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Size Determination of Gold Colloid by Nanoparticle Tracking Analysis

Application Note

Gold nano-particles are commonly used in a variety of applications such as: TEM/SEM analysis, secondary antibody/protein labels, immunoassays, biosensors, catalysis and when combined with polymeric materials as biological scaffolds.

Background

NanoSight instruments provide a unique ability to directly visualise and size nanoparticles in a liquid suspension. The visualisation of the particles allows **each particle to be simultaneously and individually sized** (fig. 1), overcoming inherent problems associated with techniques such as Photon Correlation Spectroscopy (PCS, or dynamic light scattering). The intensity of light scattered from a nanoparticle as a function of particle radius follows a power law and increases with the sixth power for a Rayleigh particle(1). Hence the **average** particle size produced by PCS (which measures total light scattered from an ensemble of particles) is heavily weighted towards small numbers of large, perhaps contaminant, particles. Electron microscopy on the other hand requires time-consuming sample preparation and imaging and only views a small area thus risking a non-representative analysis of the sample as a whole.

Whilst the NanoSight view could distinguish between particles by the amount of light they scatter, particle sizing based on light scatter requires knowledge of the refractive index of the particles. The NanoSight technique calculates a sphere-equivalent hydrodynamic radius based on the **Brownian motion** of each individual particle tracked over multiple frames and hence is totally independent of refractive index. The ability to track each particle individually allows better characterisation of poly-dispersed systems (fig. 2).

Sample preparation

The only preparation required is dilution of the sample to between 10^6 and 10^9 particles/ml dependent on sample type and size. At this dilution individual particles can be seen moving under Brownian motion and therefore can be analysed. Optimum concentration is particle and solvent dependent.

Hydrodynamic Radius

The technique measures the hydrodynamic radius of a particle. This is the physical radius of particle, plus a small (typically few nm) Helmholtz layer of tightly bound water molecules. To minimise this effect the sample should be prepared in a 1mM salt solution. Due to this effect the

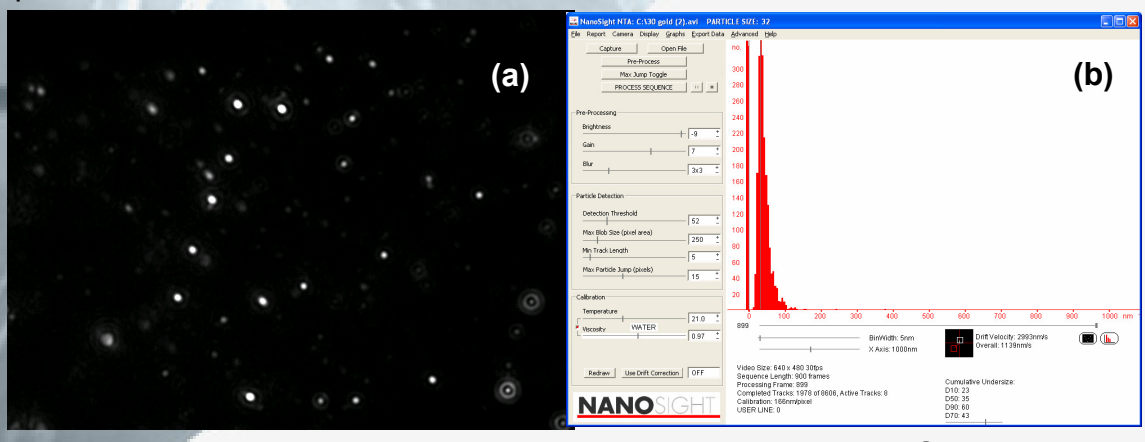

Figure 1: A typical a) image and b) distribution produced by NanoSight particle sizing equipment. The above distribution and image is for 30nm Gold Colloid(2).

(1) C. F. Bohren, D. R. Hoffman, *Absorption and scattering of light by small particles*, (Wiley, New York, 1983).

