



Label-Free Discrimination of Extracellular Vesicles

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Interferometric nanoparticle tracking analysis (iNTA) combines particle size determination with sensitive refractive index measurements at the single-particle level. This dual-parameter approach enables label-free discrimination of extracellular vesicles (EV) from co-isolates and supports reliable concentration measurements even in highly complex samples.

About Extracellular Vesicles (EV)

Virtually all types of cells secrete vesicles (EV) into the extracellular space, a process that contributes to intercellular communication and holds considerable potential for diagnostic applications. As EV are released into body fluids, they are readily accessible via liquid biopsies and can carry disease-relevant biomarkers (e.g. in cancer). In particular, EV isolation from routinely collected samples (e.g. urine, blood) is of high clinical interest.

EV characterization requires determination of size and concentration. This remains challenging, as EV cannot be completely purified by density- or size-based separation alone. Consequently, measurements of complex samples must be interpreted cautiously, since co-isolated components such as protein aggregates or lipoproteins (LP) may confound results. To address these limitations, established techniques such as conventional NTA or flow cytometry (FCM) are supplemented with fluorescence detection to increase analytical specificity.

iNTA Paves the Way for Label-free Specificity

Through the sensitive determination of individual particle refractive indices, iNTA offers a label-free approach to increase the specificity for EV in concentration measurements.

Figure 1 shows superimposed measurements of cell culture-derived EV (red) and untreated blood plasma (grey).

Particles present in plasma (predominantly LPs) exhibit markedly higher refractive indices (> 1.4) than EV (< 1.4), enabling clear discrimination between both populations.

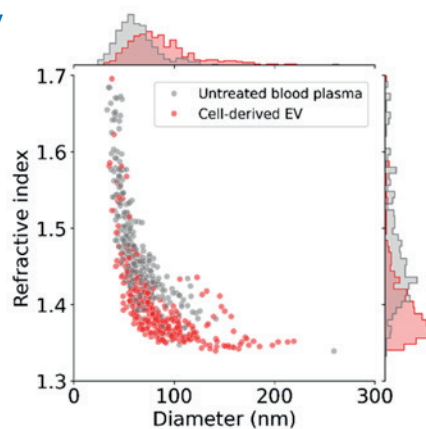


Fig. 1
iNTA measurements of untreated blood plasma and cell culture-derived EV, acquired in separate experiments and adjusted to a suitable measurement concentration using PBS.

Learning Algorithm Empowers Accurate Concentration Estimation

As particle properties can partially overlap depending on sample composition and experimental conditions, classification is not reduced to simple threshold values. Instead iNTA uses learning-based classification algorithms to identify patterns within high dimensional data sets and to assign particles probabilistically to predefined classes.

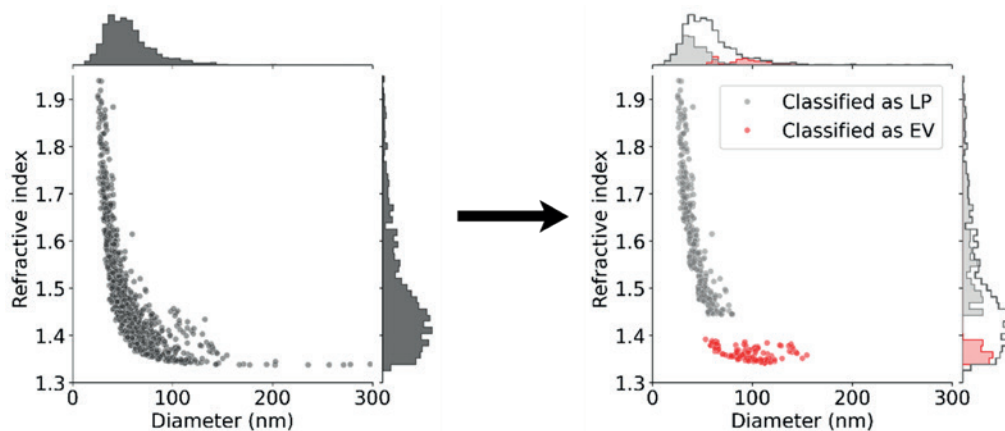


Fig. 2
iNTA measurement of a plasma sample spiked with cell-derived EV at a 3:1 ratio. The classifier was trained on reference data from Figure 1 and applied to the mixed sample (left). Classification results for EV and co-isolates are shown for particles assigned with a confidence level of $\geq 75\%$ (right).

In the example shown in Figure 2, a classification algorithm was trained using reference data sets obtained from the measurements in Figure 1. The trained classifier was then applied to the result of a plasma sample spiked with cell-derived EV at a defined ratio, enabling quantitative determination of the EV fraction within the mixture (see Figure 3).

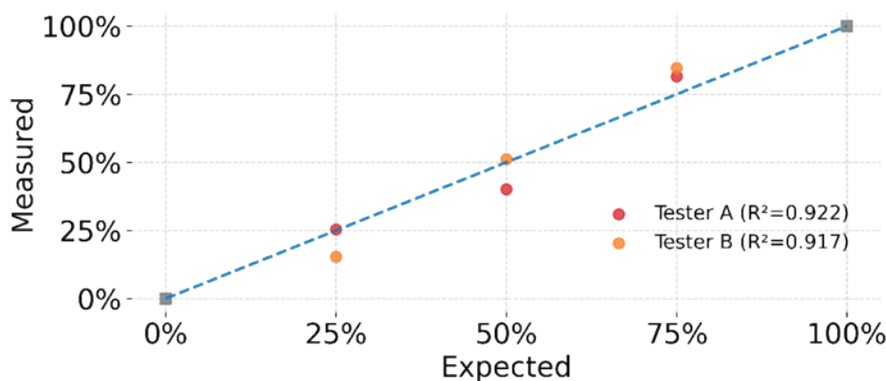


Fig. 3
Quantification of EV concentrations in plasma samples spiked with cell-derived EV at different ratios. Measurements were performed by testers of different labs iNTA.

Conclusion

iNTA enables label-free discrimination of EV from co-isolates in complex biological samples. By combining size and refractive index measurements, it overcomes the limitations of conventional techniques (e.g. NTA, FCM). iNTA is fast, sensitive, and requires minimal sample volume, making it ideal for EV research, biomarker discovery, and quality control of EV-based therapeutics. Its ability to resolve subpopulations and quantify EV even in complex samples positions iNTA as a new standard in extracellular vesicle analytics.

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