

Multi-Fluorescence Nanoparticle Tracking Assay

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MISSIONE 4
ISTRUZIONE
RICERCA



Finanziato
dall'Unione europea
NextGenerationEU



Ministero
dell'Università
e della Ricerca



Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



1506
UNIVERSITÀ
DEGLI STUDI
DI URBINO
CARLO BO



Size



Concentration



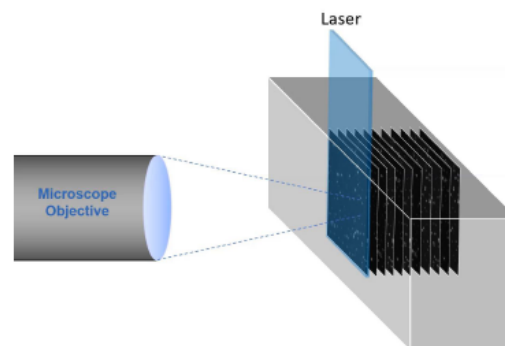
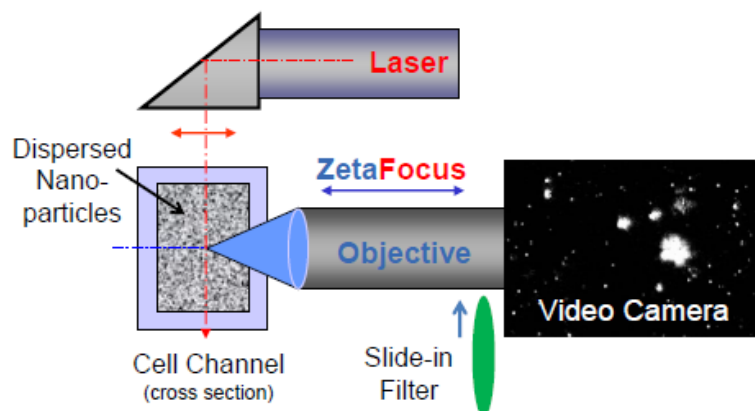
Zeta Potential



Fluorescence



Colocalization

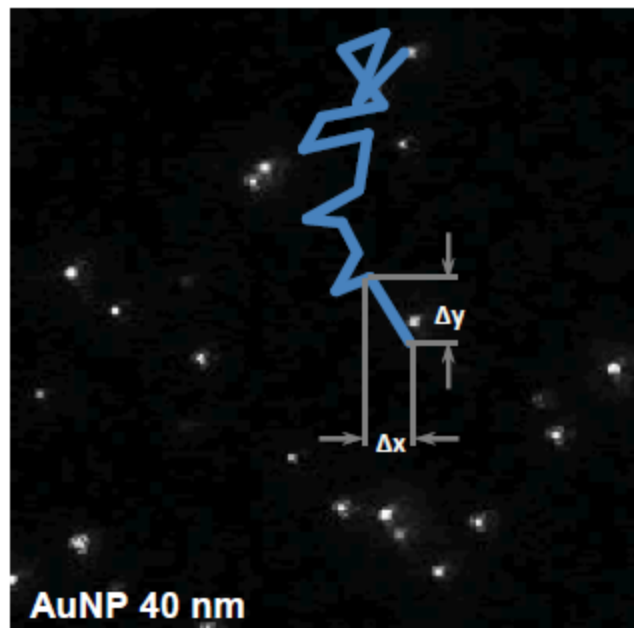


PARTICLE
MATRIX

- Automated measurement of 11 or more positions per sample
- Representative sample result with high statistical power



