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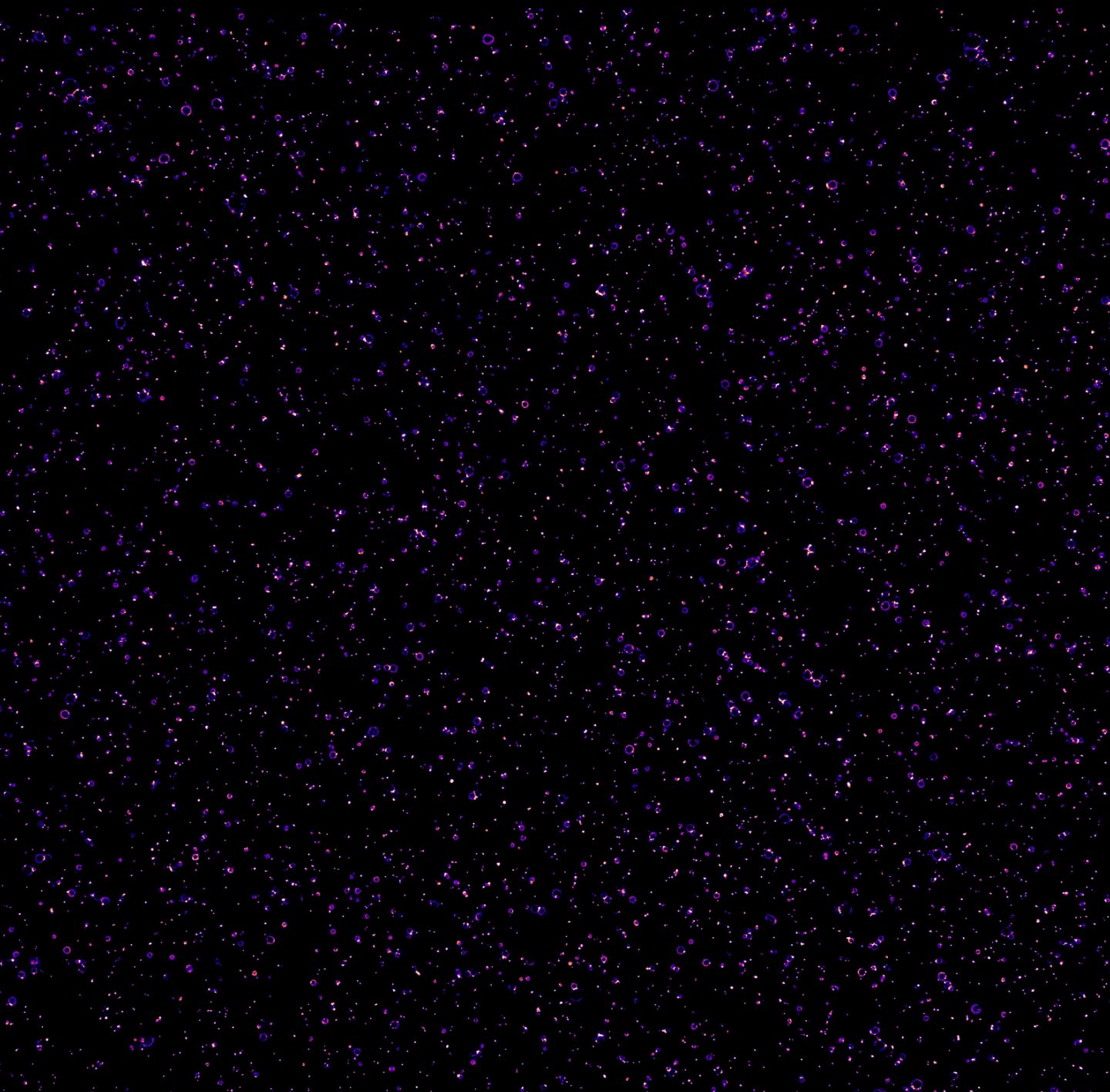
Extracellular Vesicles at the Nanoscale



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Extracellular Vesicles at the Nanoscale

Extracellular vesicles (EVs) are membrane bound particles, with nanometer-range size (20-5000 nm) that are secreted by all cell types and play a key role in cell-to-cell communication. (1-4)

EVs are extremely diverse in size and composition as they carry many bioactive compounds such as proteins, enzymes, lipids and nucleic acids. Factors such as cell growth, migration, apoptosis, disease or physiological stress can all influence the properties of secreted EVs. (5-7) Moreover, the EVs surface cargo signature will determine their fate after secretion, as it will influence their uptake and effect on the target cells. Indeed, EVs play an active role in the modulation of pathophysiological processes, like disease modulation and progression. (8)

The most widespread techniques for characterizing extracellular vesicles are nanoparticle tracking analysis (NTA) for sizing, flow cytometry (FC) and fluorescence microscopy (FM) for cargo characterization, and electron microscopy (EM) for sizing and morphology. However, none of these techniques can output size, cargo composition and morphology in one high-throughput measurement.

