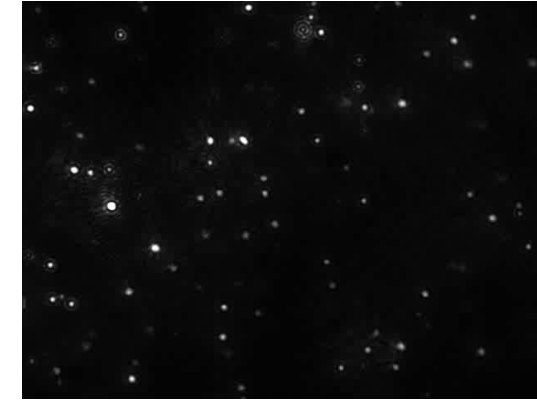


Assessment of the efficiency of encapsulation of a fluorescent drug using **Nanoparticle Tracking Analysis (NTA)**

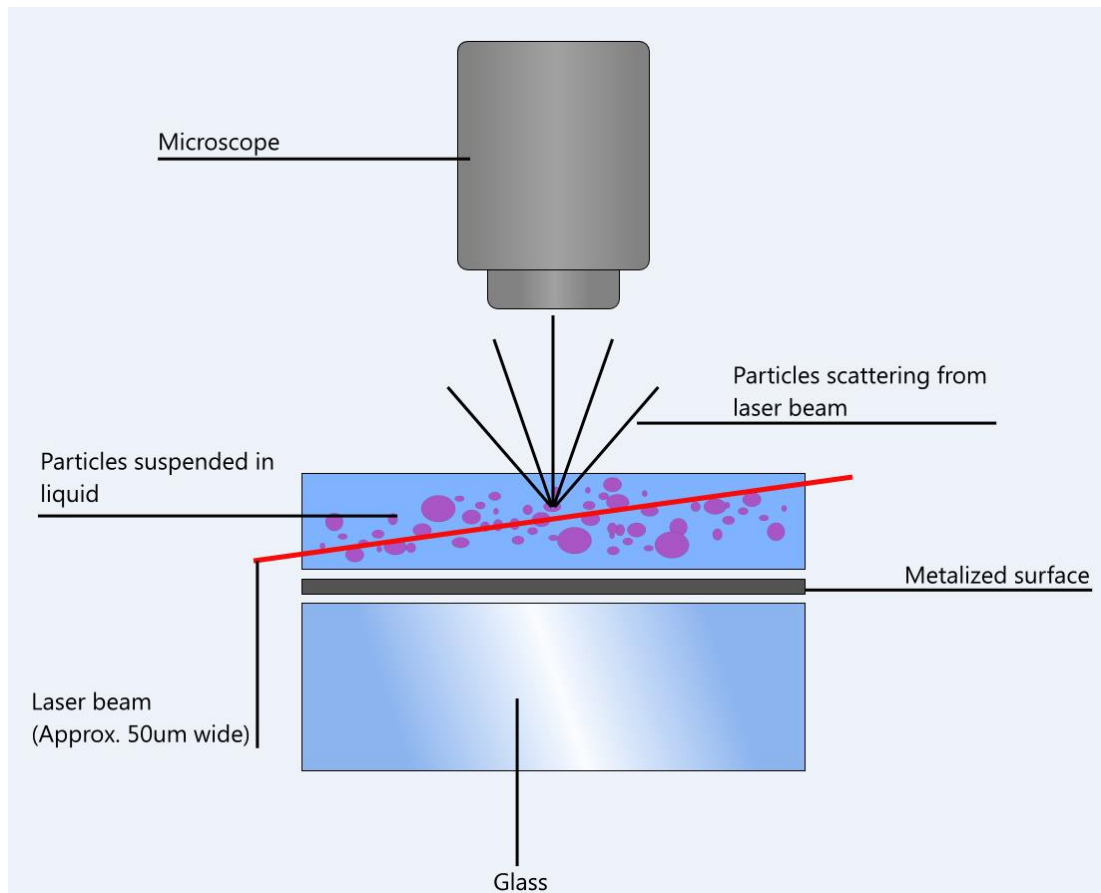
Roberto Santoliquido

A Brief Introduction to NTA

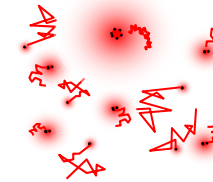
- Proprietary optical element
- Illuminated by specially configured laser beam
- Scattered light collected by camera



