

# Visualisation, Sizing and Counting of Fluorescent and Fluorescently-Labelled Nanoparticles

## Introduction

Fluorescent molecules have long been used to specifically label particular structures and features in complex mixtures or matrices to allow their presence and spatial distribution to be determined. Historically based on organic molecules, a very wide range of such fluorophores have been developed, the selection of which is determined by the application required.

## Background

Fluorescent molecules, depending on their structure and properties, exhibit specific excitation and emission spectra, have different solubilities and stabilities in different chemical/solvent environments and possess varying quantum efficiencies and resistance to photobleaching. Their ability to be discriminated from non-labelled background through the use of selective optical filters allows the user to identify and quantify almost any type of structure. This is the case whether mediated by antibody, nucleic acid fragment or other structures with a specificity for, and an ability to bind to a target analyte, or if used as a direct fluorescent 'stain' with a direct affinity for lipids, sugars, proteins, etc. Recently, a new class of luminescent semiconductor nanocrystal, called a quantum dot, which exhibits significantly enhanced luminescence, chemical stability and resistance to photobleaching has become available. Fluorophores are thus found to be used throughout the bioanalytical sciences.

## Fluorescent Nanoparticles seen by NanoSight's Nanoparticle Tracking Analysis

NanoSight's new fluorescence versions of the LM Series instrument range allow fluorescent nanoparticles to be individually tracked in real-time from which labelled particle size and concentration can be determined. Under light scatter mode, the total number of particles can be measured and subsequently compared to the concentration of labelled particles when measuring in fluorescence mode.

The NanoSight system uses either a 405nm (blue) or 532nm (green) laser source to excite suitable fluorophores whose fluorescence can then be determined using matched 430nm and 560nm long-pass filters respectively.

The 405nm laser can be used to excite fluorescein dyed polystyrene beads (which are excited at 441nm and emit at 486nm).

For example, a mixture of 100nm fluorescent (Fluoresbrite™, PolySciences Inc.) and 400nm non-fluorescent calibration polystyrene particles was measured under scattered light (Figure 1a) and through an optical fluorescence filter (Figure 1b). Under scattered light, both fluorescent and non-fluorescent particles were observed, sized and counted, while under the fluorescence filter only 100nm fluorescence particles could be visualised.

Note that it was also possible to retain concentration information on the fluorescently labelled nanoparticles for comparative labelling efficiency purposes.

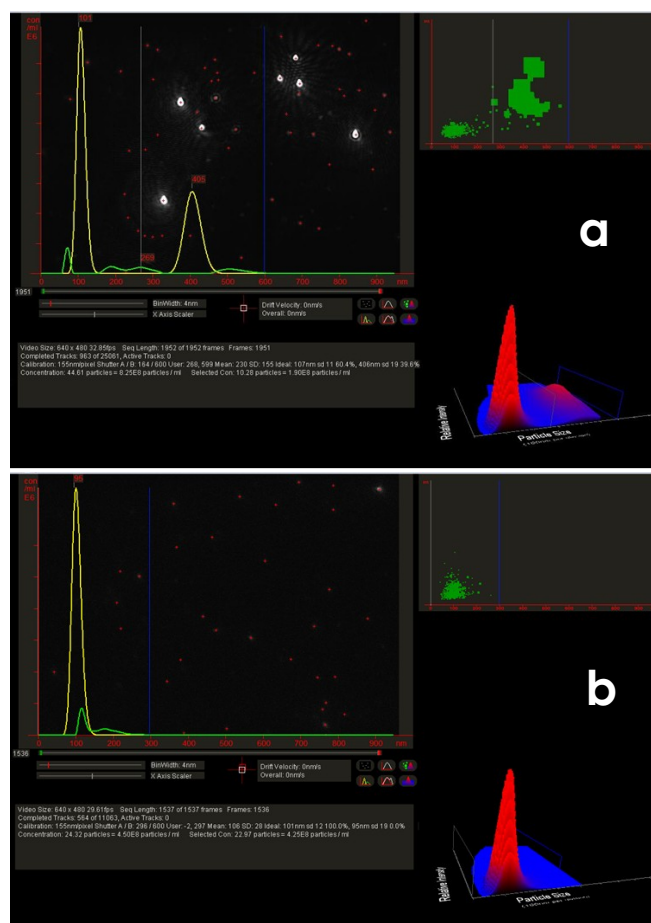


Figure 1: Particle Size Distribution profiles (yellow graph) of a mixture of 100nm fluorescent and 400nm non-fluorescent polystyrene particles analysed under a) scatter mode and b) fluorescent (optically filtered) mode.



## Fluorescence Nanoparticles Analysed by NanoSight's Nanoparticle Tracking Analysis

NanoSight's new fluorescent versions of their LM Series instrument range allow not only fluorescent nanoparticles to be individually sized in real-time but also for them to be counted at the same time.

