SIMULTANEOUS SIZING OF NANOPARTICLES BY INDIVIDUALLY VISUALIZING AND SEPARATELY TRACKING THEIR BROWNIAN MOTION WITHIN A SUSPENSION.

Bob Carr, Andrew Malloy and Patrick Hole

NanoSight Ltd, 2 Centre One, Old Sarum Park, Salisbury SP4 6BU, Wilts, UK
bob.carr@nanosight.co.uk

Introduction

The analysis of nanoparticle size is a ubiquitous requirement in a wide range of applications areas (and increasingly in the drug delivery sector) and is usually carried out by either electron microscopy or dynamic light scattering. Both techniques suffer from disadvantages; the former requiring significant cost and sample preparation, the latter generating only a population average which itself can be heavily weighted towards larger particles within the population. A new method of microscopically visualizing individual nanoparticles in a suspension allows their Brownian motion to be simultaneously analysed and from which the particle size distribution profile (and changes therein in time) can be obtained on a particle-by-particle basis.

Experimental Methods

A small (250µl) sample of liquid containing particles at a concentration in the range 106-10/ml is introduced into the scattering cell through which a finely focused laser beam (approx. 20mW at λ=635nm) is passed. Particles within the path of the beam are observed via a microscope-based system (NanoSight LM10) or dedicated non-microscope optical instrument (NanoSight LM20) onto which is fitted a CCD camera. The motion of the particles in the field of view (approx 100x100µm) is recorded (at 30fps) and the subsequent video analysed. 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 and from which can be obtained the particle's diffusion coefficient. Results are displayed as a sphere-equivalent, hydrodynamic diameter particle distribution profile (Fig 1). 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 familiar formats (diameter, surface area or volume on either linear or log scale). The instrument can be programmed to carry out repeat measurements of dynamically changing samples to analyse dissolution, aggregation and particle-particle interactions. 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.

Results and Discussion

A number of results on calibration microspheres and mixtures thereof, colloidal gold and liposomes are shown below which indicate the advantages of the technique. The minimum particle size detectable depends on the particle refractive index but for highly efficient scatterers, such as colloidal silver, 10nm particles can be detected and analysed. For weakly scattering (e.g. biological) particles, the minimum detectable size may only be >50nm. 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. (Fig 2). All particle types can be measured and in any solvent

type providing that the particles scatter sufficient light to be visible (i.e. are not too small or indexed matched).

The results shown in Fig 3 obtained from an analysis of a mixture of 200 and 300nm latex beads (overlaid with the normal particle size distribution plot) show 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 to method in this deeply sub-micron size range. Finally, the technique has been successfully applied to the analysis of a wide range of viruses and liposomes, vesicles and nano-emulsions. See [1] for more information and examples of playable videos of a variety of samples.

Conclusion

The technique is robust and low cost representing an attractive alternative or complement to higher cost and more complex methods of nanoparticle analysis such as photon correlation spectroscopy (PCS) or electron microscopy that are currently employed in a wide range of technical and scientific sectors. Finally, 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 such as PCS) and from which an independent quantitative estimation of sample size, size distribution and concentration can be immediately obtained.

REFERENCES:

[1] www.nanosight.co.uk

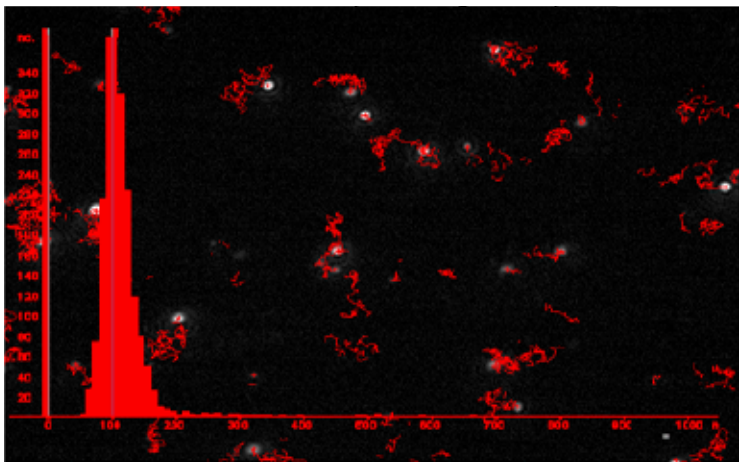

Fig 1. A still from a video of 100nm polystyrene calibration particles showing *some* (for clarity) of the Brownian motion trajectories analysed and which is overlaid with the corresponding size plot.

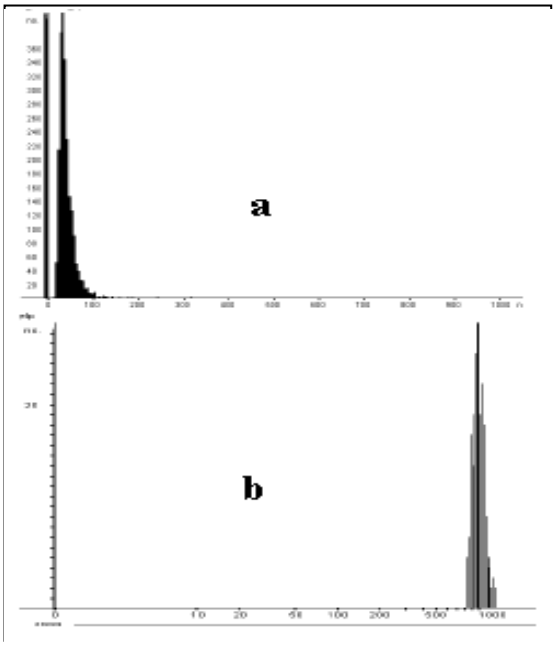

Fig. 2 Size distribution plots of a) 30nm gold colloid (linear scale) and b) 900nm polymeric (log scale) particles.

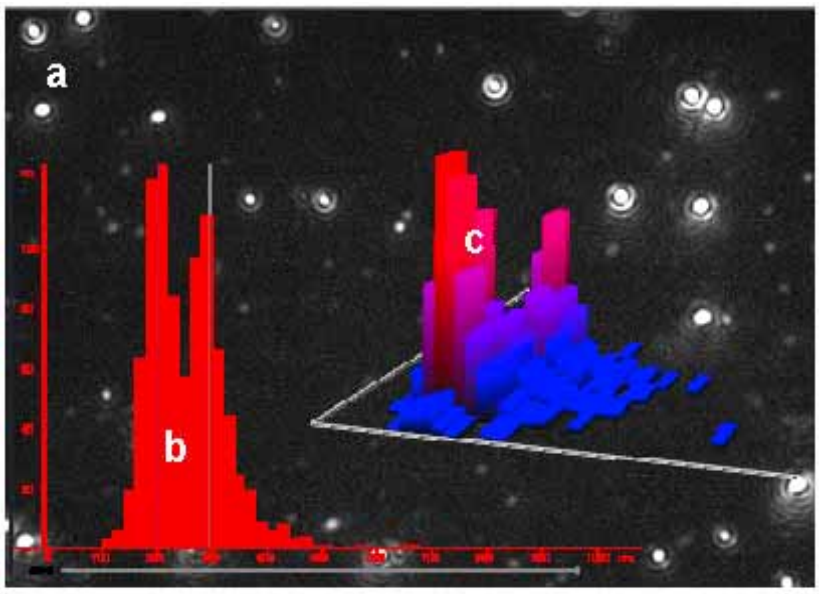

Fig. 3 A mixture of 200nm and 300nm particles; a) still image, overlaid with b) analysis plot and c) 3D number v. relative intensity v. diameter plot.