Particle Technology Labs first to Gain cGMP Accreditation for Nanoparticle Tracking Analysis

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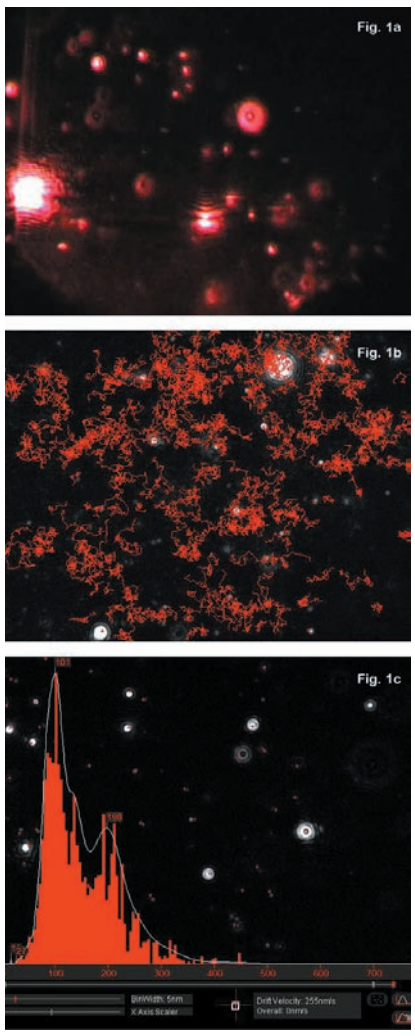
Sub-micron particle characterisation is playing an increasingly important role in many aspects of the pharmaceutical industry as researchers exploit the novel properties these sizes exhibit. At nanometre sizes existing formulations exhibit enhanced bioavailability whilst newcomers frequently look to nano formulation to address poor solubility. Insight into these properties increasingly requires new techniques for their analysis. Here we will look at an increasingly popular technique, Nanoparticle Tracking Analysis (NTA), and its use to study the size and number of particles present.

What is NTA – How Does it Work?

NTA visualises, measures and characterises virtually all nanoparticles in liquids (10-2000nm dependent on material). Particle size, concentration, zeta potential and aggregation can all be analysed while a fluorescence mode provides analysis of suitably-labelled particles. NanoSight provides real time monitoring of the subtle changes in the characteristics of particle populations with all of these analyses uniquely confirmed by visual validation.

Based on a laser-illuminated microscopy technique, the Brownian motion of nanoparticles is analysed in real-time by a CCD or CMOS camera, with each particle simultaneously but separately visualised and tracked by a dedicated particle tracking image analysis program. The NTA program simultaneously identifies and tracks the centre of each particle on a frame-by-frame basis throughout the length of the video – typically 30 seconds. The distance each particle moves in the image is automatically measured. From this value, the particle diffusion coefficient can be obtained and knowing the sample temperature and solvent viscosity the particle hydrodynamic diameter is identified. Because each particle is visualised and analysed separately the resulting particle size measurement and size distribution does not suffer from the limitations of being the intensity weighted, z-average distribution from DLS. The ability of NTA to simultaneously measure particle size and particle scatter intensity allows heterogeneous particle mixtures to be resolved and particle concentration is measured directly; the particle size distribution profile obtained by NTA being a direct number/frequency distribution. As this is an absolute method, no user calibration is required. From loading the sample into the cell to getting results can take as little as 2-3 minutes, with the ability to run batches of samples under the same conditions and directly compare results.



Figures 1a, (top), 1b (middle), 1c (bottom)

The schematic (left) shows the process of an NTA measurement. *Figure 1a* shows the particles present in liquid illuminated by the laser. *Figure 1b* shows the individual tracks of each particle. Finally, *Figure 1c* shows the distribution of the particles under study.