In the following example an approx. 50:50 mixture of fluorescently labelled (Fluoresbrite) 100nm and unlabelled 100nm polystyrene beads were analysed under light scatter mode (red line and top image) and when fluorescently filtered (white line and bottom image).

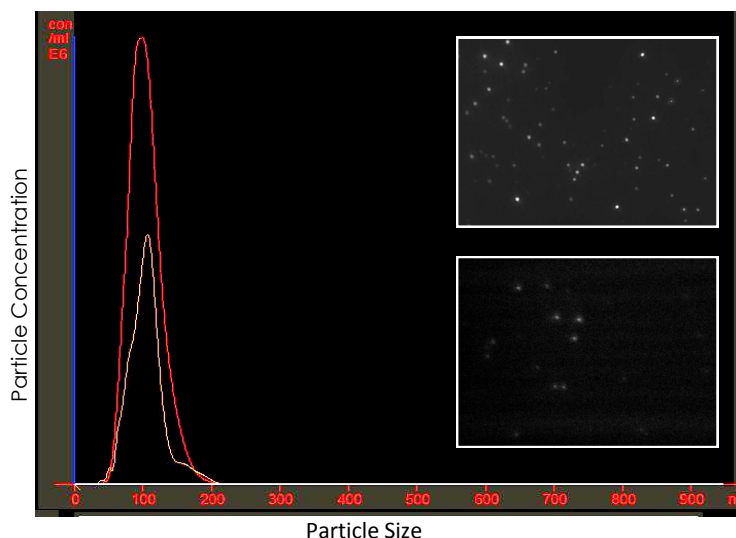


Figure 2

The table below shows that while the sizes are the same, the number of particles seen when only the fluorescently- labelled part of the population is observed through the filter, fall as expected.

Mixture	Mixture of 100 nm fluorescence and 100 nm non-fluorescence polystyrene nanoparticles	
Mode	Under scatter light	Under optical filter (fluorescence)
Particle size [nm]	100	98
Concentration [*10 <sup>8</sup> particles/ml]	8.88	3.44

Figure 3

## Unique Detection of Individual Quantum Dots (QDots®) in Solution

Semiconductor nanocrystals have recently emerged as a powerful and attractive alternative to conventional fluorescent labels due to their great chemical and optical stability and ease of use. Now commercially available as pre-functionalised kits with a choice of emission wavelengths, these interesting materials are rapidly gaining in popularity in the biosciences. While conventionally restricted to being imaged when immobilised (i.e. visualised by long exposure microscopy or when used to multiply label larger structures (e.g. Cellular structures)), **NanoSight's new fluorescent versions of their LM Series instrument allow, for the first time, quantum dots to be visualised, sized and counted when unbound and moving freely under Brownian motion in liquids.**

The following example is an analysis of a suspension of Invitrogen's non-functionalised QD655 QDot® nanocrystals in an aqueous buffer. Excited by NanoSight's 405nm (blue) laser and detected through a suitable filter, these 655nm emitting QDot® structures are visualized, sized and counted on an individual basis in less than 60 seconds.

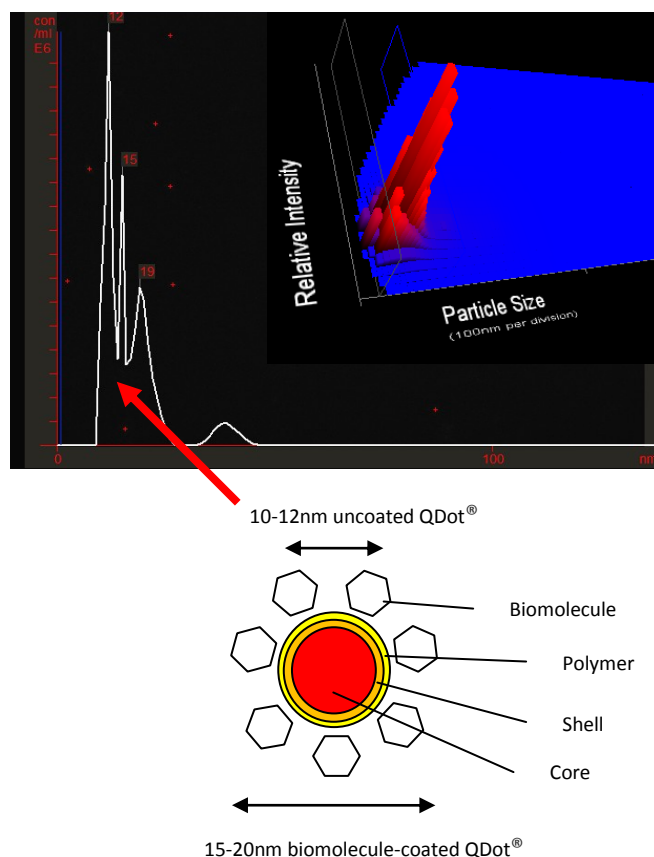


Figure 4

The following plot is of a functionalised QDot® sample in which initial particle interaction with binding ligands in the sample is evident. Note the mode of the smaller peak is compatible with the dimensions of a protein coated QDot® but aggregates (multimers) are also appearing.