EVs in suspension moving under Brownian motion



Particle tracks are determined



Stokes-Einstein equation

$$Dt = \frac{TK_B}{3\pi\eta d}$$

η = viscosity
T = Temperature

Particles Visualised Directly, in Real Time

- Particles are too small to be imaged by the microscope
- The particles seen as light points moving under Brownian motion
- This is visualisation of scatter (not a resolved image)

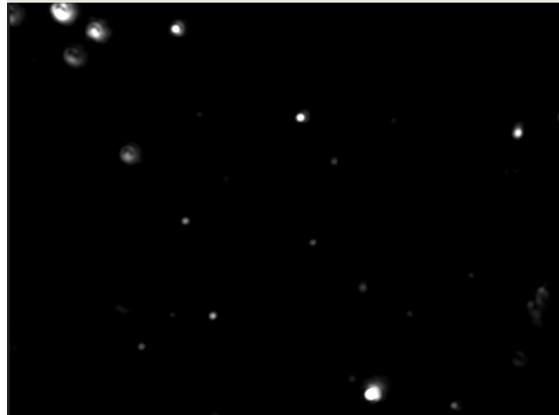


100 – 500 nm Microvesicles purified from serum, Field of view is approximately 120 x 100 microns.

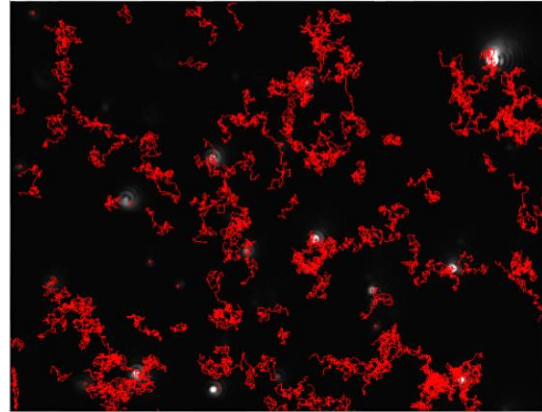
Small Particle = **Fast** Brownian motion

Large Particle = **Slow** Brownian motion

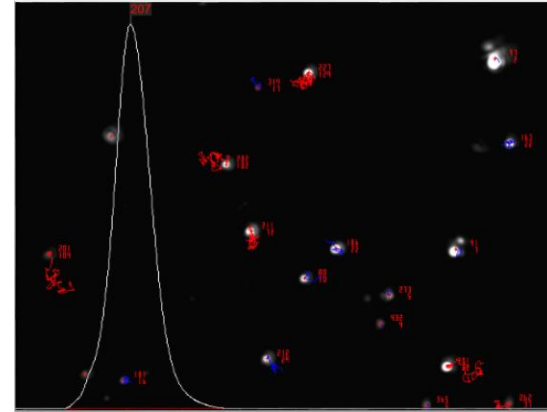
NTA steps & Identification of Different Materials



