ONI Application note

Extracellular Vesicles

Advanced EV characterization made simple

Welcome to CODI. A collaborative data analysis platform for EV research.

Introduction

Super-resolution microscopy is a powerful tool for EV imaging and characterization by allowing researchers to directly visualize single-EVs, their surface biomarkers and therapeutic cargo. However, being able to extract quantitative data from these images through clustering analysis will enable researchers to unlock the secrets of EVs which until now, hasn't been fully explored. Clustering analysis allows the user to characterize the heterogeneity of an EV sample, to determine the percentage of positive markers on the surface of the EVs, and to measure DNA cargo. In this application note, we discuss how ONI's new analysis platform provides researchers with a methodical analysis pipeline that standardizes EV characterization.

Data analysis of super-resolution microscopy: CODI

To assist EV researchers in extracting quantitative information from their super-resolution images, ONI has developed a novel analysis software termed CODI. This platform hosts a wide range of advanced image analysis tools that have been designed to aid the study of EVs and address the challenges associated with their characterization. This allows for multi-factor characterization that enables users to count thousands of EVs from a single super-resolution image, assess their morphology with 20 nm resolution and visualize multiple biomarkers with single-molecule sensitivity. EV researchers can now unveil previously invisible insights from their sample through this simple, reliable and easy to use platform. The workflow for single-EV analysis on the Nanoimager starts with the preparation of EVs and finishes with clustering analysis to extract quantitative information about the EV sample (Figure 1).

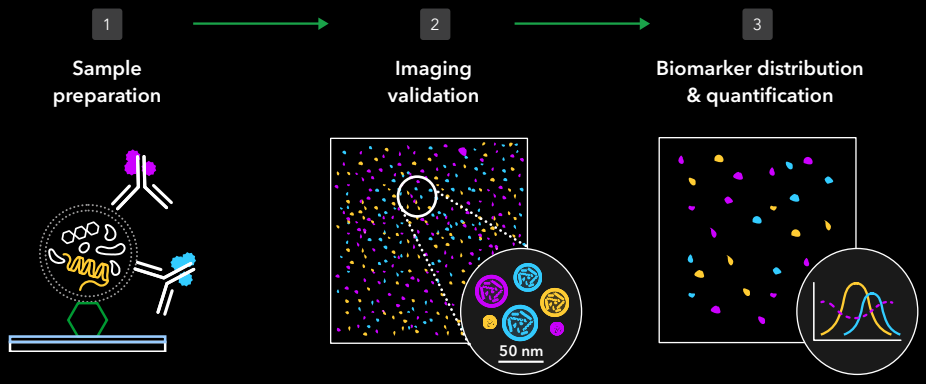

Figure 1 | Schematic representation of the EV workflow showing how EVs are first stained and adhered to the glass surface, before being imaged on the Nanoimager and analyzed using CODI.

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Firstly, EV surface proteins, membrane structures or internal cargo are labeled with a dSTORM compatible fluorescent dye and then specifically captured onto a glass surface. High resolution, dSTORM imaging is carried out, allowing for thousands of captured EVs to be directly visualized with 20 nm resolution. Lastly, the super-resolution images are subjected to clustering methods and co-localization analysis in which quantitative information detailing the different subpopulations, sizes and biomarker distributions can be obtained rapidly and automatically (Figure 2).

Quick Summary

ONI's analysis platform provides advanced tools which have been designed to aid the characterization of extracellular vesicles using streamline workflows, that allows researchers to:

- Directly visualize single-EV allowing sample validation
- Assess and count hundred to thousands of surface bound EVs
- Quantify multiple biomarkers and assess their distribution of the EV surface
- Evaluate EV morphology with 20 nm resolution
- Measure and quantify EV cargo

Figure 2 | Screenshots of CODI, demonstrating dSTORM imaging of EVs (A) and clustering analysis (B).

Clustering analysis works by identifying the localizations from the dSTORM image which corresponds to each of the tetraspanin proteins labeled on the EV surface. All localizations within a defined radius are grouped into a dense circular cluster representing a single EV. Once these clusters have been identified, they can be constrained on parameters such as size, shape, length and circularity.

