

The analysis of nanoparticle properties is an increasingly important requirement in a wide range of applications areas and size analysis is usually carried out by either electron microscopy or dynamic light scattering (DLS). Both techniques suffer from disadvantages; the former requiring significant cost and sample preparation, the latter frequently generating only a population average size, which itself can be heavily weighted towards larger particles within the population.

A new method of microscopically visualizing individual nanoparticles in a suspension, called Nanoparticle Tracking Analysis (NTA), allows their Brownian motion to be analyzed and from which the particle size distribution profile (and changes therein in time) can be obtained on a particle-by-particle basis [1-3]. The technique offers significant advantages over traditional light scattering techniques (such as DLS- and SLS-based systems) for the characterization of polydispersed populations of nano-scale particles. Independent of particle density or refractive index, NTA dynamically tracks individual particles within the range of 10 - 1,000nm and provides size distributions along with a real-time view of the nanoparticles being measured.

This technique also provides a measurement of particle count within the measured volume. By knowing the interrogated volume, this particle count can be converted to a total concentration measurement.

Additionally, the technique works equally well whether the light from the particle is scattered or fluorescence. This allows sizing of counting of either naturally fluorescent materials or of particles that have been tagged with fluorophores.

2 MEASUREMENT METHODOLOGY

A small (250µl) sample of liquid containing particles at a concentration in the range 10^6-10^{10} particles/ml is introduced into the scattering cell through which a finely focused laser beam (approximately 40mW at wavelengths appropriate to the fluorophore of interest) is passed. Particles within the path of the beam are observed via a microscope-based system (NanoSight LM10 or NS500) onto which is fitted a CCD camera.

The motion of the particles in the field of view (approx. 100 x100 µm) is recorded (at 30 frames per second) and the subsequent video analyzed. Each and every particle visible in the image is individually but simultaneously tracked from frame to frame and the average mean square displacement determined by the analytical program. From this can be obtained the particle's diffusion coefficient. Results are displayed as a sphere-equivalent, hydrodynamic diameter particle distribution profile. The only information required to be input is the temperature of the liquid under analysis and the viscosity (at that temperature) of the solvent in which the nanoparticles are suspended. Otherwise the technique is one of the few analytical techniques which is absolute and therefore requires no calibration. Results can be obtained in typically 30-60 seconds and displayed in a variety of formats.

The minimum particle size detectable under scattering mode depends on the particle refractive index but for highly efficient scatterers, such as colloidal silver, 10nm particles can be detected and analyzed. For weakly scattering (e.g. biological) particles, the minimum detectable size may only be 30-50nm. For fluorescence measurements, the minimum detectable size also depends significantly on the specific fluorophore, incident wavelength, and number of fluorphores per particle.

The upper size limit to this technique is defined by the point at which a particle becomes so large (>1000nm) that Brownian motion becomes too limited to be able to track accurately. This will vary with particle type and solvent viscosity but in normal (e.g. aqueous) applications is approximately 800-1000nm. See www.nanosight.com for details.

3 SIZE DETERMINATION BY NANOPARTICLE TRACKING ANALYSIS

Brownian motion in a Newtonian fluid is governed by the Stokes-Einstein equation. Whilst the motion clearly occurs in three dimensions, NTA observes motion only in two dimensions. It is possible to determine the diffusion coefficient from measuring the mean squared displacement of a particle in the two observed dimensions;

$$\overline{(x,y)}^2 = \frac{4TK_B t}{3\pi\eta d}$$

where the first term is the mean squared displacement, T, is temperature, K_B is Boltzmann's constant, t is the time period (here given by 1/*framerate*), η is viscosity and d is the hydrodynamic diameter.

By tracking the centers of the particles the mean squared displacement for each and every particle is calculated. This process is depicted below in figure 1. By recording a video of particles (fig 1a), tracking them (fig 1b) and compiling the resulting sizes a particle size distribution is established (fig 1c).

