# NanoParticle Tracking Analysis – The NANOSIGHT system.

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ABSTRACT

*A new technique for nanoparticle sizing that allows visualisation of nanoscale particles in liquids on an individual basis is described. The technology comprises a metallised optical element illuminated by laser beam at the surface of which nanoscale particles in suspension can be directly visualised, sized and counted in real time using only a conventional optical microscope fitted with a low cost camera and a dedicated analytical software package.*

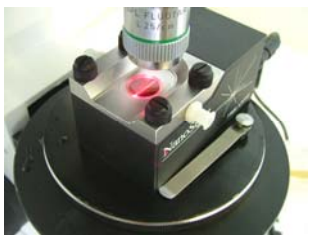
**Keywords:** nanoparticle, sizing, imaging, analysis

## 1 INTRODUCTION

The analysis of nanoparticles is a ubiquitous requirement in a broad range of industry sectors. Product performance and stability frequently depends on the ability to manufacture particle suspensions to fine tolerances without the presence of contaminants or aggregates. Foremost in such analyses is particle size and size distribution measurement for which a number of techniques are well established and commonly employed in routine quality control as well as in a research and development environment. Depending on the nature of the product and the particle characteristics sought, one or more analytical methodologies are routinely employed which include electron microscopy, dynamic light scattering, Fraunhofer scattering, single particle detection techniques, optical microscopy, etc. (Carr et al, 1987). For particles in the nanoscale, however, only the first two of these examples are used frequently.


Both have drawbacks including capital and running costs, analysis turnaround time, and limited ability to resolve particle size distribution profiles. In this report, we describe a new technique that allows the real time visualisation of nanoparticles in liquids using only a conventional optical microscope. The device described, with its analytical software (NanoSight Ltd, 2005), is currently available as a complete microscope based system

## 2 THE TECHNOLOGY



The Class 1 laser device (NANOSIGHT LM10™) comprises a small Al metal housing (92x66x47mm) containing a solid-state, single-mode laser diode (<20mW, 655nm) configured to launch a finely focussed beam through the 500µl sample chamber. An upper optical window mounted in a detachable stainless steel top-plate through which the sample is viewed down the microscope defines the chamber. The base of the chamber comprises a specially designed metallised optical flat above which the beam is caused to propagate in close proximity to the metal film. Particles in the liquid sample which pass through the beam path are seen down the microscope as small points of light moving rapidly under Brownian motion. A sample (suitably diluted if necessary) is simply introduced into the chamber by syringe via the Luer fittings and allowed to equilibrate to unit temperature for a few moments. For samples containing a high concentration of very large contaminants/aggregates, some pre-filtering may be employed.

The light scatted by the particles could be conventionally modelled by Mie theory (Kerker,1969; Bohren and Huffman, 1983) though the determination of particle size by light scattering intensities using this device would require significant a priori knowledge of the optical properties of the particle, solvent, collection optics and camera sensitivity and performance. In the size range for

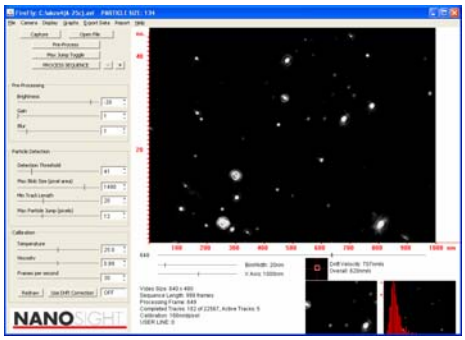
which this device is designed to operate (particles of approximately 10 to 600nm in diameter), the intensity of light scattered by a particle, $I_{scat}$, varies strongly (up to the sixth power) with particle radius, r. Accordingly, contaminating particles or particle aggregates can be clearly seen and identified visually, though ascribing a diameter to the particle on the basis of an image of its light scattered intensity can be problematic or require careful calibration with a reference or calibration standard.

