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Using Nanoparticle Tracking Analysis to Characterize Size and Concentration of Nanoparticles in Drug Delivery Systems

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- Particle size
- Particle concentration
- Stability assessment
- Speciation

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Nanoparticle Tracking Analysis to characterize nanoparticles and exosome preps

Nanoparticle Tracking Analysis (NTA) is an integral technique for nanoparticle characterization. By capturing the light scattered from particles undergoing Brownian motion, NTA provides particle-by-particle, high resolution particle size data in the 50nm to 1000nm range, along with concentration measurements for EVs and exosome preps.

Particle Brownian motion in a fluids is caused by constant collisions between solvent molecule and the suspended particles. The diffusion coefficient of the particle D_t and its size d are related via the Stokes-Einstein equation (fig.3)

Upon collecting a video of the particles undergoing Brownian motion, they are individually labelled and their displacement tracked frame by frame (fig.4). Finally the particle size distribution and concentration are attained (fig.5)

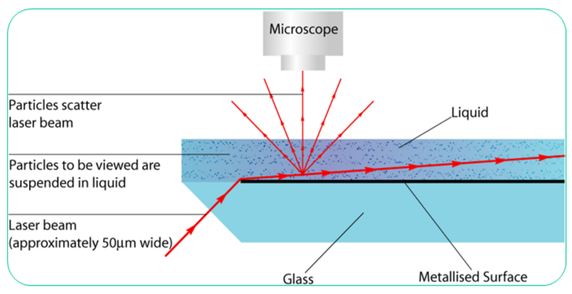


Fig 1: Schematic for the optical arrangement in NanoSight. A laser beam travels across the particle suspension and upon interacting with the particle some of the light is scattered in all direction. The cone of the scatter collected by a 20X microscope objective and visualized by a CMOS camera.

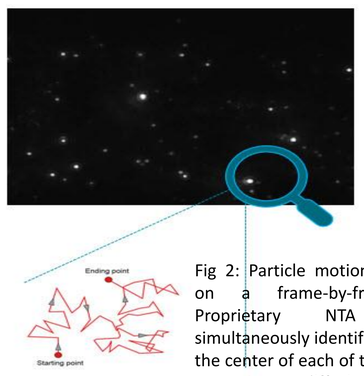
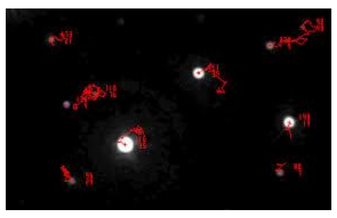


Fig 2: Particle motion is captured on a frame-by-frame basis. Proprietary NTA software simultaneously identifies and tracks the center of each of the particle to generate a diffusion coefficient (Dt).

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

K_B = Boltzmann Constant
η= viscosity
T = Temperature

Fig 3: Stokes-Einstein equation



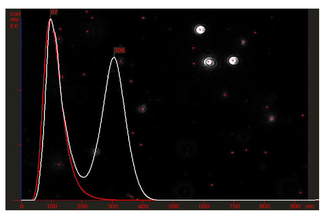


Fig 4: Labelling and tracking the particle displacement based on mean square displacement. Overlaid particle size distribution plots of a virus preparation before (white) and after (red) a final purification step.

$$msd(\tau) = \langle \Delta r(\tau)^2 \rangle = \langle [r(t+\tau) - r(t)]^2 \rangle$$

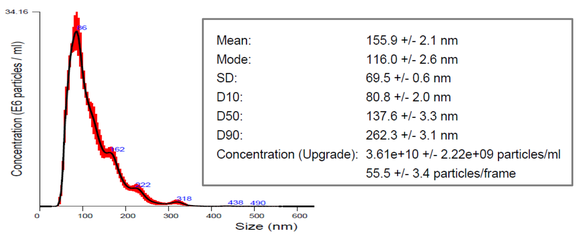
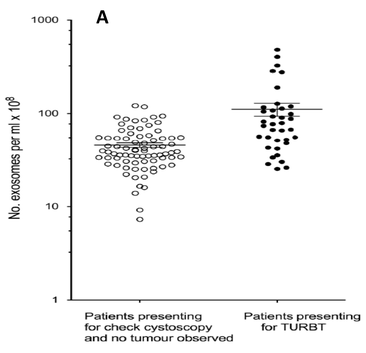
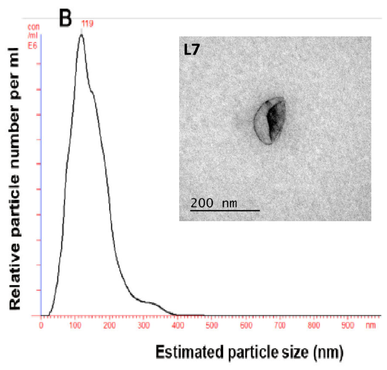


Fig 5: High resolution particle size distribution and particle concentration in minutes.