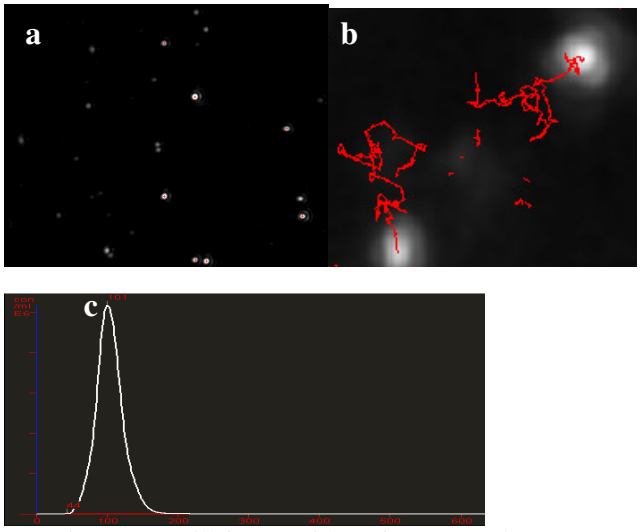

Fig 1. a) A still from a video of 100nm polystyrene calibration particles, b) showing only some (for clarity) of the Brownian motion trajectories analysed and c) subsequent particle size distribution.

4 INTENSITY DISTRIBUTION ANALYSIS

In addition to measuring the particle size for each and every particle, it is possible to extract further information about the particles. The intensity of the light scattered is strongly dependent on the particle size. Therefore simultaneously measuring the intensity of light scattered along with the particle size can further be used to gain more information about the particles. This can either infer information about the size distribution at higher resolution than diffusion rates alone or give an indication of the different particle materials used.

The results shown in Fig 3 were obtained from an analysis of a mixture of 200 and 300nm latex beads (overlaid with the normal particle size distribution plot, 3b) and shows that the two populations can be well resolved from each other. Furthermore, because the technique analyses particles on an individual basis and can collect information on their relative brightness as well as their size (measured dynamically) these two data can be combined to give an intensity v size plot (Fig 3c). This capability shares many features in common with conventional flow cytometry but is unique in this deeply sub-micron size range. [4]

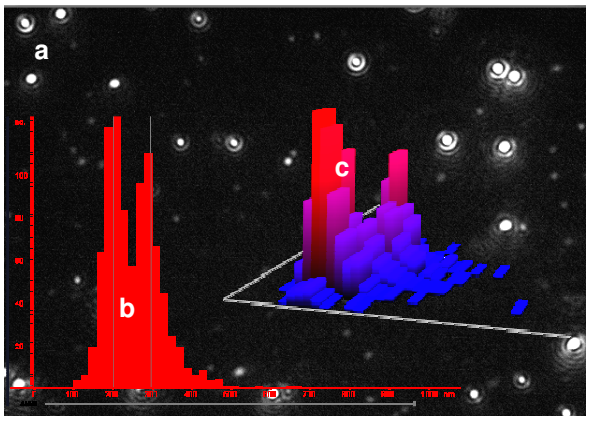

Fig. 3 A mixture of 200nm and 300nm particles; a) still image, overlaid with b) analysis plot and c) 3D number v. relative intensity v. diameter plot.

Figures 4 shows a heterogeneous mix of 50nm gold nanoparticles and 100nm polystyrene latex). Note how the intensity distributions demonstrate that the 50nm (gold) particles scatter at higher intensity than the 100nm (polystyrene latex particles). This example uniquely demonstrates the ability of the technique to differentiate species not just on their hydrodynamic size but also by their scattering ability.

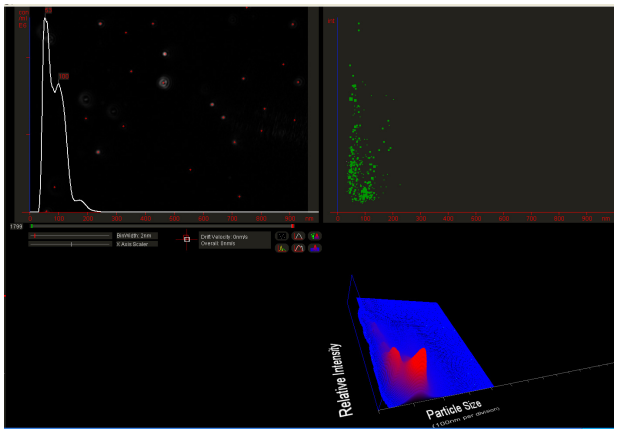

Fig. 4: Particle size distribution, intensity scatter plot, and intensity map for a 50nm gold, 100nm polystyrene mix.

5 FLUORESCENCE MEASUREMENTS

Extension of the NTA technique allows fluorescent nanoparticles to be individually tracked in real-time from which labeled particle size and concentration can be determined. Under light scatter mode, the total number of particles can be measured and subsequently compared to the concentration of labeled particles when measuring in fluorescence mode.