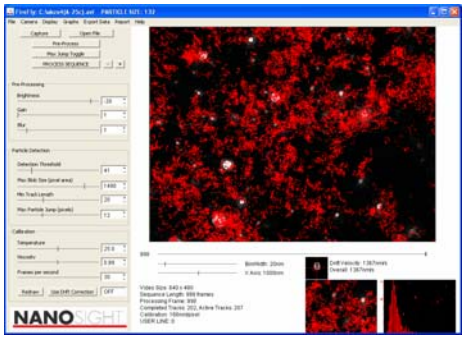
A more attractive alternative, given the ability of the NANOSIGHT system to visualise nano-scale particles in real time and in liquids, is to dynamically analyse the paths the particles take under Brownian motion over a suitable period of time (e.g. 10-20 seconds). Despite the rapidity with which particles move (in the sub-100nm size range in particular) we have shown that such motion can be readily tracked using only conventional CCD cameras. Supported on a C mount on the microscope and operating at 30 frames per second (fps), such cameras can be used to capture video clips of particle suspensions magnified x1000 when present in the 80μm diameter laser beam within the device. It should be appreciated, however, that the particles are not being imaged. For the nano-scale particle range to which the NANOSIGHT system is best suited, the particles act as point scatterers whose dimensions are far below the Rayleigh or Abbé limit, only above which can structural information and shape be resolved.

Such videos can then be analysed using a specially designed NANOSIGHT NTA analytical software programme and from which the size of each particle can be separately determined and accurate particle size distribution profiles derived accordingly.

## 3 THE ANALYTICAL SOFTWARE

The video images of the particle's movement under Brownian motion can be analysed by a single particle tracking programme (Nanoparticle Tracking Analysis [NTA] software). In the system described here, a video can be either captured directly from the camera through the programme or imported as a separate *.avi file. The first frame of the 8 bit video sequence can be user-adjusted in terms of image smoothing, background subtraction, setting of thresholds, removal of blurring etc. to allow particles of interest to be tracked without interference from stray flare or diffraction patterns which can occasionally occur with non-optimum sample types.




Having selected suitable image adjustment settings, the remainder of the video is similarly treated allowing particles to be identified and located on a frame-by-frame basis. Through use of specific selection criteria, movement of individual particles can be followed through the video sequence and the mean squared displacement determined for each particle for as long as it is visible. The user can further select for trajectories whose lifetimes are sufficiently long to ensure statistically accurate results, ignoring those which are so short (e.g. below 5 or 10 frames) that the estimation of diffusion coefficient is statistically unacceptable. Similarly, the occurrence of trajectory cross-over can be accounted for thereby minimising error. From these values, the diffusion coefficient (Dt) and hence sphere-equivalent, hydrodynamic radius ($r_h$) can be determined using the Stokes-Einstein equation:

$$Dt = \frac{K_B T}{6\pi\eta r_h} \qquad (1)$$

where $K_B$ is Boltzmann's constant, T is temperature and $\eta$ is viscosity.

Given that each and every visible particle is separately tracked, it is possible to generate particle size distribution profiles that reflect the actual number of particles thus seen and which is a

significant advance on those distributions that are obtained by other dynamic light scattering techniques such as Photon Correlation Spectroscopy (PCS) in which a large ensemble of particles are collectively analysed and from which only a z-average particle mean is obtained as well as a polydispersity quotient indicating the width of the particle size distribution (Pecora, 1985). As all particles are measured simultaneously in PCS, it is frequently the case that a relatively small number of highly scattering larger particles (e.g. contaminants or aggregates) can effectively obscure the presence of the bulk of the smaller particles that may be present (hence the limitation to an intensity weighted, z-average). It is possible, however, through the application of various de-convolution algorithms, to extract particle size distribution structure (e.g. a bimodal distribution) from the results obtained but this approach is reliable only if the two populations are not too polydisperse themselves or too close together in size.

# 4 RESULTS

## 4.1 Measurement accuracy and reproducibility

The accuracy of results obtained by the NANOSIGHT NTA NanoParticle Tracking system is dependent on a number of factors, primarily particle concentration analysed, the length of time over which the sample is analysed and the size of the particles present. While the sample chamber is approximately 250μl in volume, the section of the laser beam visualised (the scattering volume) is very small (a cylinder approx 70μm diameter x 80μm length). Accordingly, for a statistically significant number of particles to be present on the beam, sample concentrations should lie between $10^5$ and $10^{10}$ particles/ml. Higher concentrations (e.g. $10^{10}$/ml) improve measurement accuracy at shorter analysis See Fig 1). Similarly, observing a sample for 10 seconds (300 sequential images at 30 fps) will result in better statistical accuracy than an analysis of 1 second. The following (Table 1 and Fig 1) show the effects of increasing particle concentration and analysis time for a suspension of 96nm polystyrene latex calibration nanoparticles (Polymer Laboratories, Batch No PSS797W).