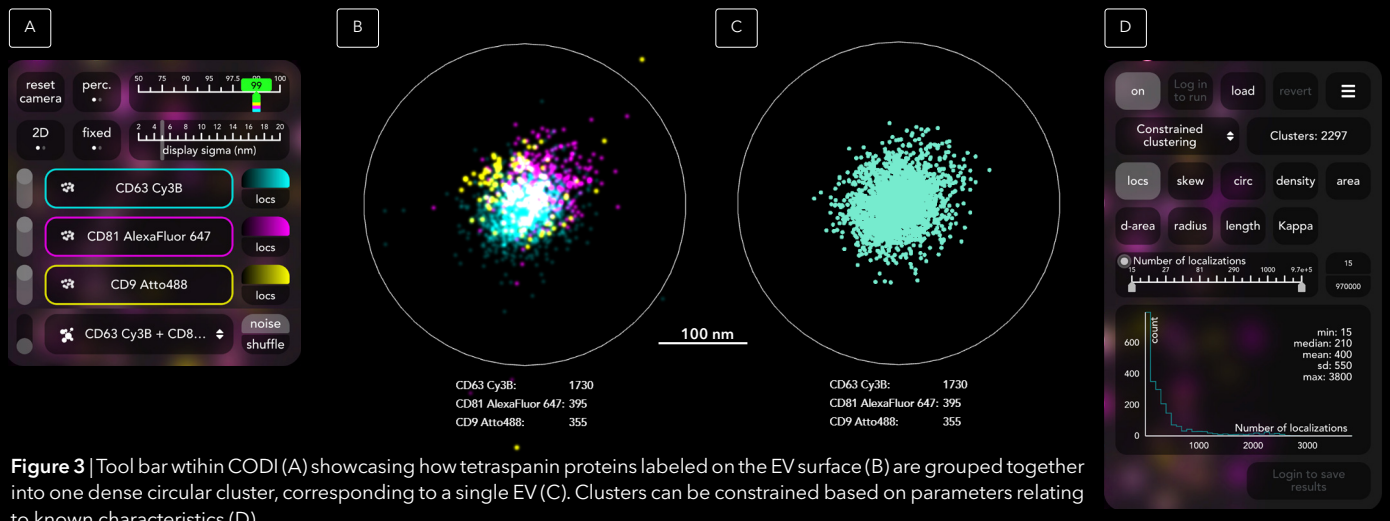

Figure 3 | Tool bar wtihin CODI (A) showcasing how tetraspanin proteins labeled on the EV surface (B) are grouped together into one dense circular cluster, corresponding to a single EV (C). Clusters can be constrained based on parameters relating to known characteristics (D).

This ensures that EV clusters can be distinguished from any background or aggregates in the field of view in the field of view and removes them from the final results, allowing the user to have additional confidence that the clusters being analysed are in fact EVs (Figure 3).

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These constraints can be saved, exported and applied to other datasets to facilitate reproducible analysis when comparing between different datasets. Once these clusters are defined, information on specific characteristics of the EV population can be extracted. For example, the size distributions can be calculated for the global population of EVs in the sample (Figure 4). This allows users to assess the homogeneity of their EVs, and identify specific sub-types of EVs within the sample. Additionally, other features of an EV can be quantified such as density, shape and morphology, features which are key in allowing us to understand more about the EV sub-populations.