CAPTURE



TRACK

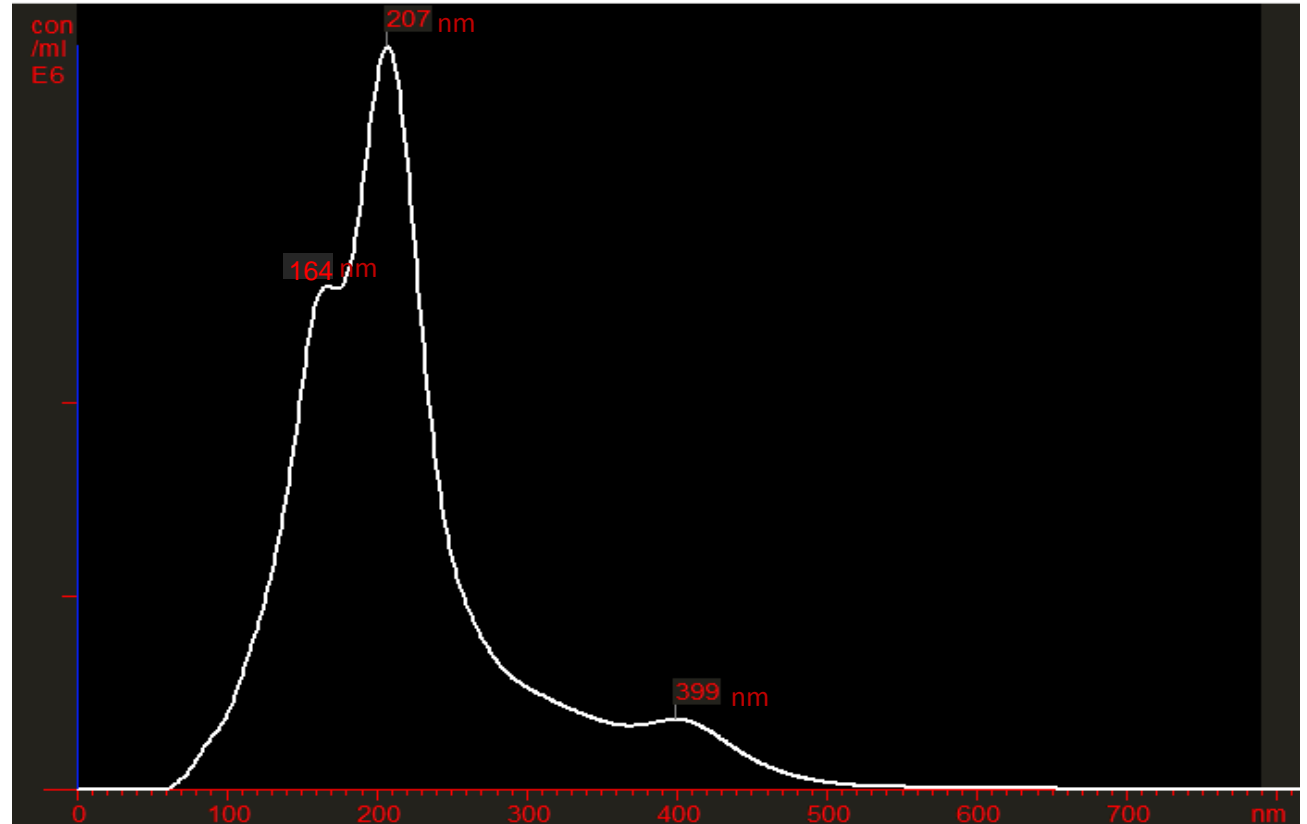


ANALYSE

- The scatter intensity is not related to D_h , only tracked Brownian speed is used to measure size
- Scatter intensity gives useful information on particle identity – **Different materials = Different scattering properties**
- Scattering intensity used to ID particles in complex formulations

High-Resolution Size Distribution Profile

- NTA can **accurately track multiple sub-populations**
- **Size** and **concentration** calculated through tracking of scattering spots