**Table 1. Particle size estimation as a function of particle concentration and analysis time.**

| **Particles per ml** | **$10^8$** | **$10^9$** | | **$10^{10}$** | |
|---|---|---|---|---|---|
| **Analysis time (s)** | **10** | **2** | **10** | **2** | **10** |
| **Run number** | | | | | |
| 1 | 143.1 | 125.5 | 105.4 | 103.1 | 102.4* |
| 2 | 115.2 | 129.1 | 123.6 | 101.4* | 99.5 |
| 3 | 120.3 | 135.4* | 111.7 | 100.5 | 102.6 |
| 4 | 140.9 | 142.9 | 127.5 | | |
| 5 | 138.3 | 179.5 | 115.2 | | |
| 6 | 125.7 | 161.7 | 117.6 | | |
| 7 | | 103.2 | 111.3 | | |
| 8 | | 116.6 | 101.1 | | |
| 9 | | 114.5 | 108.2 | | |
| 10 | | 98.8 | 104.2 | | |
| **Average** | **130.6** | **130.7** | **112.6** | **101.7** | **101.5** |
| **SD** | **11.7** | **25.3** | **8.5** | **1.3** | **1.7** |

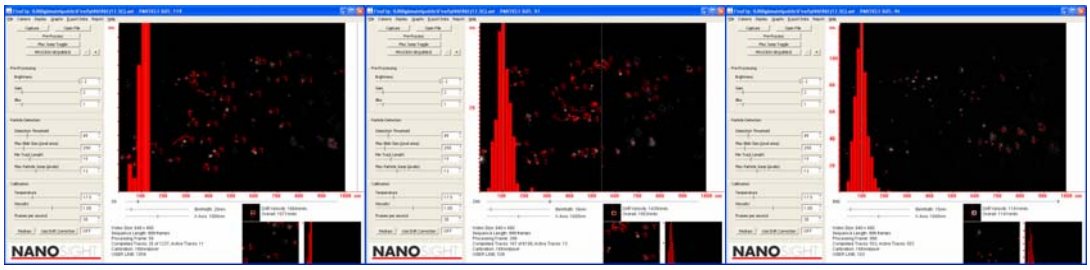


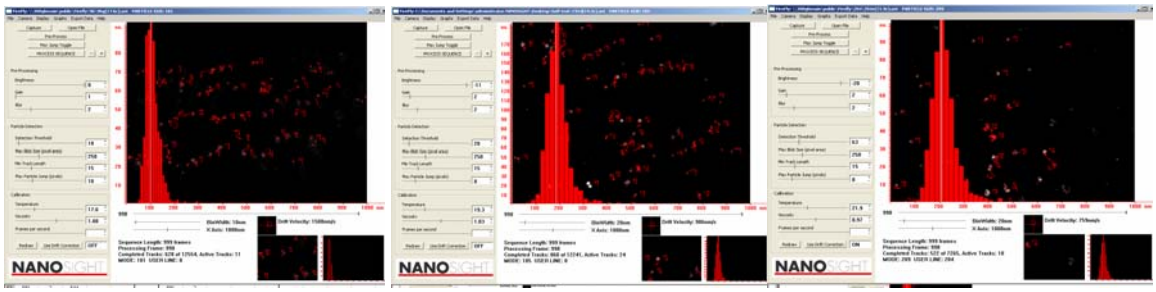
**Fig 1. Particle size distribution profiles for 96nm particles at analysis times of 2, 10 and 30 seconds respectively.**

Larger sized particles become increasing difficult to size accurately. This is demonstrated (Table 2 and Fig 2) as the standard deviations become increasingly large as particle size increases and the apparent particle size distribution width increases. When particles approach and exceed approximately 600nm; a) the amount of light scattered increases significantly leading to camera overload (pixel blooming), b) diffraction rings appear around each particle causing particle centering, and hence tracking, problems and c) Brownian motion becomes increasingly restricted and slower thereby reducing the accuracy with which $D_t$ can be determined.