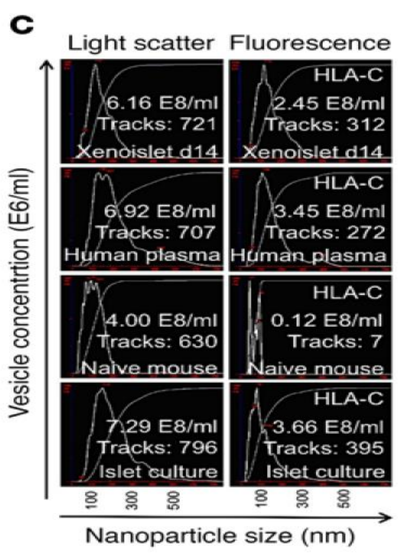
EVs in the Clinical Diagnostic Space

Exosomes can be found in most bodily fluids making them very attractive for diagnostic applications.
An example of exosome diagnostic application is below [Bladder 2015 Vol. 2(3)]. In this study exosomes isolated from urine have shown the potential to be used as early detection for bladder cancer. Graph A shows exosome quantification assessed via NanoSight in healthy vs diseased patients. A significant increase in vesicle concentration is detected for the cohort with bladder tumor (TURBT) . Graph B is a typical size profile of urinary exosomes from a patient obtained via NanoSight and an inlet with a TEM image (L7) of the purified exosomes highlighting its characteristic cup-shaped morphology.





Exosomes were shown to be a valuable tool in monitoring immunologic rejection following transplant [jci.org 2017 Vol. 127 (4) 1375-1391]. It was found that transplanted tissues release donor-specific exosomes into recipient circulation and that the quantitation and profiling of donor exosomal cargoes may constitute a biomarker platform for monitoring rejection.



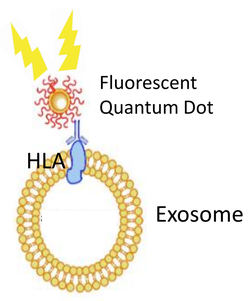
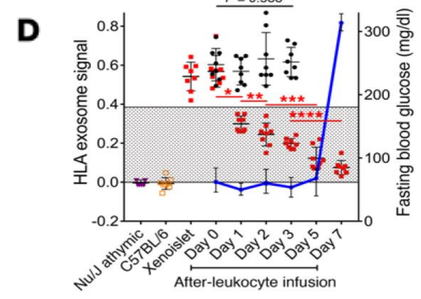


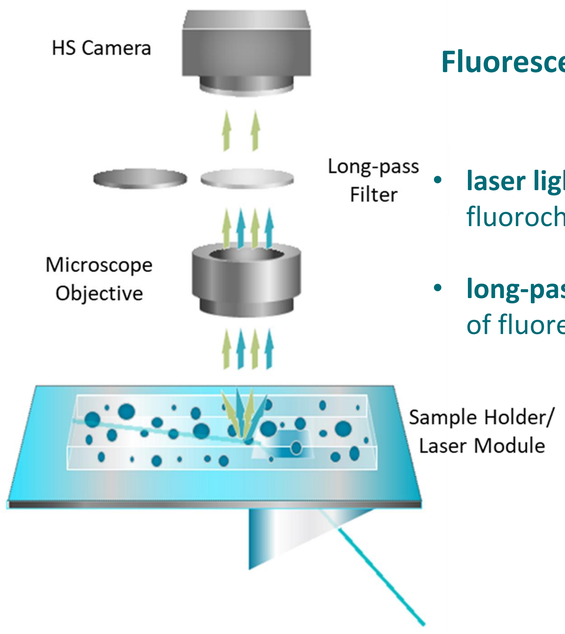
Fig 6: Schematic illustrating the labelling of exosome with fluorescent quantum dots conjugated to an antibody, that specifically bind to HLA.

An animal model of islet transplantation was used to validate the biomarker potential of MHC (Major histocompatibility complex) antigens expressed on exosome surface like the human leukocyte antigen (HLA).
On graph C the recipient plasma was analyzed for total exosomes via NanoSight NS300 in scatter and fluorescence mode to extract total exosome and donor specific MHC signals using anti-HLA–C quantum dot (Fig.6). The trend of HLA exosome signal during the evolution of acute rejection is shown in graph D.



The study utilizes NanoSight both in scatter and fluorescence mode to diagnose, following specific proteomic signature on exosomes (that are tissue specific), the status of the transplant tissue and to diagnose rejection in recipients.