The NanoSight LM10 system uses either a 405nm (blue) or 532nm (green) laser source to excite suitable fluorophores whose fluorescence can then be determined using matched 430nm and 560nm long-pass filters respectively.

For example, a mixture of 100nm fluorescent (Fluoresbrite™, PolySciences Inc.) and 400nm non-fluorescent calibration polystyrene particles was measured under scattered light (Figure 5a) and through an optical fluorescence filter (Figure 5b). Under scattered light, both fluorescent and non-fluorescent particles were observed, sized and counted, while under the fluorescence filter only 100nm fluorescence particles could be visualized.

Note that it was also possible to retain concentration information on the fluorescently labeled nanoparticles for comparative labeling efficiency purposes.

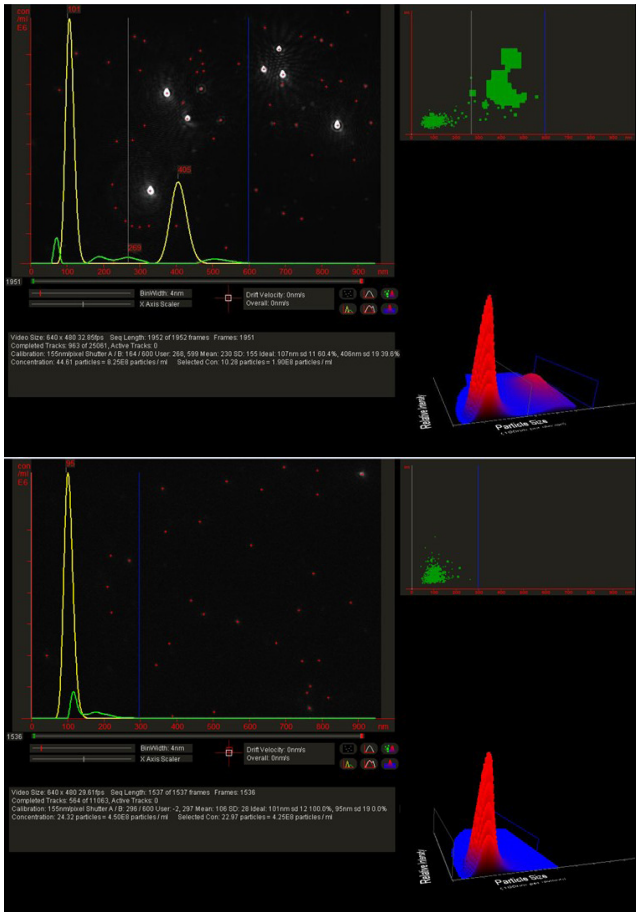

Fig. 5: Particle Size Distribution profiles (yellow graph) of a mixture of 100nm fluorescent and 400nm non-fluorescent polystyrene particles analyzed under a) scatter mode and b) fluorescent (optically filtered) mode.

In the following example an approx. 50:50 mixture of fluorescently labelled (Fluoresbrite) 100nm and unlabelled 100nm polystyrene beads were analyzed under light scatter mode (red line and top image) and when fluorescently filtered (white line and bottom image). The size result was the same for both results, but the difference in the y-axis shows the ability to accurately measure concentration differences.

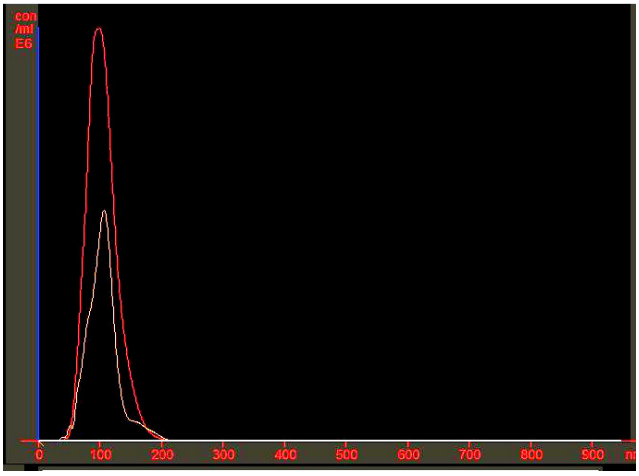

Fig. 6 Mixture of 100nm polystyrene and fluorescent polystyrene, showing difference in concentration in fluorescence measurement mode.

