ONI Application note

Extracellular Vesicles

EV cargo: Visualizing and quantifying molecular DNA in single-EVs

Introduction

Extracellular Vesicles (EVs) are membranous particles that enable cell-cell communication via their surface components and cargo which includes proteins, lipids and genetic material such as DNA, mRNA and miRNA. While most of the transported material is carried within the lumen of EVs, a number of publications have identified a significant amount of genetic material coating the outer surface of vesicles (Figure 1). It is believed that the luminal contents of EVs are directly linked to their functional effect at recipient cells however, less is known about the role of DNA on the EV surface.

Studies have demonstrated that the presence of surface DNA modified EV adhesion properties[1], contributed to the overall net negative charge of EVs[2] and mediated horizontal DNA gene transfer[3]. Furthermore, on EVs derived from cancer cells, surface DNA contained oncogenic mutations that may imply a role of EV DNA in modulating the tumour microenvironment[4]. Therefore, spatial visualization of DNA and association with vesicles of discrete sizes or molecular signatures may contribute to a better understanding of the mechanisms and distinct functions of luminal and surface DNA in disease.

Challenge

A persistent challenge with imaging EVs is their small size (the smallest sub-category measuring between 40-200 nm in diameter) which falls below the resolution limits of conventional light microscopy. These diffraction-limited techniques restrict the user's ability to accurately size EVs, detect and quantify biomarkers or cargo, or to distinguish the fluorescent signal of an intact EV from that of fragments or isolated proteins. With super-resolution imaging techniques such as dSTORM, we can overcome this resolution limit and visualize EVs with single-molecule sensitivity.

Summary

The Nanoimager enables visualization and detection of multiple proteins and genetic material simultaneously both within the lumen and on the surface of individual EVs with single-molecule sensitivity.

This type of research supports the:

- Characterization of sub-populations of EVs possessing one or more biomarkers
- Distinction of molecules within the lumen or on the surface of EVs
- Association of unique molecular signatures to EVs of a specific size
- Investigation of the function of DNA on the EV surface
- Understanding the unique role of EV subtypes in cancer and other diseases

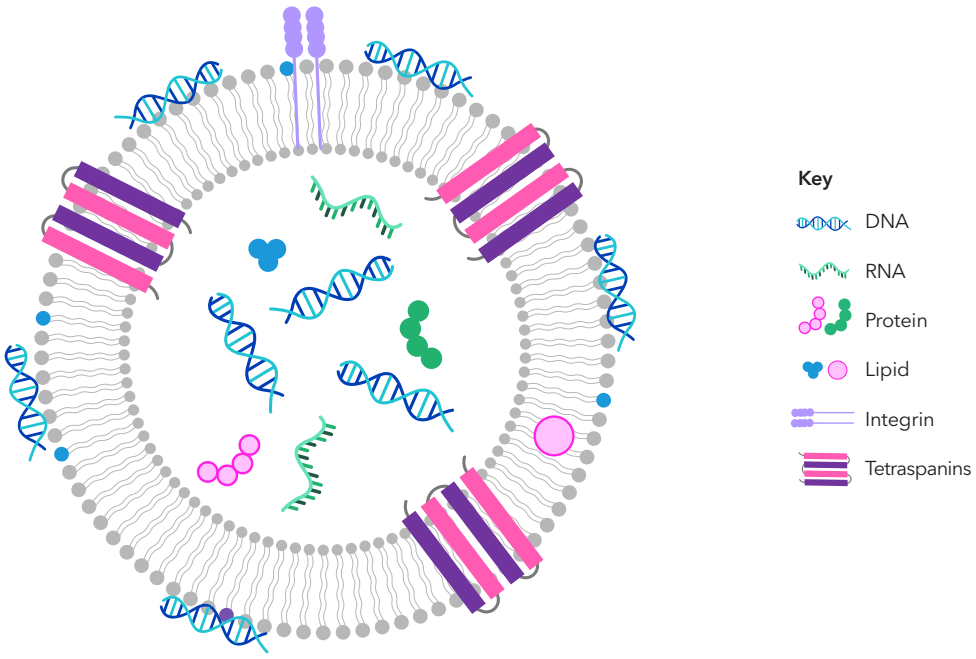

Figure 1 | Schematic representation of an EV showing the presence of DNA on both the EV surface and within the vesicle lumen.

