DAISY nanoparticle tracking analysis for extracellular vesicle size, concentration, and purity

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Introduction

DAISY nanoparticle tracking analysis (DAISY-NTA) is perfectly suited to rapid and convenient characterisation of extracellular vesicle samples. Alongside accurate size and concentration quantification, DAISY reveals refractive index and geometry details of your particles. This allows you to discern extracellular vesicles from potential contaminants, such as lipoproteins and protein aggregates, with ease. All of this information can be gathered irrespective of the liquid in which your sample is contained.

Extracellular vesicles are nano-sized vesicles (50 to over 1000 nm diameter) secreted into the extracellular environment by a host of cell types. The discovery that extracellular vesicles contain biomolecules, effectively protecting them from degradation, has spurred huge and growing interest in both their fundamental biological functions and their potential bio-tech and bio-medical applications[1]. The small size and relatively low refractive index of extracellular vesicles has proven challenging in terms of analysis. DAISY-NTA provides unprecedented detail on EV samples, greatly assisting characterisation, evaluation, and reproducibility in extracellular vesicle sample generation.

Contents:

Highlights

- DAISY reliably quantifies extracellular vesicles, down to 50 nm in diameter
- Enables differentiation and quantification of extracellular vesicles and large lipoprotein particles in mixed samples
- Direct optical sizing provides reliable size distributions independent of sample media and viscosity
- DAISY provides information on internal mass distribution of particles allowing discrimination between large particles and aggregates

Figure 1. Size range of extracellular vesicles and common contaminants

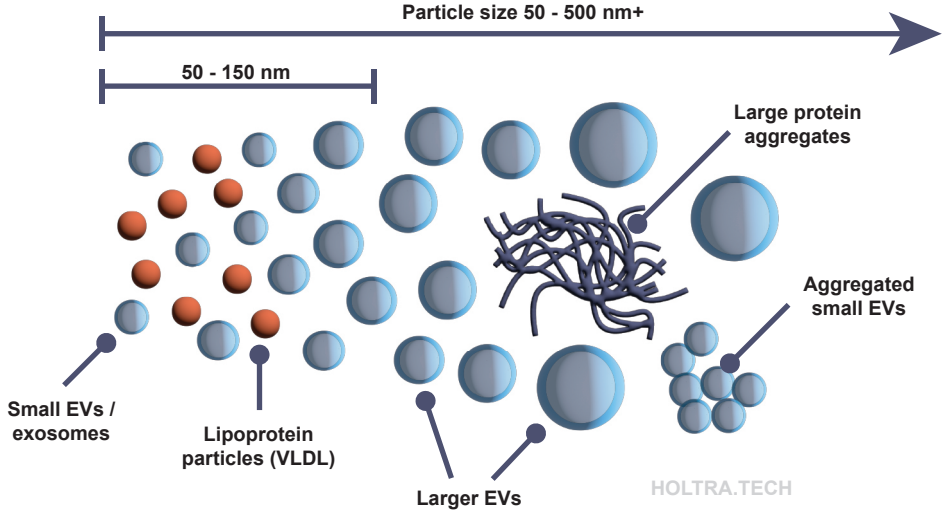
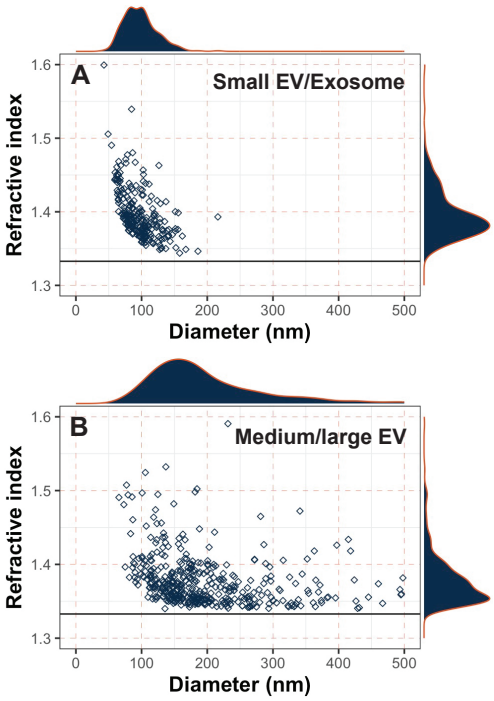


Figure 1. Extracellular vesicles exist as a mixed population of vesicles from a variety of sources. EVs have a wide size distribution that overlaps with a number of potential contaminants. This makes accurate determination of EV size and purity both important and challenging.