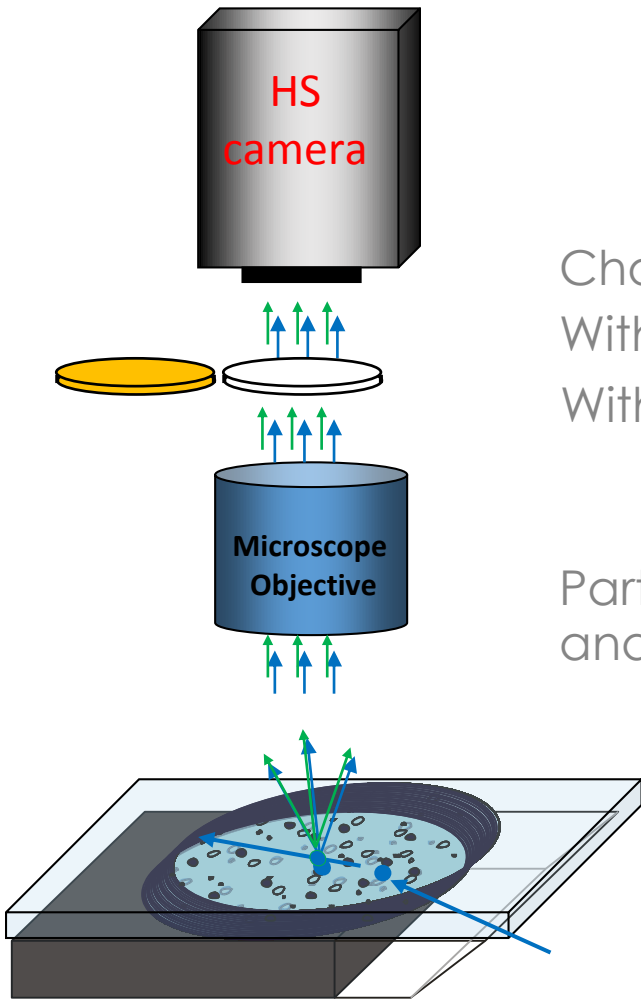
NTA Fluorescent Measurement

405nm

488nm

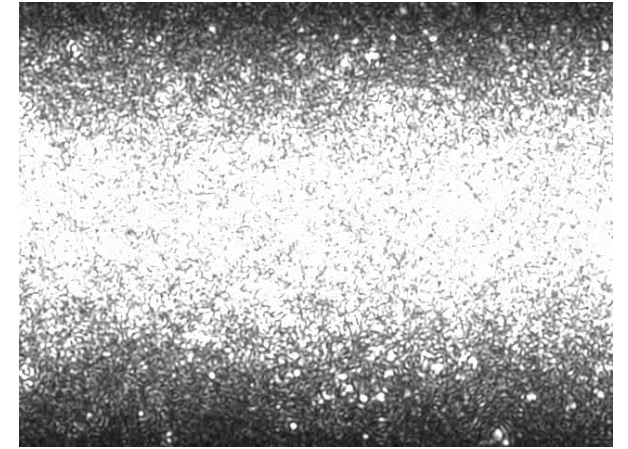
532nm

642nm



Choice of clear or fluorescent filter:
 With clear filter, all light transmitted
 With fluorescent filter, scattered light blocked