Note also there is slight evidence of the presence of non-functionalised QDots® at 14nm. The veracity of this peak would need to be confirmed with further analysis.

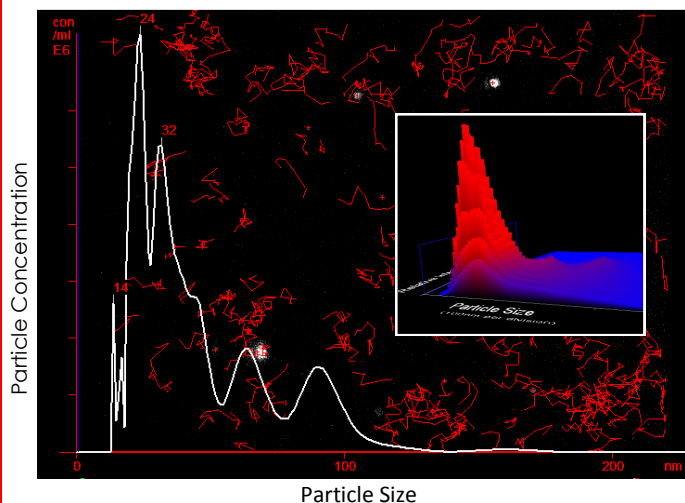


Figure 5

## Analysis of Fluorescently Labelled Sub-Micron Biological Structures

Finally, in the plot shown in Figure 6 below, a sample of clinically significant cellular micro and nanovesicles were specifically labelled with an appropriate fluorescent label attached by antibodies raised against molecular antigens on the micro-vesicle. The red plot is of the micro-vesicle population as viewed under fluorescence analysis, the green plot is of the entire population of labelled and unlabelled structures.

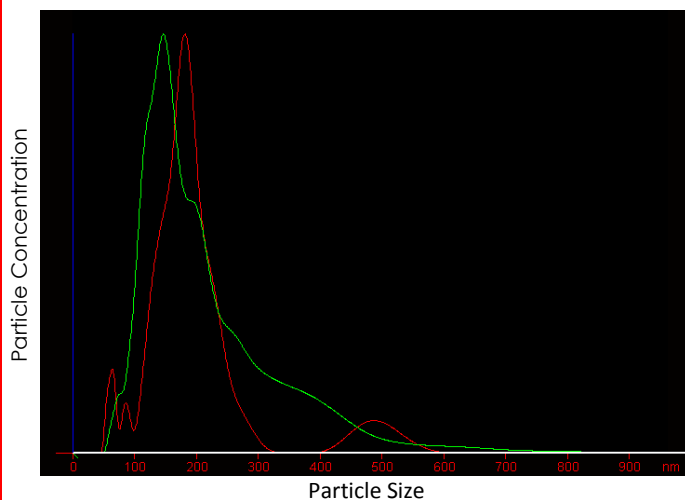


Figure 6

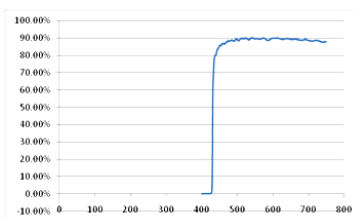
## Key Features

- Particles can be measured, sized and counted under two modes: scattered light and optical filter (fluorescence)
- Small sample volume required
- Low cost of instrument
- Visualisation of individual fluorescent particles
- Ability to rapidly analyse time-dependent factors such as agglomeration and stability

## Fluorescence Filters Available for 405nm and 532nm Laser Sources\*

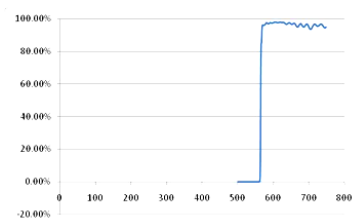
\*Contact NanoSight for alternative filter sets.

### 430nm Longpass



Filter- Cut-on: 430+/-3nm  
 Trans: >=90% avg  
 80%min 440-750nm  
 Block: >=OD4 @ 420nm  
 Size: 22 +0/-0.25mm  
 Thick: </=3.5mm

### 565nm Longpass



Filter- Cut-on: 565 +/-3nm  
 Trans: >=90% avg  
 80%min 570 -750nm  
 Block: >=OD4 @ 550nm  
 Size: 22 +0/-0.25mm  
 Thick: </=3.5mm

## Contact Details

For further information, contact NanoSight or your local distributor, listed at [www.nanosight.com](http://www.nanosight.com)