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Results

Through multi-color dSTORM imaging on the Nanoimager, it is possible to fluorescently label and visualize DNA molecules on the surface of EVs and within the lumen, and quantify the association of the genetic material with vesicles of varying molecular signatures. Here, in collaboration with Dr. Franz Ricklefs from UMC Hamburg, EVs were purified from primary glioblastoma cells from patients. Prior to fluorescent labeling of CD63 and CD81 with Alexa Fluor® 555 and 647 respectively, and DNA molecules with the cell-impermeable SYTOX™ Green dye, EVs were subjected to one or a combination of the following conditions (Table 1 and Figure 2):

Table 1 | Conditions EVs were subjected to.

Sample	1. DNase treatment	2. Permeabilization	3. DNA labeling	Outcome
A	✓	✗	✗	Negative control
B	✓	✓	✓	Luminal DNA alone
C	✗	✗	✓	Surface DNA alone
D	✗	✓	✓	Luminal and Surface DNA

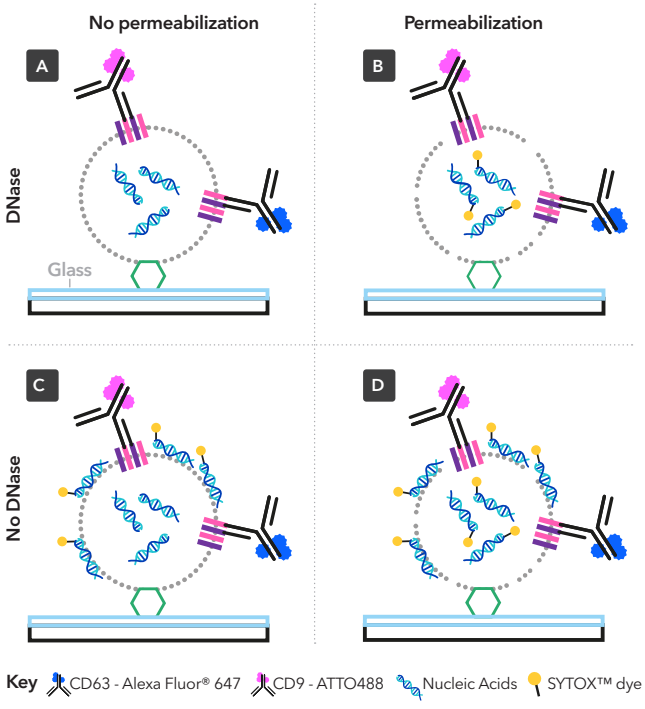

Figure 2 | Schematic representation of corresponding experiemental outcome of samples A-D.

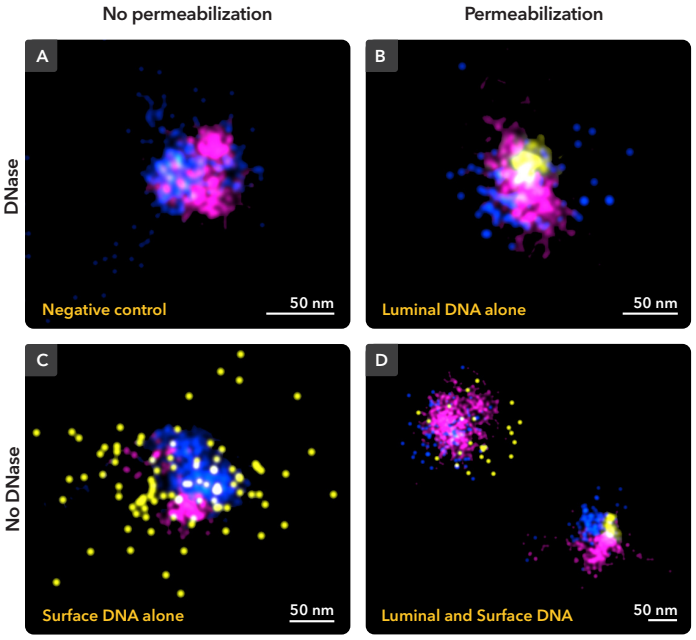

Figure 3 | Detection of DNA on the surface and within the lumen of EV samples (A-D) isolated from primary glioblastoma cells using dSTORM. DNA (yellow), CD63 (blue) and CD81 (magenta). *Dr. Franz Ricklefs, UMC Hamburg.*

Clusters of tetraspanin proteins and DNA on individual EVs were visualized by super-resolution dSTORM imaging (Figure 3) and subsequently analyzed through cluster analysis. The results illustrate that DNA molecules are present both within and on the surface of glioblastoma-derived EVs and the number of localizations of SYTOX™ signal differs greatly depending on treatment conditions (Figure 4). The use of additional techniques to further characterize the EV-associated DNA may provide insight into the role of these EVs in tumor progression or strengthen them as biomarkers for the identification and classification of tumors in clinical samples.