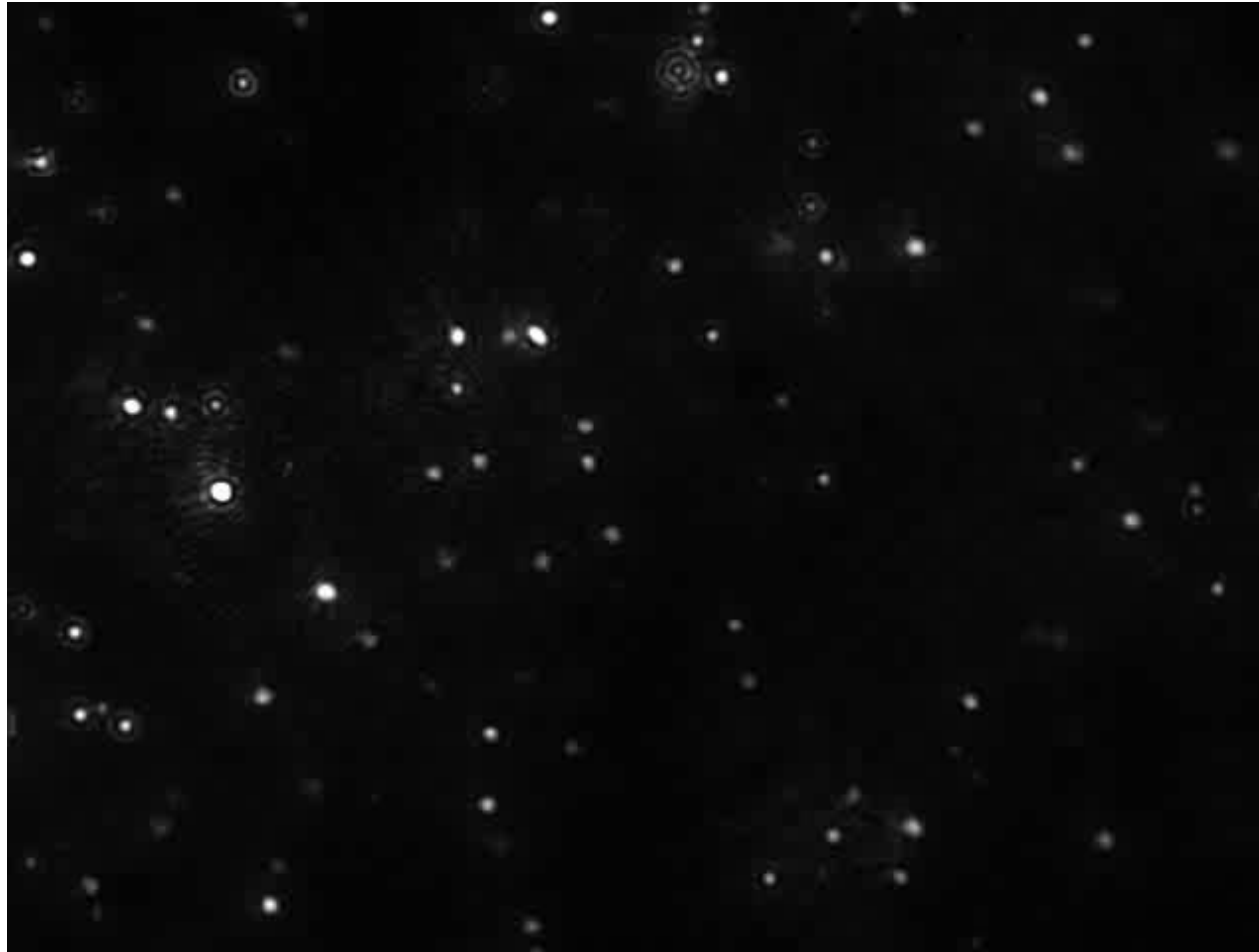
Particles scatter the laser beam
 and if fluorescent will also fluoresce



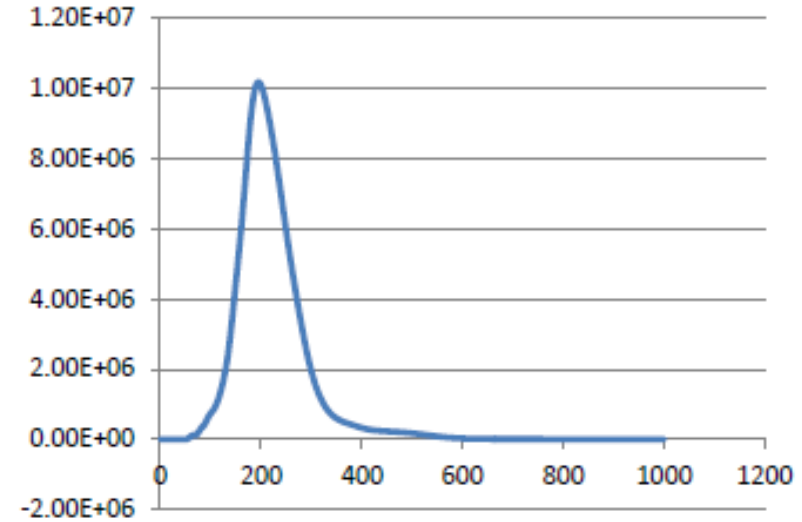
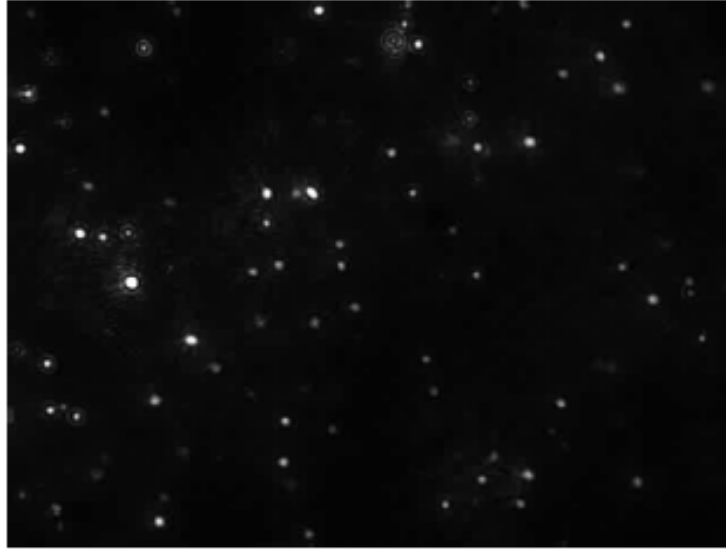
The experiment