**Table 2. Particle size estimation of polystyrene calibration spheres when compared to quoted values (analysis performed for 30 seconds with each sample).**

| Particle | Exp. No. | Mode | Average | S.D. | Particle | Exp. No. | Mode | Average | S.D. |
|---|---|---|---|---|---|---|---|---|---|
| 96 | 1 | 102 | | | 309 | 1 | 306 | | |
| 96 | 2 | 103 | | | 309 | 2 | 309 | | |
| 96 | 3 | 97 | | | 309 | 3 | 288 | | |
| 96 | 4 | 102 | | | 309 | 4 | 311 | | |
| 96 | 5 | 104 | | | 309 | 5 | 309 | | |
| 96 | 6 | 105 | | | 309 | 6 | 291 | | |
| 96 | 7 | 101 | | | 309 | 7 | 299 | | |
| 96 | 8 | 103 | | | 309 | 8 | 285 | | |
| 96 | 9 | 103 | | | 309 | 9 | 316 | | |
| 96 | 10 | 105 | 102.5 | 2.3 | 309 | 10 | 306 | 302 | 10.6 |
| 191 | 1 | 179 | | | 384 | 1 | 408 | | |
| 191 | 2 | 177 | | | 384 | 2 | 387 | | |
| 191 | 3 | 185 | | | 384 | 3 | 380 | | |
| 191 | 4 | 185 | | | 384 | 4 | 363 | | |
| 191 | 5 | 183 | | | 384 | 5 | 362 | | |
| 191 | 6 | 187 | | | 384 | 6 | 384 | | |
| 191 | 7 | 183 | | | 384 | 7 | 354 | | |
| 191 | 8 | 175 | | | 384 | 8 | 380 | | |
| 191 | 9 | 183 | | | 384 | 9 | 366 | | |
| 191 | 10 | 183 | 182 | 3.8 | 384 | 10 | 364 | 374.8 | 16.1 |
| 204 | 1 | 207 | | | 591 | 1 | 582 | | |
| 204 | 2 | 207 | | | 591 | 2 | 544 | | |
| 204 | 3 | 210 | | | 591 | 3 | 554 | | |
| 204 | 4 | 211 | | | 591 | 4 | 544 | | |
| 204 | 5 | 209 | | | 591 | 5 | 552 | | |
| 204 | 6 | 203 | | | 591 | 6 | 562 | | |
| 204 | 7 | 211 | | | 591 | 7 | 554 | | |
| 204 | 8 | 210 | | | 591 | 8 | 568 | | |
| 204 | 9 | 200 | | | 591 | 9 | 568 | | |
| 204 | 10 | 208 | 207.6 | 3.6 | 591 | 10 | 547 | 557.5 | 12.3 |