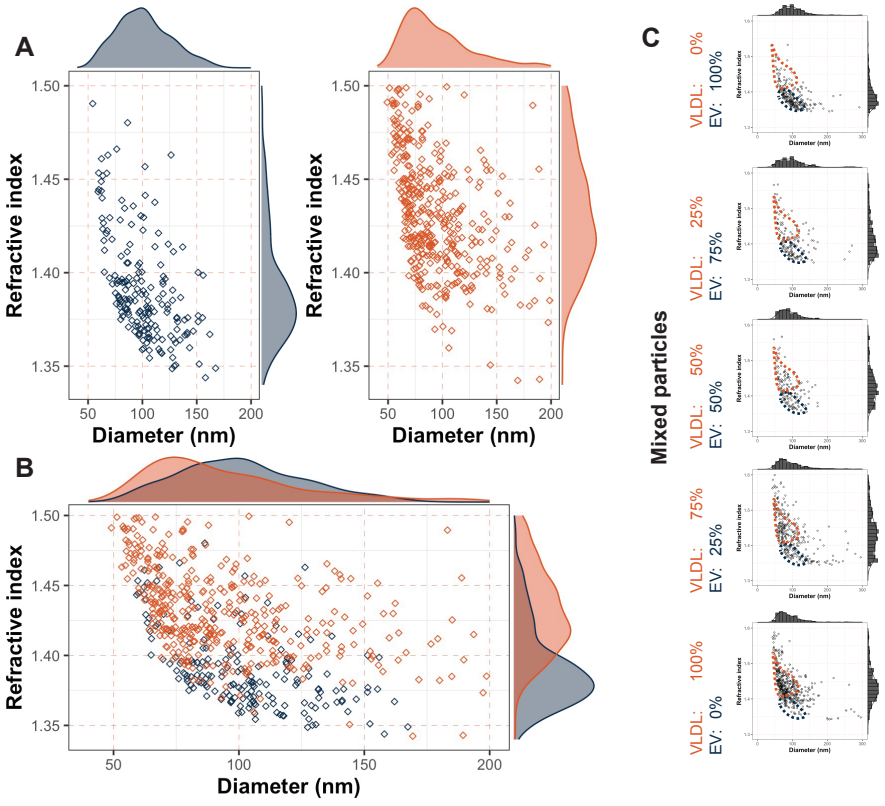
Figure 2. Size and refractive index of extracellular vesicle populations



Extracellular vesicles exist in many forms, with differing biosynthesis pathways, content, and biophysical properties. Due to the inherent difficulty of identifying the sources of distinct extracellular vesicle subpopulations, the current guidance is to characterise EV subtypes based primarily on their physical or biochemical attributes, such as size and surface protein composition. Therefore, accurate and multi-dimensional characterisation of EV preparations is an essential step in ensuring reproducible and comparable EV research.

Vesicle size is used as a primary classifier of extracellular vesicle class, DAISY-NTA can accurately measure vesicles ranging from 50 nm to over 1 µm in diameter. Alongside size, DAISY-NTA provides a particle by particle measurement of refractive index. Refractive index measurement is invaluable in differentiating particle types. The plots to the left demonstrate simultaneous analysis of size and refractive index of samples of purified small EVs (**A**) and medium/large EVs (**B**). This shows how refractive index scales with EV size, due to the decreasing contribution of the membrane to total EV mass as size increases.