Size Measurement



Stokes-Einstein equation: $D = \frac{k_B T}{6\pi\eta r}$

D = Diffusion coefficient [m^2/s]

k_B = Boltzmann constant

T = Temperature

η = Viscosity

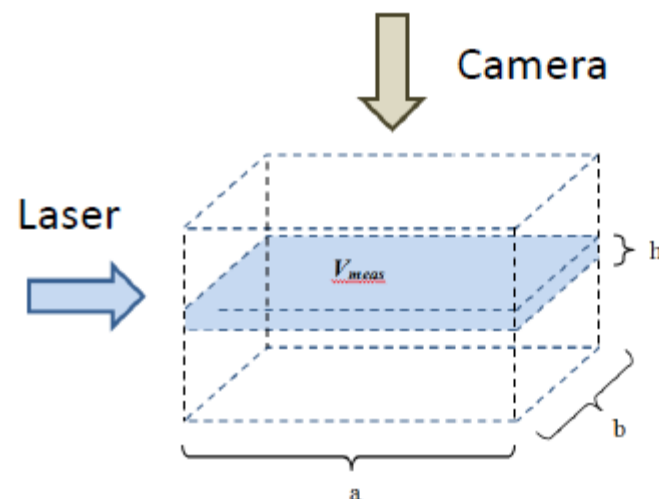
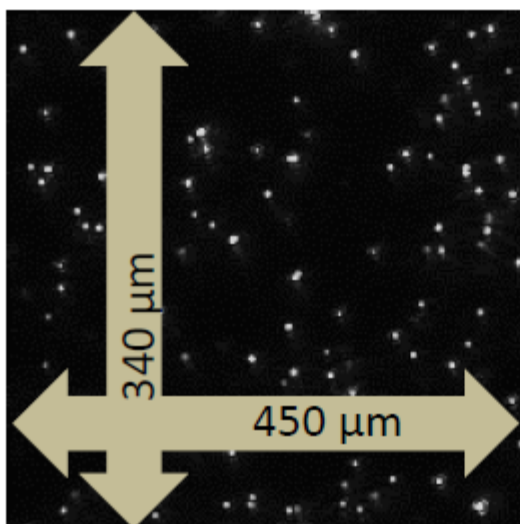
r = Particle radius



Size is calculated by the measurement
of the Brownian Motion



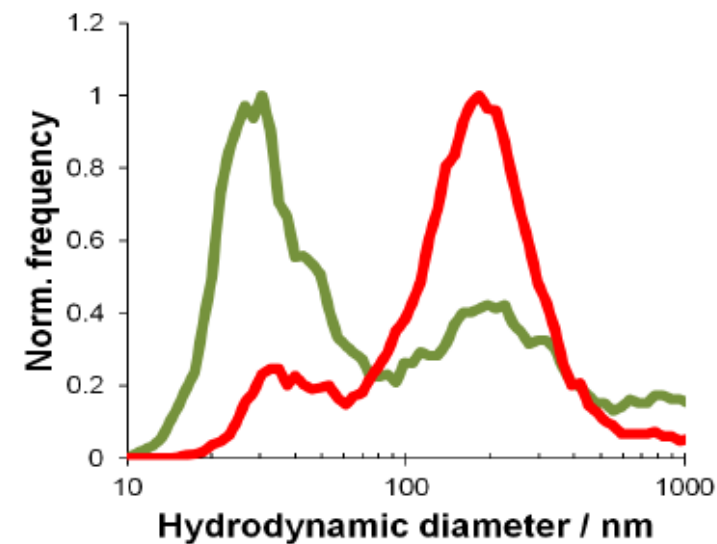
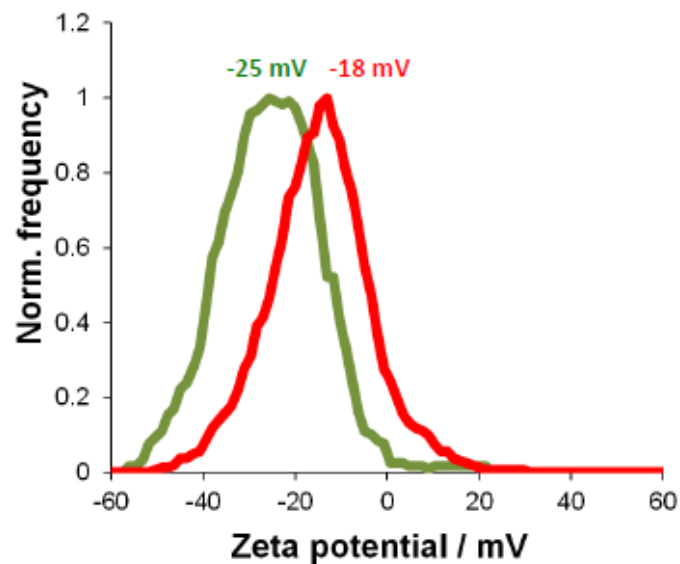
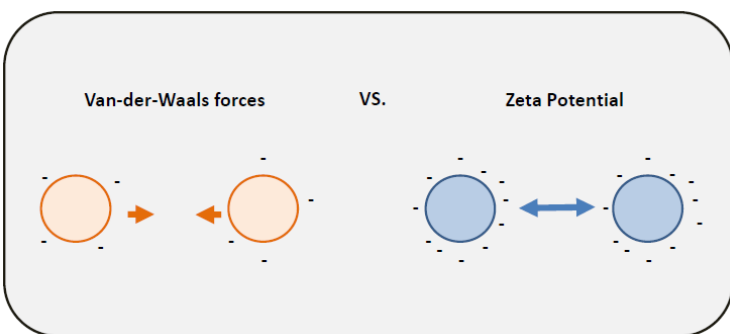
Concentration Measurement