**96nm:** **191nm:** **204nm:**



**309nm:** **384nm:** **591nm:**

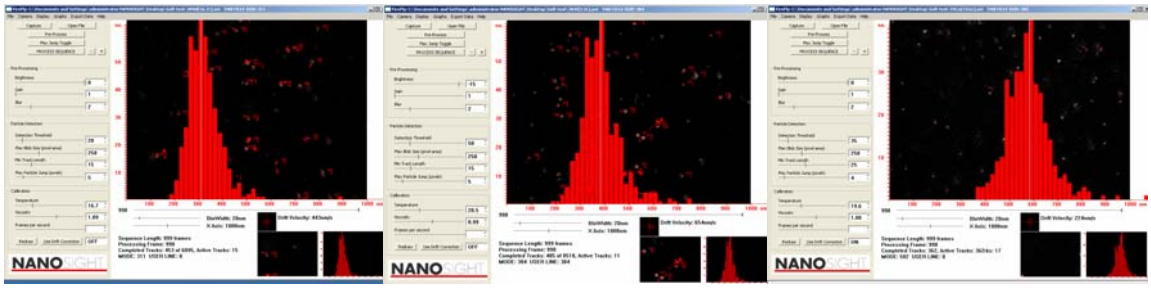


**Fig 2. Representative particle distribution profile for each particle size.**

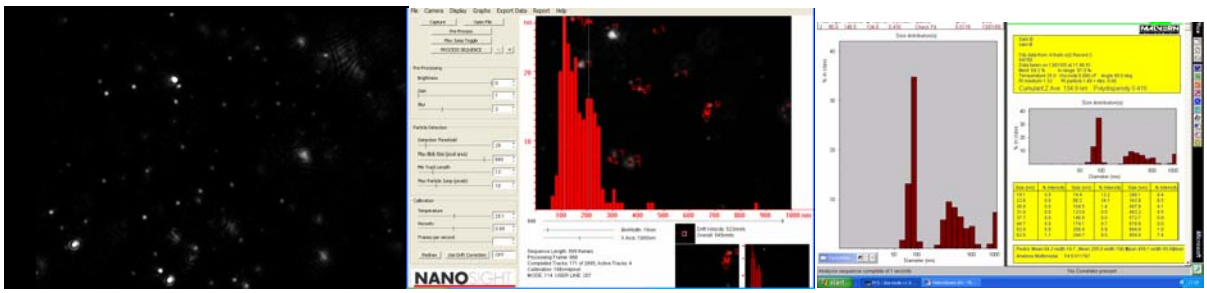
Extending analysis times can help improve accuracy, as can image smoothing but, in general, such sizes represent the practical upper limit for the system described here. Advantageously, however, this upper limit of 600nm represents the bottom of the size range for which other particle sizing techniques can be used (e.g. conventional microscopy, Fraunhofer, etc.).

## 4.2 Comparison with Photon Correlation Spectroscopy

One of the specific advantages offered by the technique described here is the ability to identify, track and analyse small particles even when they are present in heterogeneous samples containing high numbers of larger particles. Fig 3 shows a comparison between the results of a 30 second analysis of a suspension of 100nm polystyrene latex micro-spheres containing a low number of 200nm particles (Fig 3a). It can be seen that analysis by PCS (Malvern Instruments $4700_{CE}$) generates a particle size distribution profile (Fig 3c) that, through the intensity bias of the technique, can over-estimate the concentration of larger particles present, though this can be adjusted for. The NANOSIGHT system also shows the presence of the 200nm particles (Fig 3b) but which more accurately reflects their true concentration. Similarly, it should be noted that the NANOSIGHT system allows the number of particles of any given size that were seen during the analysis to be counted directly representing an advantage over conventional PCS which cannot generate such information.

**Fig 3. Particle size distributions obtained from a video (a) of a suspension of 100nm particles containing a low number of 200nm particles when analysed by b) the NANOSIGHT Single Particle Tracking system and by c) PCS**

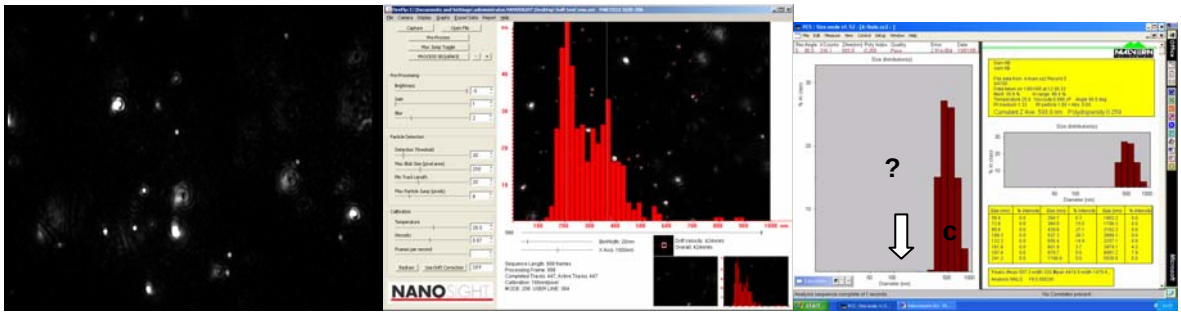
**a)** **b)** **c)**



In Fig 4 can be seen the benefit of being able to analyse particles for size by Brownian motion but without relying on an intensity weighted average of a large ensemble of particles as in PCS. In this case a suspension of 200nm particles was spiked with a low number of 400nm particles and analysed conventionally by PCS and for only 30 seconds by NANOSIGHT. The signal generated in PCS by the larger particles effectively obscures that generated by the smaller particles leading to loss of data relating to the smaller particle peak (as marked in Fig 4c). PCS has a very limited ability to produce particle size distributions from a poly-dispersed sample. Only in ideal situations where the level of light scattered from each particle population is matched, the two populations are each of narrow size distribution and the two particle populations are sufficiently dissimilar in diameter (e.g. >2:1), will PCS be able to produce accurate bimodal distributions. Due to the unique ability to see and subsequently ascribe particle size on an individual particle basis, the Nanosight NANOSIGHT system does not suffer from these limitations.