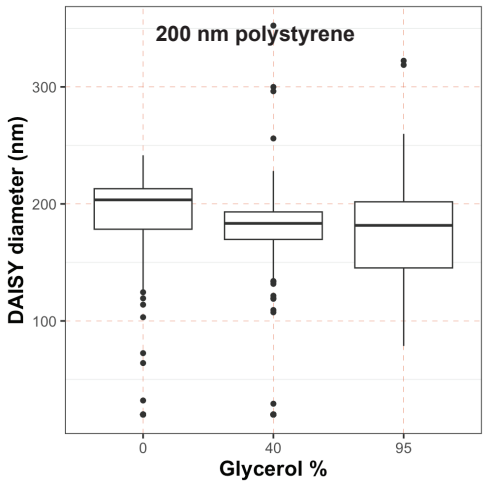
Figure 3. Identifying lipoprotein contaminants in EV samples



Plasma is a readily accessible and commonly used source of EVs, particularly in clinical studies[2]. EVs are purified from plasma using a number of size dependent filtration and enrichment approaches. Plasma is also rich in lipoprotein complexes, the largest of which, VLDL (very low density lipoprotein) have a size distribution (50-80 nm) overlapping that of small EVs (50-150 nm)[2,3]. Small EVs have a relatively low refractive index, generally below 1.4 dependent upon diameter, while VLDL has a higher refractive index, generally above 1.4[4].

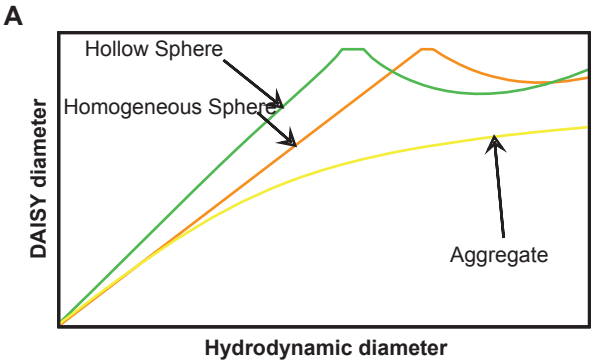
DAISY-NTA simultaneously records the size and refractive index of individual particles. This enables differentiation of EVs and VLDL within the same size range based on their different refractive indices (**A**, **B**). In a mixed sample, prepared form enriched tissue culture derived EVs and purified VLDL, the levels of lipoprotein in the sample are visible in the distribution of particle size vs refractive index (**C**).

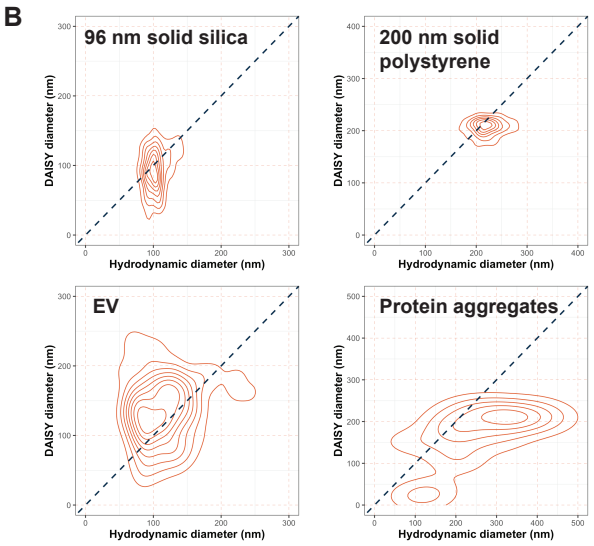
Figure 4. Optical sizing of particles independent of sample media



Nanoparticle tracking analysis relies upon accurate determination of particle diffusion through the media. This makes the approach highly sensitive to sample media viscosity. When the exact viscosity is known, straightforward corrections can be made to determine particle size. However, the viscosity of biological samples may be inconsistent or uneven, potentially having local differences in viscosity throughout the sample. This makes accurate hydrodynamic sizing of particles a challenge. DAISY-NTA provides an optical diameter, determined entirely from light scattering characteristics. This enables accurate size determination of particles in media of either unkown or variable viscosity. The plot to left shows the optical size determination of 200 nm polystyrene beads dispersed in media of varying glycerol concentration. Higher glycerol concentration greatly increases the viscosity of the media. The data show highly accurate size determination of this sample even in media containing 95% glycerol by volume.