- › Polylactic Acid (PLA) nanoparticles have been **designed for drug delivery** purposes. A drug of interest has been conjugated to a fluorescent label : Coumarin6
- › The purpose of this experience is to **evaluate the efficiency of encapsulation** of this drug into the PLA nanoparticles.
- › Thanks to its ability to **track, count and measure nanoparticles in liquids**, the NTA technic has been used to analyze this preparation both under scatter light and a fluorescence filter.
- › The device used was an **LM10 HSBF comprising a sCMOS camera, 405nm laser and 430nm long-pass filter**. Analysis has been performed under NTA software 2.3.5 (Nanoparticle Tracking Analysis)

Light scattered by the PLA particles moved by the Brownian motion.



Counting of PLA nanoparticles under scatter mode (All particles)



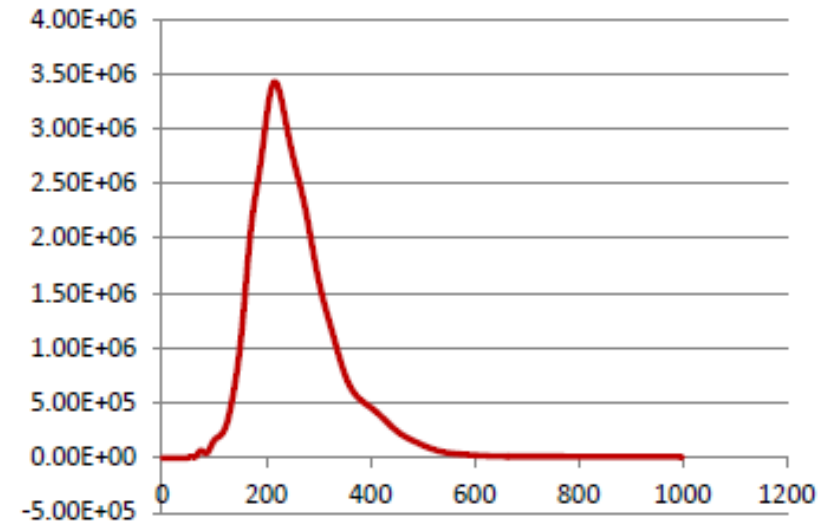
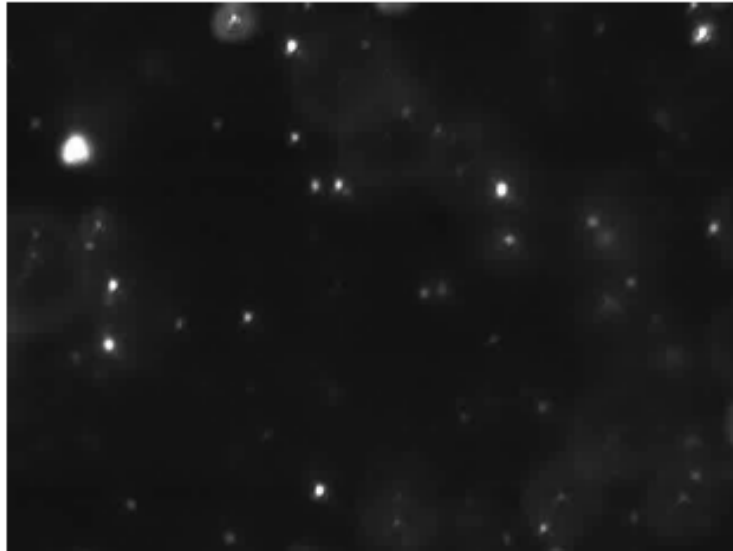
Mean: 214 nm
 Mode: 199 nm
 Measured concentration: 1.23e+009
 particles/ml
 Dilution factor: 100 times