Why Single Molecule Localization Microscopy?

By separating in time and space the emission of single fluorophores, Single Molecule Localization Microscopy (SMLM) is the fluorescence-based microscopy technique which offers the highest spatial resolution among super-resolution techniques (below 20 nm in lateral resolution), achieving true molecular resolution.

Single molecule localization microscopy is a powerful technique for EVs characterization: with SMLM it is possible to stain EVs for specific biomarkers and image them with nanoscopic resolution, making possible to distinguish the nanometric features of single EVs and obtain information about size, biomarker composition and morphology for each single vesicle.

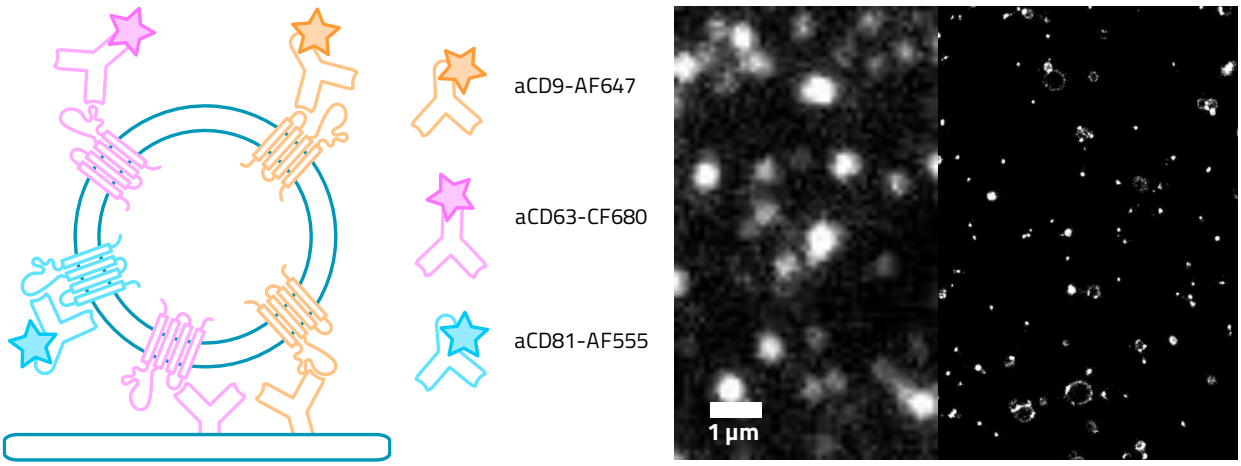

Figure 1 Extracellular vesicles and single molecule localization microscopy. Left: sample preparation for SMLM: EVs (either isolated or from unpurified medium) are immobilized on an immunocapture chip and stained for CD9, CD63 and CD81 in three different channels. Right: a snippet of a diffraction-limited image versus a super-resolution reconstruction of extracellular vesicles from hASC culture.

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In practice

Abbelight offers a complete solution from sample preparation to data management for analyzing your extracellular vesicles.

- **Abbelight™ Smart EVs Kit**: A ready-to use and optimized kit for immobilizing and imaging your EV samples
- **Abbelight™ SAFe Platform**: A state-of-the-art SMLM imaging platform which is robust, reliable, and easy to use
- **Abbelight™ NEO Analysis**: A dedicated software suite for analyzing and visualizing your data

Workflow in this note :

PREPARATION

EVs from hASC culture (EVerZom) were immobilized using **Abbelight™ Smart EVs Kit** on a WGA/ConA or anti-tetraspanins surface and stained with anti-tetraspanins-AF647 or aCD9-AF647, aCD63-CF680 and aCD81-AF555.

IMAGING

The sample was imaged on a **Abbelight™ SAFe MN360** with a large field of excitation using Abbelight's **STORM 2D, ultimate 3D imaging and simultaneous multi color imaging with spectral demixing** modality.

ANALYSIS

The single molecule switching events were localized with **Abbelight™ NEO Analysis**, the localization coordinate tables were segmented with **DBSCAN clustering** to isolate single EVs.

