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Characterisation of Nanoparticle Size and Concentration for Toxicological Studies

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The assessment of the complete distribution of nanoparticle sizes within a suspension is notoriously difficult to carry out. We demonstrate the Nanoparticle Tracking Analysis (NTA) technique that sizes nanoparticles in suspension, based on their Brownian motion. This technique has found significant use in the field of nano- and eco-toxicology, in several research groups showing of the technique to assess a range of engineered nanoparticles including gold, SiO_2, TiO_2 and polystyrene. This capability shares many features in common with conventional flow cytometry but is unique in this deeply sub-micron size range. NTA is a direct and fast technique by which nanoparticles in their natural solvated state in a liquid can be rapidly detected, sized and counted. The technique can be used to complement existing techniques for the sizing of nanoparticles (e.g., DLS, PCS) allowing data obtained from these methods to be validated by direct microscopical observation of the sample.

Keywords: Size Distribution, Characterization, Concentration, Aggregation, Suspension.

1. INTRODUCTION

Assessing the complete distribution of nanoparticle sizes within a suspension is notoriously difficult to carry out. This is especially the case for engineered nanoparticles in the environment which are frequently polydispersed. Accurate determination of the range of sizes is critical to properly understanding transport phenomena and fate of nanoparticles. For example, The Royal Society of Chemistry suggested that 100 nm is the cut-off above which nanoparticles will not enter cells via receptor mediated processes[1] and there are other cut-offs for entering nucleus. Here we demonstrate the Nanoparticle Tracking Analysis (NTA) technique that sizes nanoparticles in suspension, based on their Brownian motion. Unlike classical light scattering techniques, NTA allows nanoparticles to be sized on a *particle-by-particle* basis. This results in a higher resolution and therefore a better understanding of aggregation than ensemble methods (such as dynamic light scattering, DLS) and it also yields directly a count/concentration measurement. Additionally, analysis of scattering intensity allows sub-populations of nanoparticles with varying characteristics to be resolved in a complex mixture. Changes in one or more of these properties can be followed both *in situ* and in real time.

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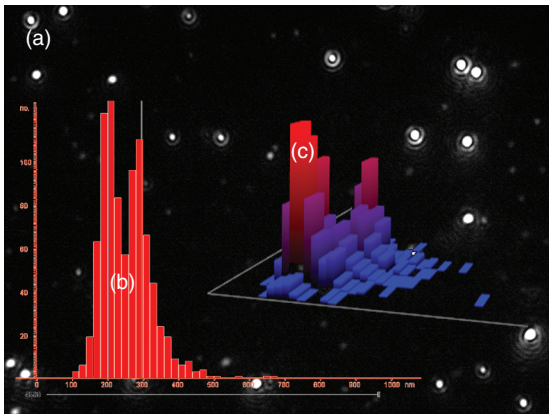
2. MATERIALS AND METHODS

In practice the NTA technique requires a 70 μl sample of liquid containing particles at a concentration in the range 10^{7-9}/ml to be introduced into a scattering cell through which a focused laser beam (approx. 40 mW at $\lambda = 635$ nm) is passed. Particles within the path of the specially configured beam are observed via a microscope-based system or a dedicated non-microscope optical instrument onto which is fitted a CCD camera.[4] The motion of the particles in the field of view (approx. 100×100 μm) is recorded (at 30 fps) and the subsequent video analysed. Each and every particle visible in the image is individually but simultaneously tracked from frame to frame and the mean square displacement is determined by the analytical program from which the particle's diffusion coefficient can be obtained. Results are displayed as an equivalent hydrodynamic diameter particle distribution, calculated from the measured diffusion coefficient. The only information required to be input is the temperature of the liquid under



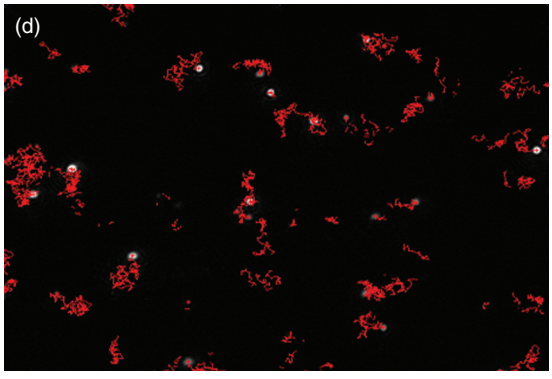


Fig. 1. A mixture of 200 nm and 300 nm particles; (a) still image, overlaid with (b) analysis plot, (c) 3D number versus relative intensity versus diameter plot and (d) A still from a video of 100 nm polystyrene calibration particles showing only some (for clarity) of the Brownian motion trajectories analysed and with the subsequent size plot shown.

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. Notably, because the instrument visualizes particles on an individual basis, particle number concentration is recoverable. Once analysed, the sample is simply withdrawn from the unit for re-use, if required.

3. RESULTS AND DISCUSSION

The results shown in Figure 1 were obtained from an analysis of a mixture of 200 and 300 nm latex beads (overlaid with the normal particle size distribution plot, 1(b)) 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 versus size plot (Fig. 1(c)). Figure 1(d) shows the tracks of the particles' motion with time. This capability shares many features in common with conventional flow cytometry but is unique in this deeply sub-micron size range. NTA is a direct and fast technique by which nanoparticles in their natural solvated state in a liquid can be rapidly detected, sized and counted. While limited to particles of 10–20 nm and above and to concentration ranges between 10^7–10^9 particles per ml, the ability to simultaneously visualise and analyse nanoparticles on an individual basis allows for much improved resolution of polydisperse and/or heterogeneous sample types. The technique can be used to complement existing techniques for the sizing of nanoparticles (e.g., DLS, PCS) allowing data obtained from these methods to be validated by direct microscopical observation of the sample.

References and Notes

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