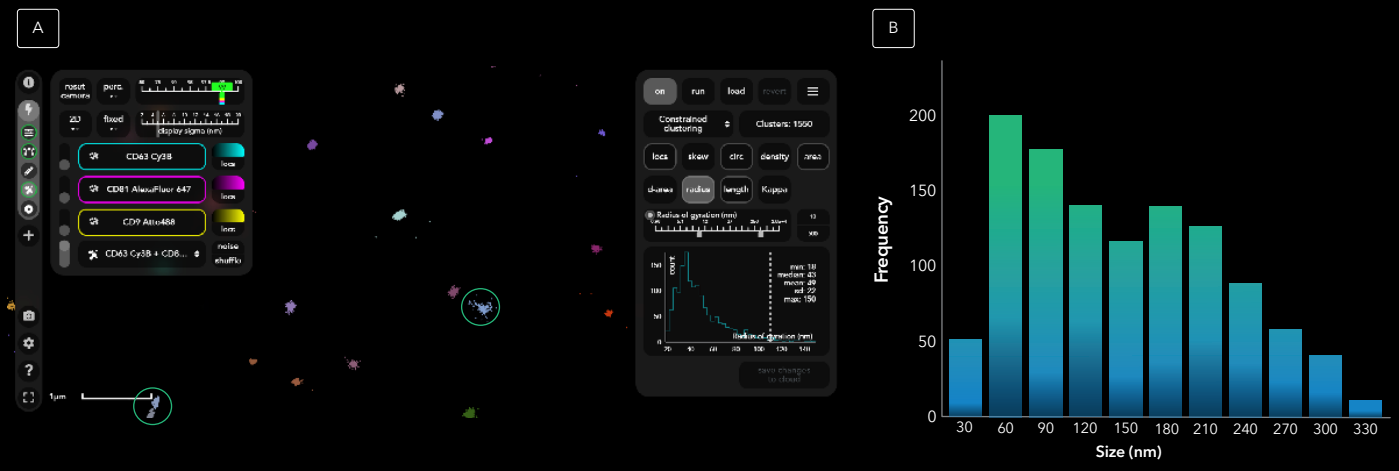

Figure 4 | Screenshot of CODI demonstrating the ability to inspect clusters, and remove localizations that do not correspond to set parameters (green circles) (A). Histogram representing the sub-populations of EVs within a sample based on size analysis (B).

These clustering tools can be used to quantify surface biomarkers on single-EVs and assess the heterogeneity within the entire sample. This enables the percentage of single, double and triple positive EVs to be distinguished and counted for an enhanced characterization. Additionally the amount of DNA present on the surface and within EVs can be visualized and measured, thus allowing these clustering tools to enable researchers to rapidly attain an understanding of the population distributions in samples and evaluate their therapeutic cargo.

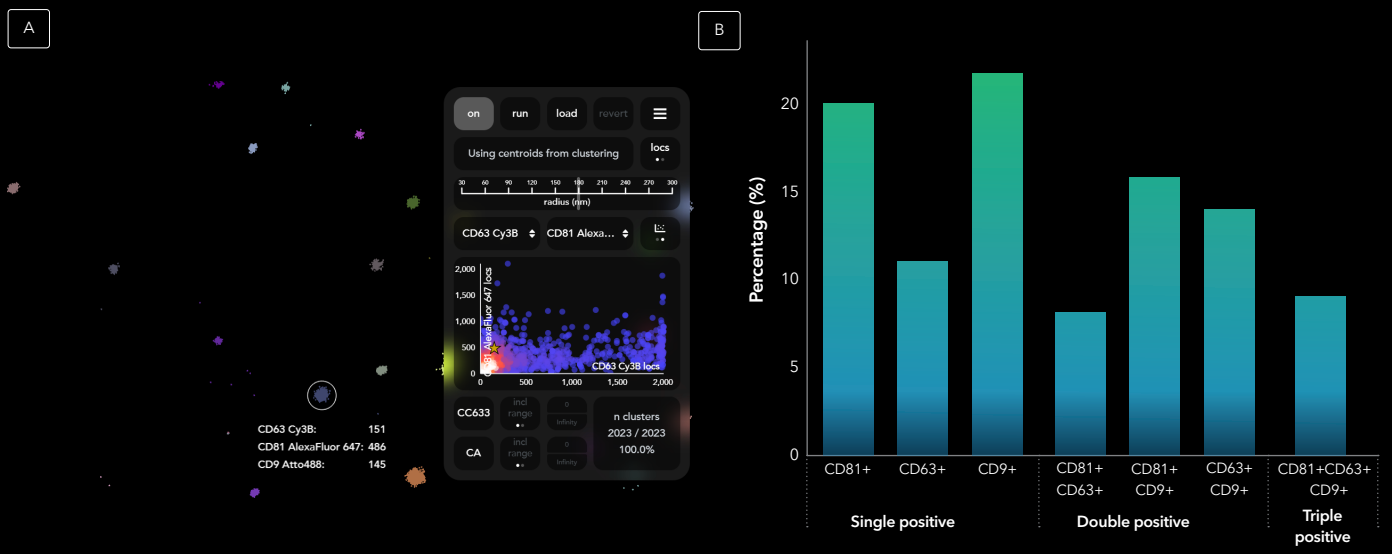

Figure 5 | Quantification of biomarkers on single-EVs to assess the heterogeneity within the entire sample (A). Histogram displays the percentage of single, double and triple positive EVs (B).

Solution with CODI

Understanding the unique molecular signatures of sub-populations of EVs and their association with specific cargo can enhance our understanding of how EVs function in cell signalling pathways and give deeper insights into the phenotypic consequences they enact at their target sites. The Nanoimager and CODI provide a complete solution for multi-color imaging and analysis of EV biomarkers including proteins, lipids and genetic material with up to 20 nm resolution for complete characterization and sizing of vesicle populations. For more information visit **oni.bio/extracellular-vesicles**

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