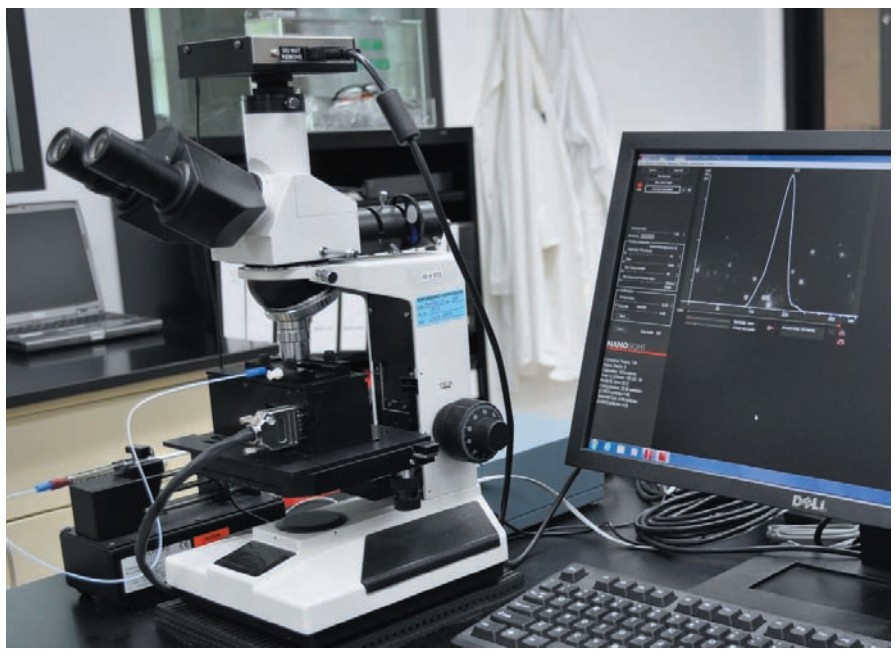
Applications in Pharmaceuticals

Case Study – certification of NTA as cGMP

Particle Technology Labs, Inc, (PTL), is a leading particle characterisation research and advisory company in the United States providing assistance to a wide range of industries. Nanoparticle Tracking Analysis, NTA, has been adopted for both research based projects and as a quality control tool for regulated industries. PTL's NanoSight LM10-HSB instrument is certified cGMP as of 4 June, 2013. cGMP refers to the Current Good Manufacturing Practice regulations enforced by the US Food and Drug Administration (FDA). With cGMP compliance established, PTL's NTA is now fully available to the pharmaceutical industry.

NTA technology will broaden PTL's analytical capabilities by allowing quantification of particle concentrations in the submicron range. In addition, NTA provides an orthogonal or alternate technique to traditional dynamic light scattering (DLS), which until now was PTL's sole submicron sizing technique.

While suitable for many samples, as with any particle sizing technique, DLS does present some specific limitations. Traditional DLS instruments typically have relatively high particle concentration requirements—a minimum of 100 to 150 ppm. The LM10-HSB, being two to three times more sensitive than DLS, can analyse concentrations of particles as low as 50 ppm. In addition, as an instrument with an intensity-weighted measurement, DLS distributions may be biased towards the upper end by just a few large particles. NTA can overcome these limitations through its individual particle-by-particle counting. Furthermore, PTL's access to NTA as an orthogonal technique will provide the ability to validate a hypothesis or questionable finding provided by the results of the DLS instruments obtained in-house.



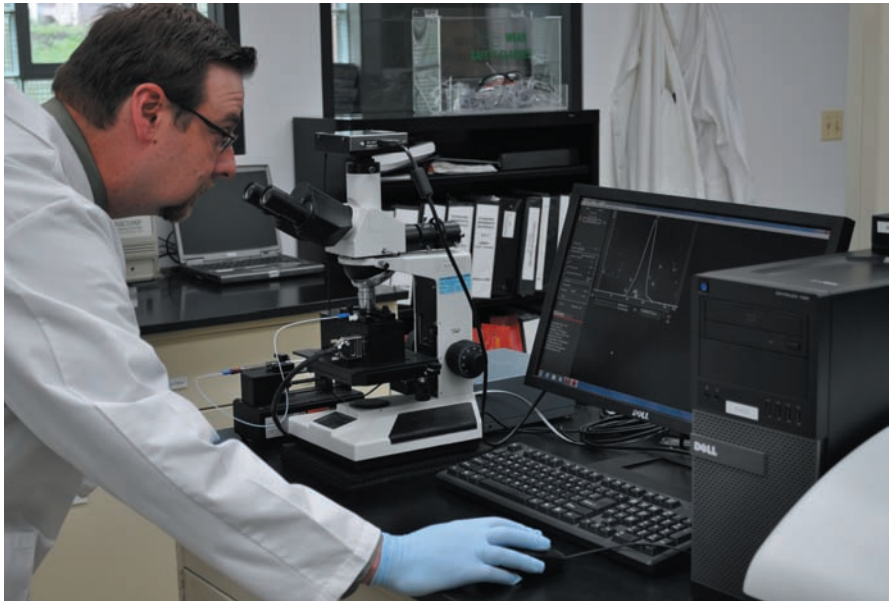
For the concentration measurements, PTL measured 160 tracks using the LM10 system coupled to a syringe pump as shown in the image above.