Figure 5. differentiation of large particles and aggregates

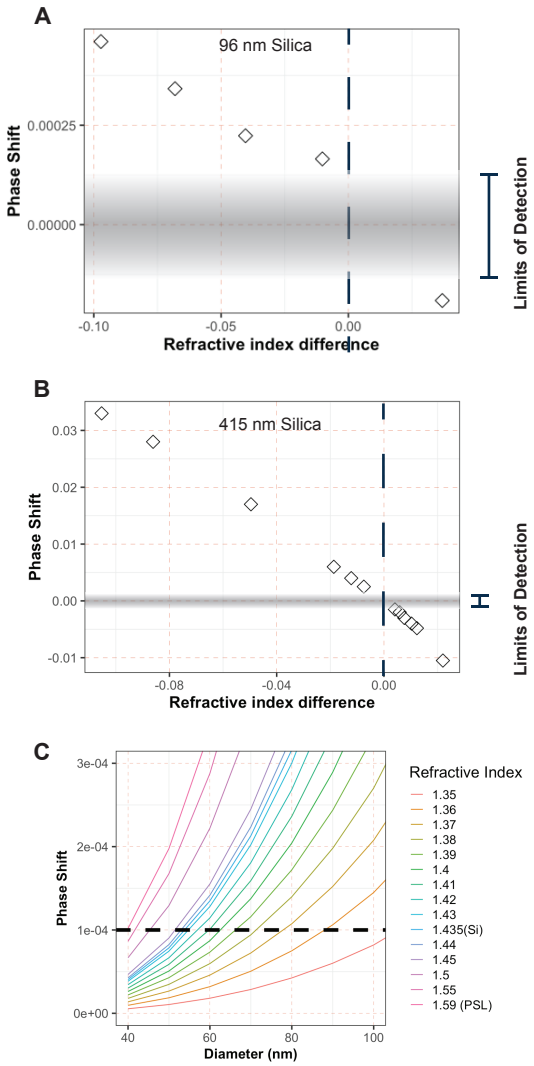




DAISY-NTA is unique in measuring both hydrodynamic size and purely light scattering-derived optical size (the DAISY diameter). The relationship between these two size measurements follows a strict 1 to 1 relationship for particles with homogenous internal mass distributions, i.e. solid spheres. However, when particle mass and geometry differ from this solid sphere model the relationship between DAISY diameter and hydrodynamic diameter deviates in predictable and quantifiable ways (**A**). Particles such as vesicles with a mass distribution biased towards the periphery will scatter light more intensely, giving a measured DAISY diameter higher than the hydrodynamic diameter. Conversely, particles with a relatively lower mass density at the periphery, such as aggregates of proteins or vesicles will scatter light less strongly than a solid sphere of the same hydrodynamic diameter.

The relationship between DAISY diameter and hydrodynamic diameter for a range of particles is shown in panel **B**. EVs, with a mass distribution biased towards the periphery, report a higher DAISY diameter than hydrodynamic diameter. Protein aggregates, with relatively lower mass at the periphery follow the opposite distribution.

Figure 6. Detection limits



As with any light scattering microscopy-based approach, the detection limits of DAISY are related to particle size and refractive index. Altering the refractive index of the surrounding media, and therefore the effective refractive index of the particles, objectively demonstrates the detection limits of our instrument.

Our experimentally determined detection limit, in terms of refractive index difference, for 96 nm particles is ~0.01 refractive index unit (**A**). For larger particles, such as 415 nm silica (**B**) this detectable refractive index difference drops to 0.005 refractive index units or below.

Combining this experimentally derived information with well validated optical simulations (**C**) demonstrates detection limits in terms of size for particles with a wide range of refractive indices.

For the detection of extracellular vesicles, which usually have a refractive index in the region of 1.38 to 1.4 (dependent upon their source), DAISY-NTA has a lower size detection limit in the region of 60 nm.

Rigorous experimental determination of precise detection limits allows researchers to make a fully informed choice about the suitability of DAISY-NTA for their samples.

References

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