SD: 53 nm
 D10: 164 nm
 D50: 204 nm
 D90: 262 nm

Final concentration : 1.23e+011 particles/ml

Counting of PLA nanoparticles under fluorescence mode

(drug containing particles).



Mean: 259 nm
 Mode: 223 nm
 Measured concentration: 5.31e+008
 particles/ml
 Dilution factor: 100 times

SD: 66 nm
 D10: 194 nm
 D50: 242 nm
 D90: 344 nm

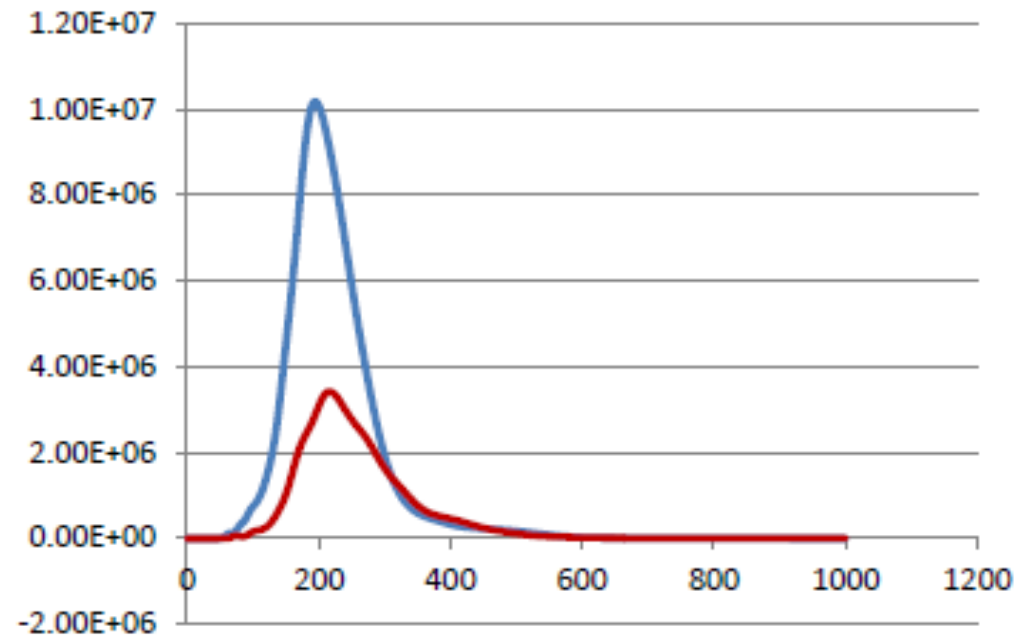
Final concentration : 5.31e+010 particles/ml