(2) http://www.bbigold.co.uk/dignostics.htm

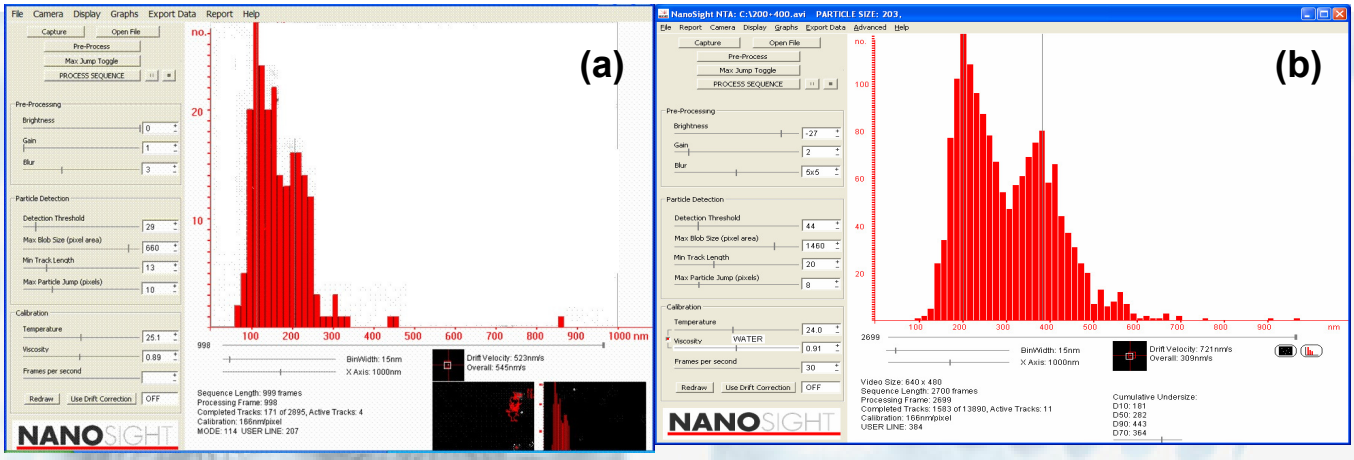

Figure 2. Particle size distributions for (a) a mix of 100 and 200nm latex particles and (b) a mix of 200 and 400nm latex particles.

size measured in a water-based measurement will always be a few nanometers larger than measurements taken by TEM and quoted by the manufacturers of the gold standards (see Table 1).

Temperature Measurement

Correct temperature measurement is very important as an incorrect reading of sample temperature leads to an incorrectly calculated viscosity. A 1°C error in temperature reading gives

Sample	Experiment No.	Mode (nm)	Average Mode (nm)	Standard Deviation	D10 (nm)	D50 (nm)	D90 (nm)
19.6nm±1.6	1	26			17	31	61
19.6nm±1.6	2	24			16	29	59
19.6nm±1.6	3	24			16	29	58
19.6nm±1.6	4	24			17	29	58
19.6nm±1.6	5	24	24.4	0.89	16	30	58
29.6nm±2.4	1	31			23	34	58
29.6nm±2.4	2	32			23	35	60
29.6nm±2.4	3	32			24	36	62
29.6nm±2.4	4	33			24	37	63
29.6nm±2.4	5	32	32	0.82	24	35	58
40.1nm±3.2	1	39			27	47	95
40.1nm±3.2	2	41			27	48	99
40.1nm±3.2	3	41			29	51	99
40.1nm±3.2	4	38			29	52	102
40.1nm±3.2	5	39	39.6	1.3	29	52	101
47.6nm±3.8	1	50			36	53	82
47.6nm±3.8	2	47			36	53	80
47.6nm±3.8	3	50			37	53	82
47.6nm±3.8	4	48			38	53	83
47.6nm±3.8	5	49	48.8	1.3	37	52	78
59.8nm±4.8	1	60			47	64	90
59.8nm±4.8	2	61			47	64	92
59.8nm±4.8	3	59			47	63	90
59.8nm±4.8	4	62			46	64	90
59.8nm±4.8	5	61	60.6	1.1	47	65	95

Table 1. Modal size and D10, D50 and D90 values for BBI International Gold Colloid compared to the quoted values measured by TEM for a range of particle sizes.

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a 2.7% error in sizing for aqueous systems. Due to the small volume required in the NanoSight instrument (<500µl) the temperature equilibration takes only a few minutes and can be directly read during the analysis.

Key features

- **Particles can be measured in their natural state (no drying/vacuum conditions required).**
- **Ability to size a sample with greater polydispersity due to the insensitivity of the technique to light scattering intensity.**
- **Small sample volume.**
- **Low cost of unit.**
- **Visualisation of individual particles without any pre-treatment such as labelling.**
- **Ability to rapidly analyse time dependent factors such as agglomeration/ stability.**

Contact details

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...seeing is believing