**Fig 4. Particle size distributions obtained from a video (a) of a suspension of 200nm particles containing several larger 400nm particles when analysed by b) the NANOSIGHT Single Particle Tracking system and by c) PCS**

**a)** **b)** **c)**




## 4.3 Analytical limitations

In the current configuration, particle Brownian motion is measured only in 2 dimensions while, of course, the particles are free to move in 3 dimensions within the sample. While the mathematical algorithm on which this technique is based has been modified to accommodate this fact, there exists possibility that, for particles whose tracks are monitored over shorter timescales, some tracks exhibit a greater degree of motion in the non-measured z-plane than in the tracked x-y plane. This undetected z-plane motion is misinterpreted by the analysis programme as a limited x-y plane movement typical of a larger particle. This problem can adequately compensated for by ensuring that all particles are tracked for a sufficiently long time (>10 frames).

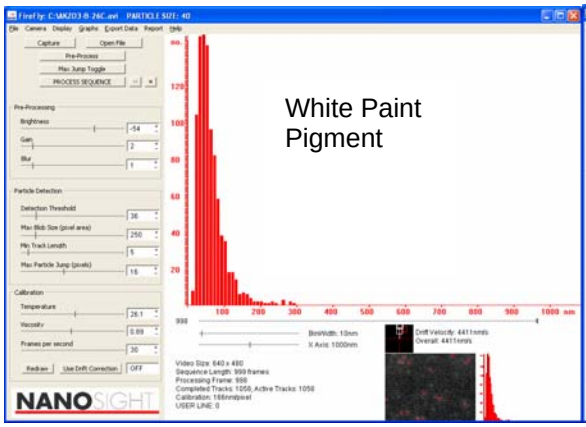
Furthermore, because the technique captures information from a finite focal plane in which particles are in focus (defined by the microscope objective and magnification of the system) particles are free to move under Brownian motion in and out veiw. As smaller particles move a greater distance and more rapidly under Brownian motion than larger particles and hence are more likely to move in and out of the field of view more frequently within any given time period, results from the technique can be number weighted towards the smaller particles within a polydisperse sample.

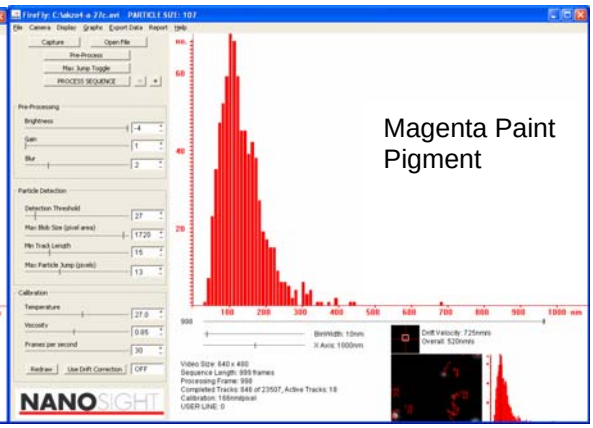
Finally, because the technique allows each and every particle within the scattering volume to be separately analysed in terms of its dynamic behaviour, it is possible to obtain information about the numbers of particles of any given size present in the sample. Simply counting the particles visible within the scattering volume at any given time allows particle number concentration per unit volume to be recovered. However, it should be appreciated that the laser beam in which the particles are present exhibits a Gaussian profile and accordingly, large particles at the less intense beam edge may remain visible while the intensity of light scattered from smaller particles in this region may have dropped below the detection threshold selected by the user. Similarly, high refractive index particles, being more efficient scatterers of light, will also remain visible at larger distances from the beam centre. The effective scattering volume is therefore particle size and Ri dependent and

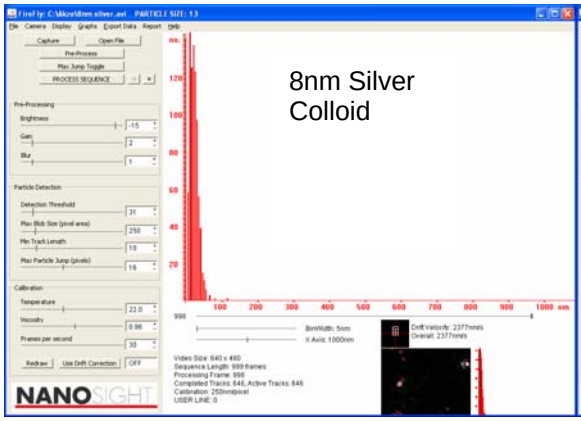
particle counts may vary accordingly. It should be noted, however, that this is only a limitation arising in one axis of the 3 dimensional scattering volume, dimensions in the imaged x and y planes being accurately defined by the field of view of the detection optics.