Comparing both measurements

Concentration of labelled particles :
5.31e+010 particles/ml

Total Concentration :
1.23e+011 particles/ml

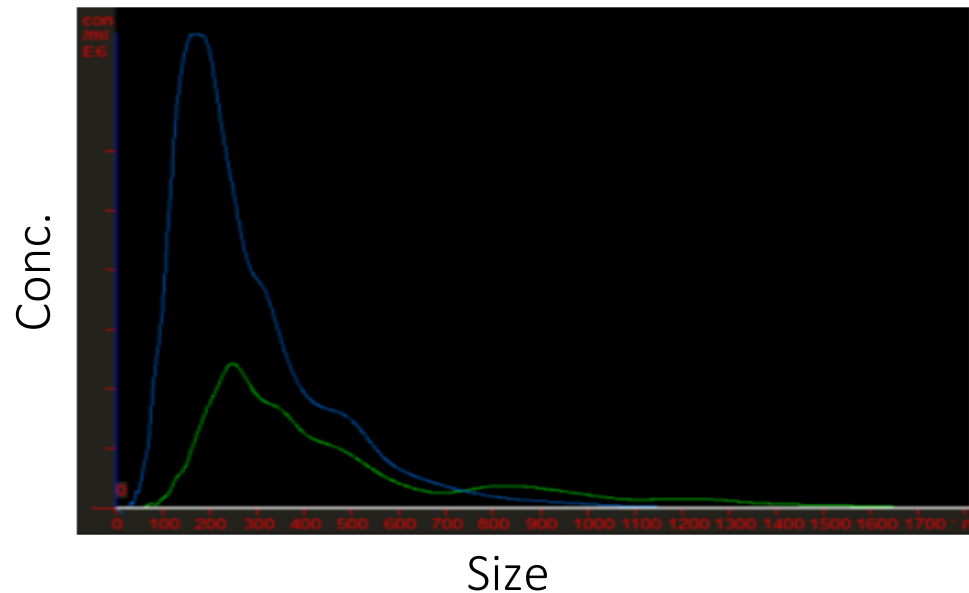
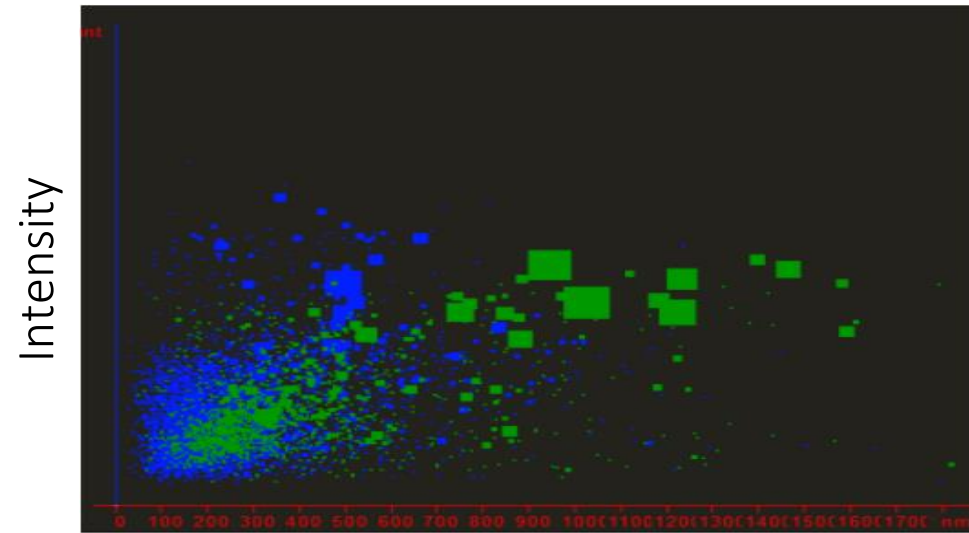
Ratio : 43.17 %



- Under the Fluorescence mode, the distribution of the labelled population (red line) shows a slightly bigger mode at 223nm (the blue line mode is 199nm).
- The NTA allows a fast evaluation of **the amount of particles labelled** amongst the total population and as a **result the efficiency of encapsulation** of this drug into polylactic acid particles.

Fluorescent labelling of Microvesicles

- Mixture of microvesicles, some of which contain **Rhodamine B**
- **Light scattering mode shows a polydisperse mixture**
- **Fluorescence mode analyses only the Rhodamine B containing vesicles**
- Particles can be focussed on depending on their fluorescent properties



In conclusion

Nanosight NTA

- High Resolution **Size** (Rh)
- Concentration (Number)
- Fluorescence
- Relative Scattering



GRAZIE PER L' ATTENZIONE