Semiconductor nanocrystals have recently emerged as a powerful and attractive alternative to conventional fluorescent labels due to their great chemical and optical stability and ease of use. Now commercially available as pre-functionalized kits with a choice of emission wavelengths, these interesting materials are rapidly gaining in popularity in the biosciences. While conventionally restricted to being imaged when immobilized (i.e. visualized by long exposure microscopy or when used to multiply label larger structures (e.g. Cellular structures)), NanoSight's new fluorescent versions of their LM Series instrument allow, for the first time, quantum dots to be visualised, sized and counted when unbound and moving freely under Brownian motion in liquids.

The following example is an analysis of a suspension of Invitrogen's non-functionalised QD655 QDot® nanocrystals in an aqueous buffer. Excited by NanoSight's 405nm (blue) laser and detected through a suitable filter, these 655nm emitting QDot® structures are visualized, sized and counted on an individual basis in less than 60 seconds.

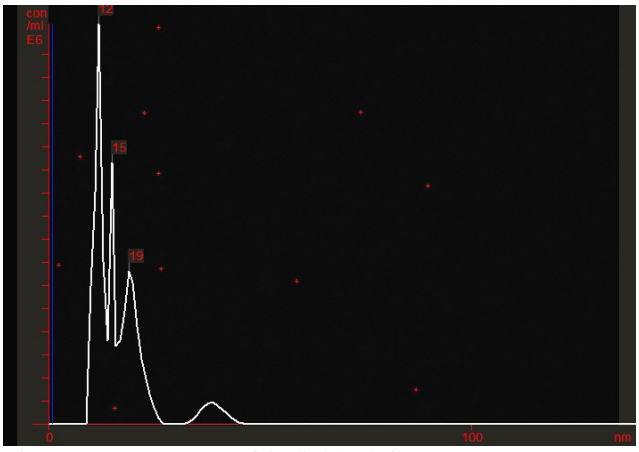

Fig. 7 Measurement of individual fluorescent quantum dot particles.

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5 CONCLUSION

The NTA technique is a robust and direct method for characterizing particle size distributions and concentrations for a wide range of particulate materials. It represents an attractive alternative or complement to higher cost and more complex methods of nanoparticle analysis such as light scattering or electron microscopy that are currently employed. The technique uniquely allows the user a simple and direct qualitative view of the sample under analysis (perhaps to validate data obtained from other techniques) and from which an independent quantitative estimation of sample size, size distribution and concentration can be immediately obtained [5-7]. In addition, fluorescence experiments can be conducted to isolate either fluorescing materials or fluorescently-tagged populations in a heterogeneous mixture.

REFERENCES

[1] CARR, R (2005), NanoParticle Detection and Analysis, 27th International Fine Particles Research Institute (IFPRI) Meeting, June 25-29th, 2006, SANTA BARBARA, USA

[2] CARR, R., DIAPER, T. and BARRETT, E. (2005) NanoParticle Tracking Analysis – The Halo system. Abs Proc 9th Particulate Systems Analysis Conference 2005, September, Stratford-upon-Avon, UK

[3] WARREN, J and CARR, R (2006) 'A Novel Method for Characterisation of Nanoparticle Dispersions', Nanoparticelle: sintesi e caratterizzazione, Politecnico di Torino, Italy 8 March 2006

[4] van der SCHOOT, A (2007) "Sizing of nanoparticles by visualising and simultaneously tracking the Brownian motion of nanoparticles separately within a suspension" ChinaNANO2007 –Abs. International Conference on Nanoscience & Technology, June 4, 2007, Beijing, China.

[5] GHONAIM, H., LI, S., SOLTAN, M.K., POURZAND, C. and BLAGBROUGH, I. S. (2007) "Chain Length Modulation in Symmetrical Lipopolyamines and the effect on Nanoparticle Formulations for Gene Delivery", British Pharmaceutical Conference, Manchester, 10th Sept, 2007

[6] SAVEYN, H., De BAETS, B., HOLE, P., SMITH J. and VAN DER MEEREN, P. (2008) Accurate particle size distribution determination by Nanoparticle Tracking Analysis based on 2-D Brownian dynamics simulation in Abs PSA2008, Stratford on Avon, September, 2008

[7] MONTES-BURGOS, I., SALVATI, A., LYNCH, I. and DAWSON K. (2007) "Characterization techniques for nanoparticle dispersion" at European Science Foundation (ESF) Research Conference on Probing Interactions between Nanoparticles/Biomaterials and Biological Systems, Sant Feliu de Guixols, Spain, 3 November 2007