Measurement volume: $\sim 3\text{nl/position}$



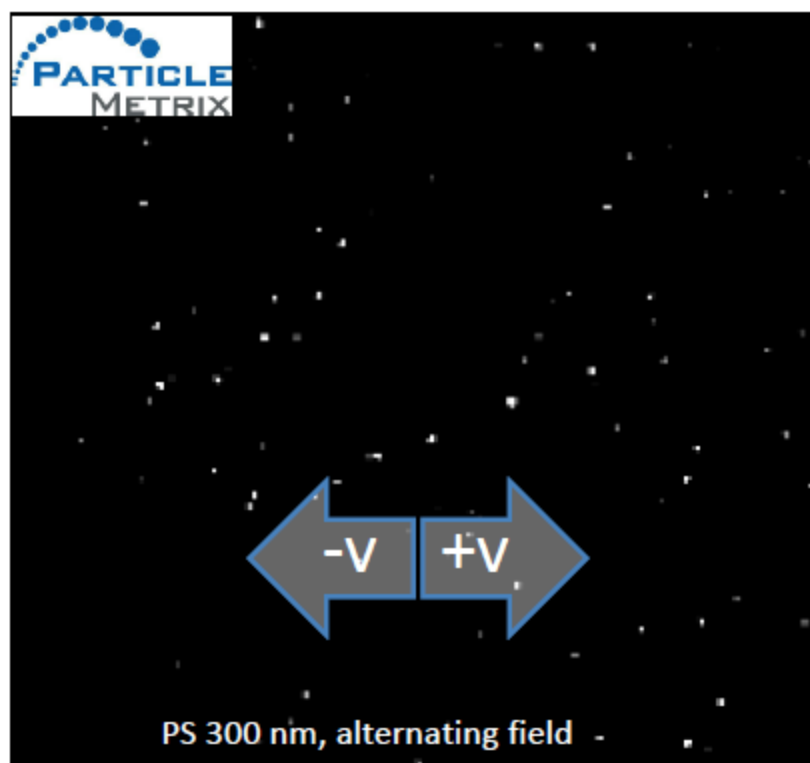
Zeta Potential Measurement



Zeta Potential can be used as a quality criteria



Zeta Potential Measurement



Helmholtz-Smoluchowski equation:

$$\zeta = \frac{4\pi\eta}{\varepsilon} f(\kappa a) \cdot \mu_e$$

ε = Dielectric constant

η = Viscosity of medium

$f(\kappa a)$ = Debye function

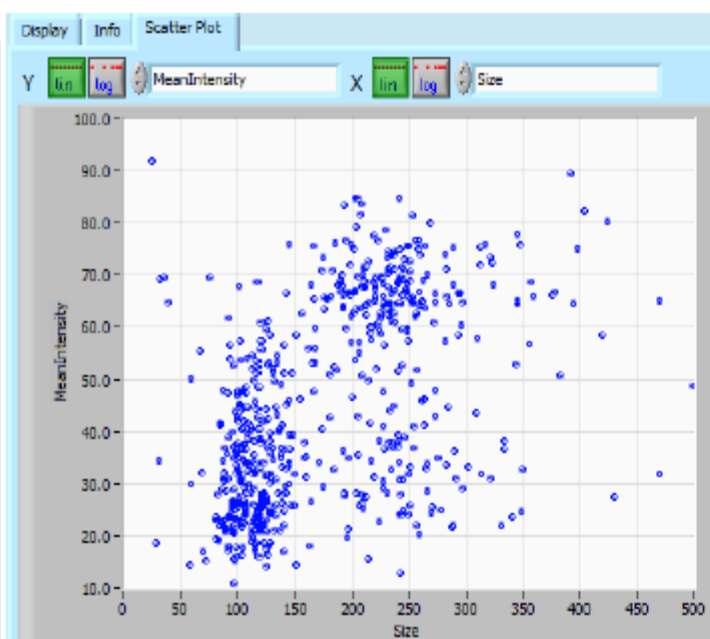
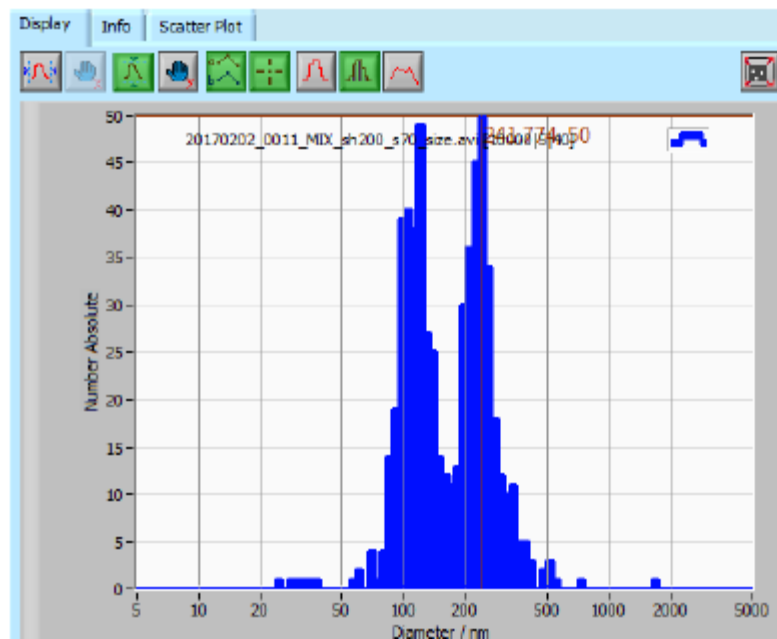
ζ = Zeta potential

μ_e = Electrophoretic mobility

➔ Zeta Potential is calculated by the measurement of the movement of the particles in a electrical field