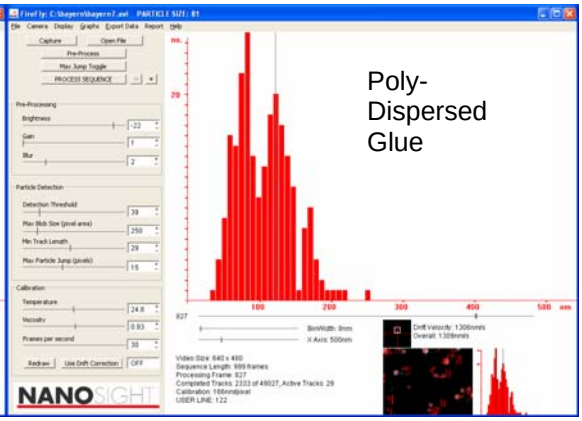
## 5 EXAMPLES OF SAMPLE TYPES MEASURED

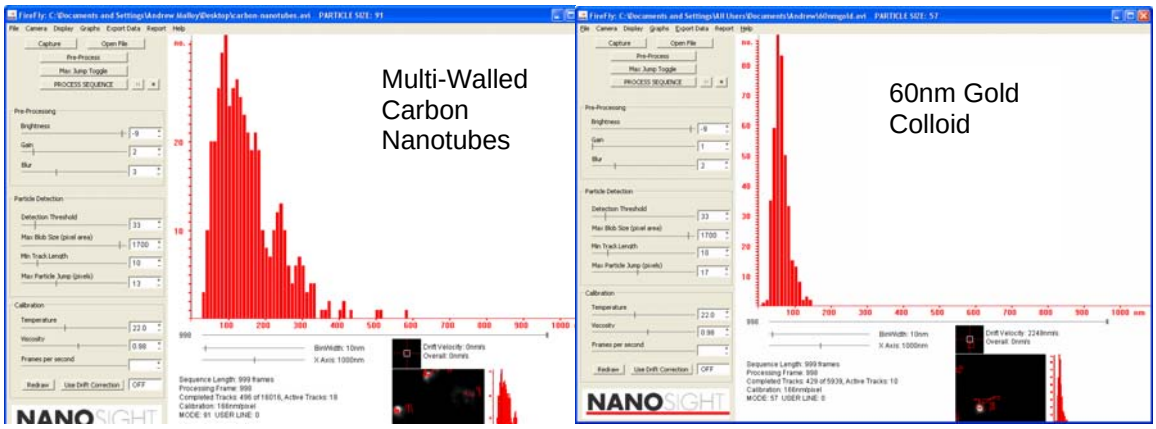
Notwithstanding the limitations outlined above, the NANOSIGHT system can be usefully applied across a wide range of sample types and examples of materials analysed by this method include; viruses (adenovirus, herpes, lambda phage), a wide range of pigments in inks and paints (e.g. TiO2), ferritin molecules, metal oxides (in magnetic storage media), precursor chemicals for wafer fabrication, multi-walled C nanotubes, fuel additives (ZnO2), cosmetics and healthcare products (cream and shampoo formulations), foodstuffs (microemulsions), ceramics, quantum dots and polymers and colloids of many different types. Many of these materials have been suspended in non-aqueous solvents such as toluene and heptane.






## 6 CONCLUSION

The nanoparticle tracking analysis (NTA) system described allows nanoscale particles to be individually visualised (but not imaged) in liquids and from which higher resolution particle size distribution profiles can be obtained compared to other light scattering techniques. Sample pre-treatment is minimal requiring only dilution with a suitable solvent to an acceptable concentration range (between $10^5$ and $10^{10}$ per ml depending on sample type). Accurate and reproducible analyses can be obtained from video of only a few seconds duration and the results allow particle number concentration to be recovered. Given the close to real-time nature of the technique, particle-particle interactions are accessible as is information about sample aggregation and dissemination. 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 indexed matched). The minimum detectable size measurable depends on particle refractive index but can be as low as 9-15nm for high refractive index materials such as colloidal silver.

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 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.

## 6 REFERENCES

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