William Kopesky, PTL's Director of Analytical Services, has driven the process to be the first US laboratory with a cGMP compliant NTA. "We are excited to have this new technology in our laboratory. As a cGMP qualified instrument, the system expands our capabilities to our regulated clients providing them with an accurate particle concentration and size device in the submicron range."

NTA technology is for submicron particle counting. As a counter, it delivers significantly better resolution than DLS instruments. According to PTL qualification results, the NTA is able to partially resolve peaks as close as a factor of 1.5; i.e., 200nm versus 300nm, and fully resolve peaks as close as a factor of 1.75; i.e., 200nm versus 350nm. The alternative technique, DLS has a recognised low and well-documented resolution that has the ability to partially resolve peaks that differ by a factor of greater than 3; i.e., 200nm versus 600nm. In addition to the NanoSight, PTL has several other counting instruments (e.g., AccuSizer, Elzone, and HIAC Royco) but these instruments can only detect particles above 0.5 - 1 micron in size.

The ability of NTA to count particles in the submicron range constitutes a paradigm shift. Access to NTA technology is a benefit for several applications. One example is California Proposition 65, which has caused companies to test their products and materials for the existence of nanosized particles. Thanks to the sensitivity of NTA technology, PTL can now detect an even lower level of submicron particles. A second example is in the analysis of proteins and polymers. These often occur as monomers, dimers, trimers, etc. In theory, if the monomers are large enough, using NTA, PTL can observe separate populations of monomers, dimers, trimers, etc., granting the ability to not only provide a snapshot of the equilibrium concentrations, but possibly the ability to obtain kinetic agglomeration data.

These aggregation studies used a proprietary protein below the 10nm detection limit of NTA. The studies were in fact of a protein matrix of aggregated forms of albumin and hence the measurements were following the size of these aggregates.



A third application benefiting from the introduction of NTA technology is filtration studies, particularly for filter manufacturers and water remediation locations. Using DLS, PTL has only been able to tell a client the mean particle diameter of particles before and after filtration, but the client's ability to determine the efficiency of their filtration was limited due to the lack of concentration data. Furthermore, DLS data is skewed towards the larger end of the distribution because of the intensity-weighting. Through the use of NTA, PTL will now be able to measure the particle concentration before and after filtration, thus providing a clear picture of the filtration efficiency. NTA will also provide results unbiased by intensity-weighting.

PTL Director of Analytical Services, William Kopesky, uses the cGMP- certified NanoSight LM10-HSB system for nanoparticle characterisation.

With more than 800 peer reviewed papers published where NTA is referenced, there is obviously a wealth of material available for the user to study. Within the pharmaceutical industry, the following topics are currently attracting the most attention in the literature.

- Environmental impact of nanoparticles including nanoparticle toxicology
- Drug delivery and targeting
- Drug nanocarrier design and formulation
- Exosomes and microvesicles: characterisation
- Exosomes and microvesicles: cancer studies
- Exosomes and microvesicles: diagnostics and therapeutic potential
- Viruses and viral vaccines
- Protein aggregation
- Design and formulation of pharmaceutical macromolecules and nanoparticles
- Nanoparticle production for pharmaceutical use

To look at a complete and current list of papers published citing results using NTA, visit: www.nanosight.com/publications/third-party-papers-

About NanoSight

NanoSight delivers the world's most versatile and proven multi-parameter nanoparticle analysis in a single instrument. The company has installed more than 600 systems worldwide with users including BASF, GlaxoSmithKline, Merck, Novartis, Pfizer, Proctor and Gamble, Roche and Unilever together with the most eminent universities and research institutes. NTA is validated by 800+ third party papers citing NanoSight results. NanoSight's leadership position in nanoparticle characterisation is consolidated further with publication of an ASTM International standard, ASTM E2834, which describes the NTA methodology for detection and analysis of nanoparticles.

About PTL

PTL is the leading particle characterisation research and advisory company in the United States. The company works with major organisations across the private, public and scientific research sectors. Their scope and expansive knowledge of particle technology allows PTL to address problems that no one else can, and provide insight and perspective necessary for clients to make the right decisions. PTL has deep functional expertise in industrial sectors such as pharmaceutical, environmental, petroleum-chemical, beauty and cosmetics, manufacturing, and food and beverage. The company is the recognised leader in particle characterisations, providing clients with a broad range of expert services in particle size and count distribution, surface area and image analysis, thermal analysis, powder flowability, and method development, verification, and validation.

Acknowledgment: NanoSight would like to thank Dr William Kopesky and his colleagues at PTL for sharing their experiences in the testing and adoption of NTA in their routine laboratory characterisation procedures.