Resolution and Scatter plots

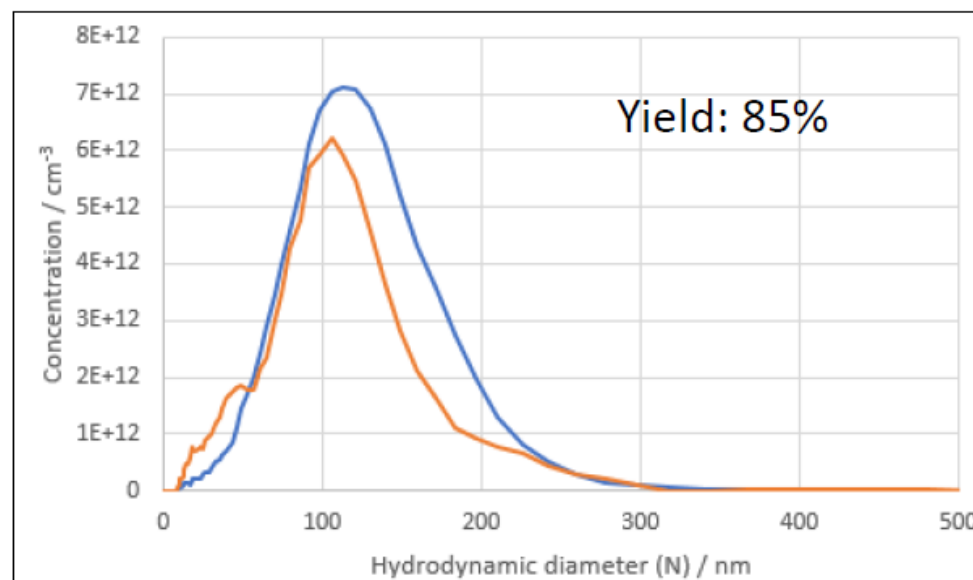
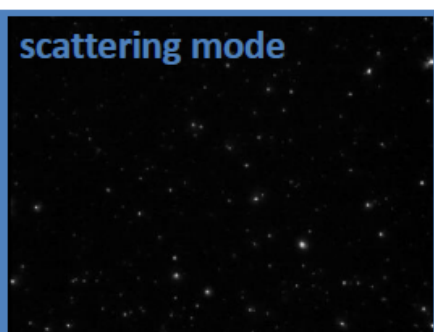


Multiple Variables

- Intensity
- Area
- Shape
- Trajectory data
- ...