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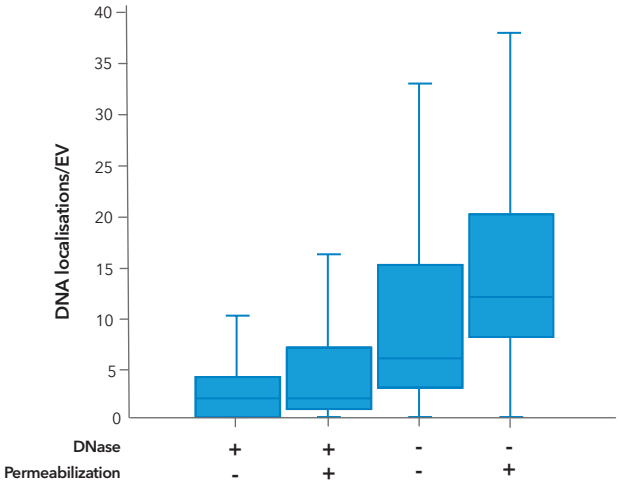
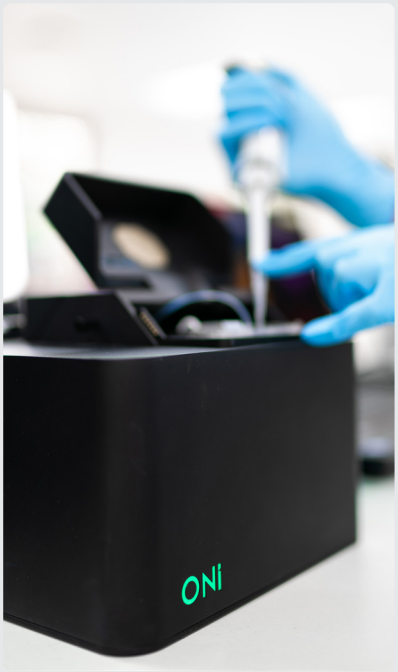


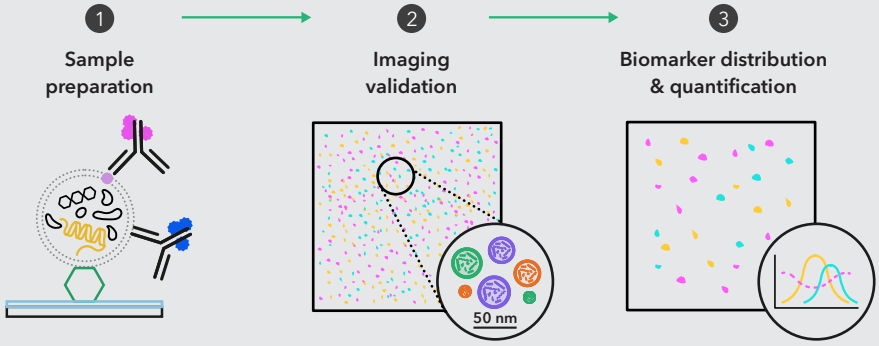
Figure 4 | Quantification of mean number of DNA localizations per EV pre-treated with either DNase I, permeabilization, neither or both, showing significant differences in localization number across conditions.



Solution with the Nanoimager

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 provides the complete solution for multi-color imaging 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



References

1. Németh A, Orgovan N, Sódar BW, et al. Antibiotic-induced release of small extracellular vesicles (exosomes) with surface-associated DNA. *Sci Rep.* 2017;7(1):8202.
2. Shelke G., Jang S. C., Yin Y., Lässer C., Lötvall J. Human mast cells release extracellular vesicle-associated DNA. *Matters (Zürich),* 2016. https://doi.org/10.19185/matters.201602000034.
3. Fischer S, Cornils K, Speiseder T, et al. Indication of Horizontal DNA Gene Transfer by Extracellular Vesicles. *PLoS One*. 2016;11(9):e0163665.
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