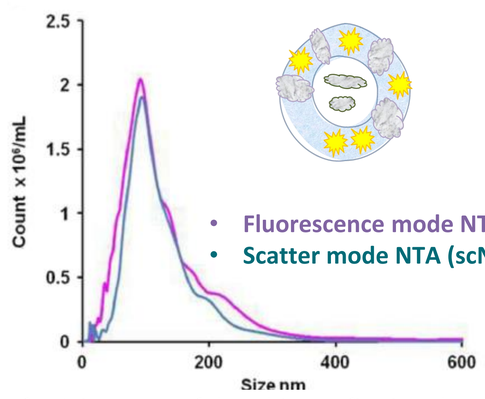
Fluorescence Speciation Analyses for Purity, and Enrichment of Nanoparticles



Fluorescence-NTA Optical Set-up Utilizes:

- **laser light** - enabling specific fluorochrome excitation
- **long-pass filter** - selective detection of fluorescently labeled EVs

EVs Surface Fluorescence Labelling

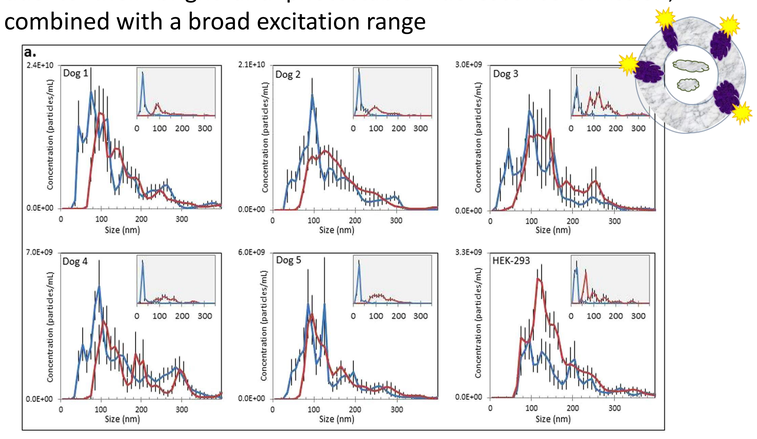


- **Fluorescence mode NTA (fNTA)**
- **Scatter mode NTA (scNTA)**

- fNTA is a powerful tool in confirming the presence and purity of EVs and subpopulations.
- Figure above shows labeled EVs in samples with CellMask™ Orange Plasma membrane dye. EVs were characterized using both fNTA (EVs membrane specific) and scNTA (total particle) modes confirming the purity of the prep.

DOI: doi.org/10.1016/j.nano.2011.04.003

Quantum dots (Qd) are ideally suited to NTA applications due to their bright and photostable fluorescence emission, combined with a broad excitation range

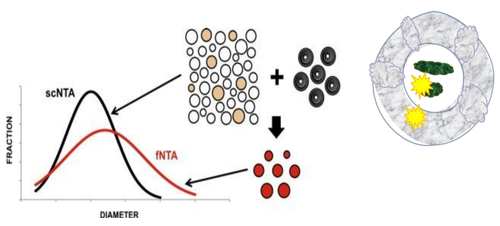


- Particle size distribution of canine and HEK293 cell-derived EV samples assessed using light scatter (red) and fluorescent (blue) modes. Immunolabeling using CD9-biotin/Qd655 Streptavidin (inset: sample's control).
- Indirect fluorescent immunolabeling utilizing Qd resulted in reproducible detection of individual labelled EV using NTA.

DOI: doi.org/10.1038/s41598-019-48181-6

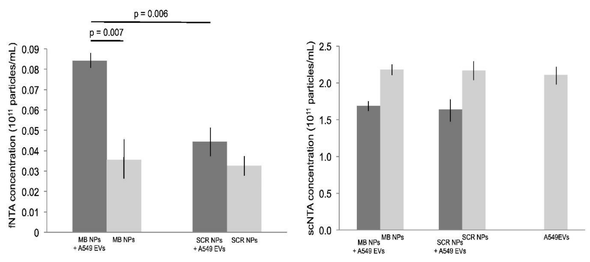
Phenotyping of EVs

EV Cargo-based Phenotyping



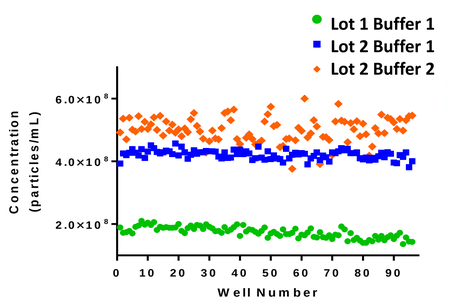
Based on an assay for detecting nanoparticle *mi*RNA by hybridization to fluorescently labeled-specific molecular beacons (MB).

DOI: doi.org/10.1016/j.nano.2016.10.013



R: Nanoparticle concentrations for *mi*RNA-21-specific MB and A549 EVs, solutions of the nanoparticles alone and scrambled MB (SCR) for background fluorescence (fNTA).
L: Nanoparticle concentrations plus the average concentration of the A549 EVs alone (scNTA)
Threshold number of MB molecules/nanoparticle is sufficient to track that EVs subpopulation of containing significant levels of *mi*RNA-21.

Reproducibility with minimal hands on time



- Walk away usage with Sample Assistant for up to 96 samples per run
- Continuous sample flow mode for better sampling statistics and improved fluorescence tracking
- Ideal for long term stability studies
- Equipped with concentration upgrade for better reproducibility

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