ZetaView Mono laser NTA – Membrane staining of EVs

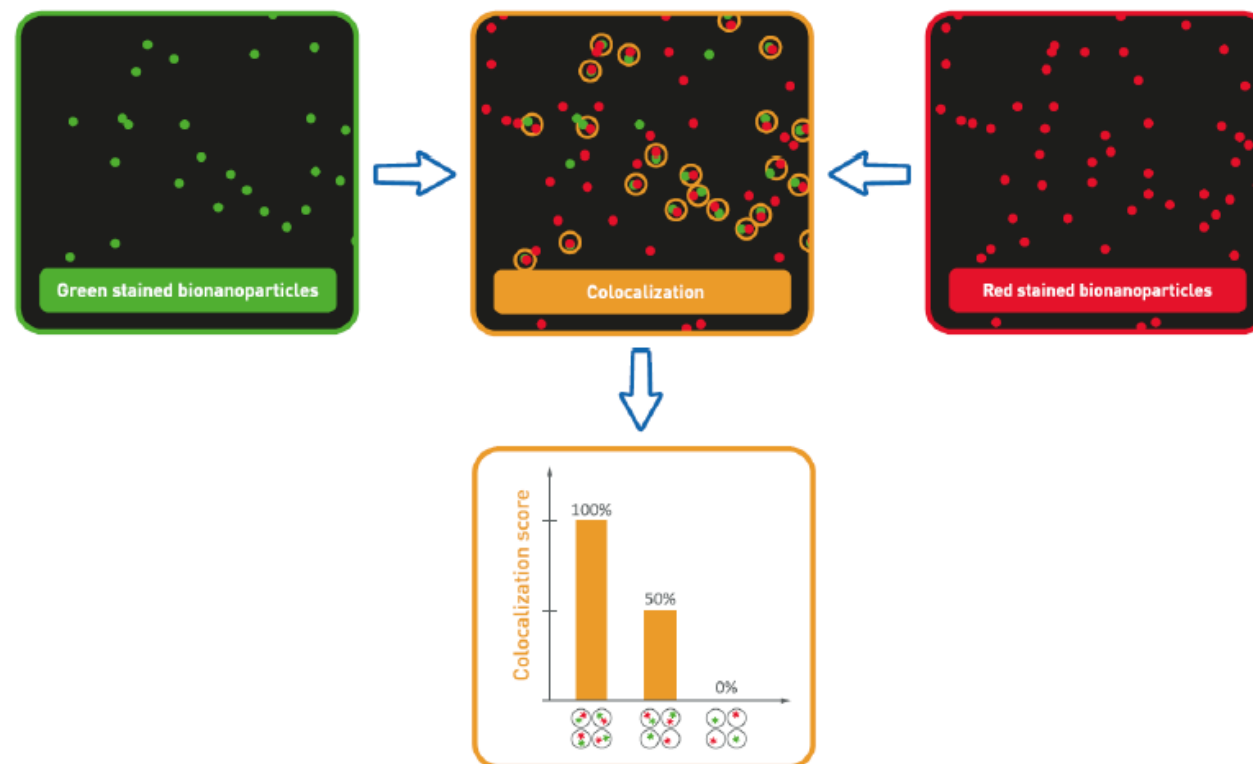


Scatter

Fluorescence



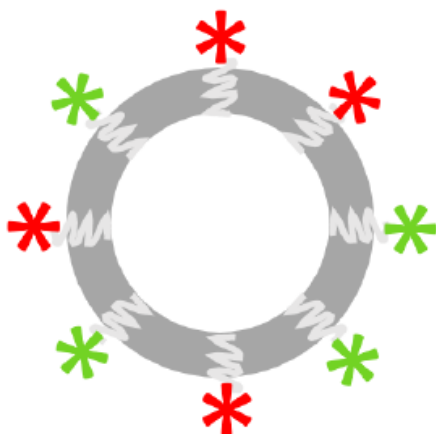
Colocalization NTA



Colocalization with fluorescent-NTA

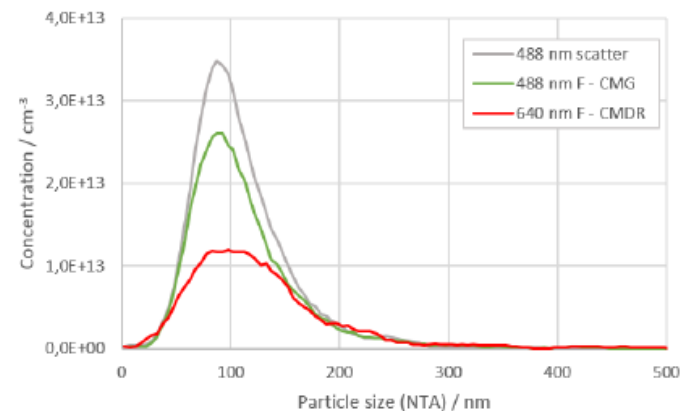
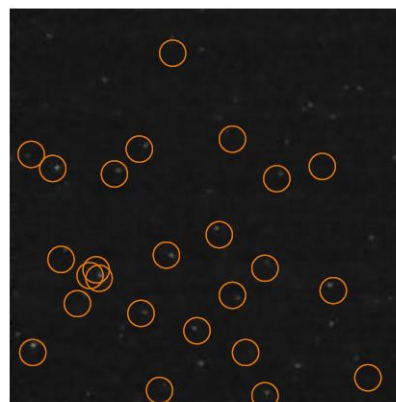


double stained liposomes



CMG CMDR

Membrane dye



Colocalization ratio about 75%

red + green positive



Typical applications of the ZetaView®



- ✓ Bionanoparticles
- ✓ F-labeled bioNP
- ✓ Liposomes + Micelles
- ✓ Drug delivery
- ✓ Proteine agglomeration
- ✓ Virus like particles (VLP)
- ✓ Virus samples / Viral titer
- ✓ Extracellular vesicles (EV)
- ✓ Emulsions
- ✓ Polymers
- ✓ Nanometals
- ✓ Nanobubbles
- ✓ Irregular particles
- ✓ Quantum dots