Quickly image thousands of EVs :

Thanks to Abbelight's patented large field of excitation (ASTER) technology, it is possible to image thousands of vesicles in a single acquisition, enabling robust EV population statistics informed by single-particle nanoscale resolution.

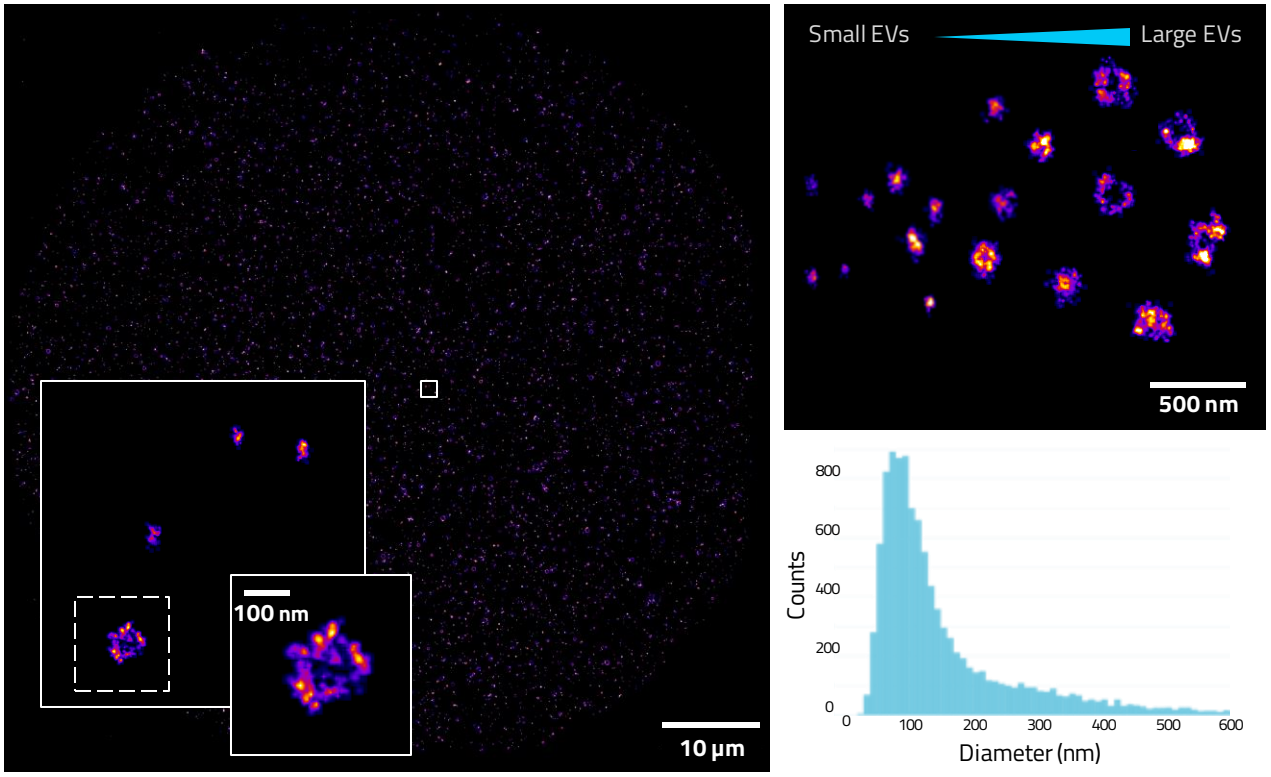

Figure 2 Extracellular vesicles imaged with a large field of excitation. Left: large-field nanoscopic reconstruction of 10112 EVs immobilized on an immunocapture surface and imaged in 5 min, the insets demonstrate how SMLM allows to distinguish the nanoscopic features of single EVs. Top right: collage of EVs of different sizes, showcasing the high size heterogeneity of the sample. Bottom right: size distribution obtained by SMLM of the sample.

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Investigate EV morphology in 3D:

Abbelight solutions also unlock the third spatial dimension in EV characterization. With Abbelight's Ultimate 3D, researchers can perform 3D SMLM up to a depth of 1.2 µm, achieving a lateral resolution below 20 nm and an axial resolution below 50 nm.

Figure 3 showcases EVs from hASC culture stained for tetraspanins in one channel and imaged using Abbelight's Ultimate 3D imaging. The distribution of tetraspanins on the EV membrane is not at all the same across different particles, these proteins can be distributed homogeneously on the entire vesicle, can cluster in "hotspots", or can create ring-like structures around the equator of the EV.

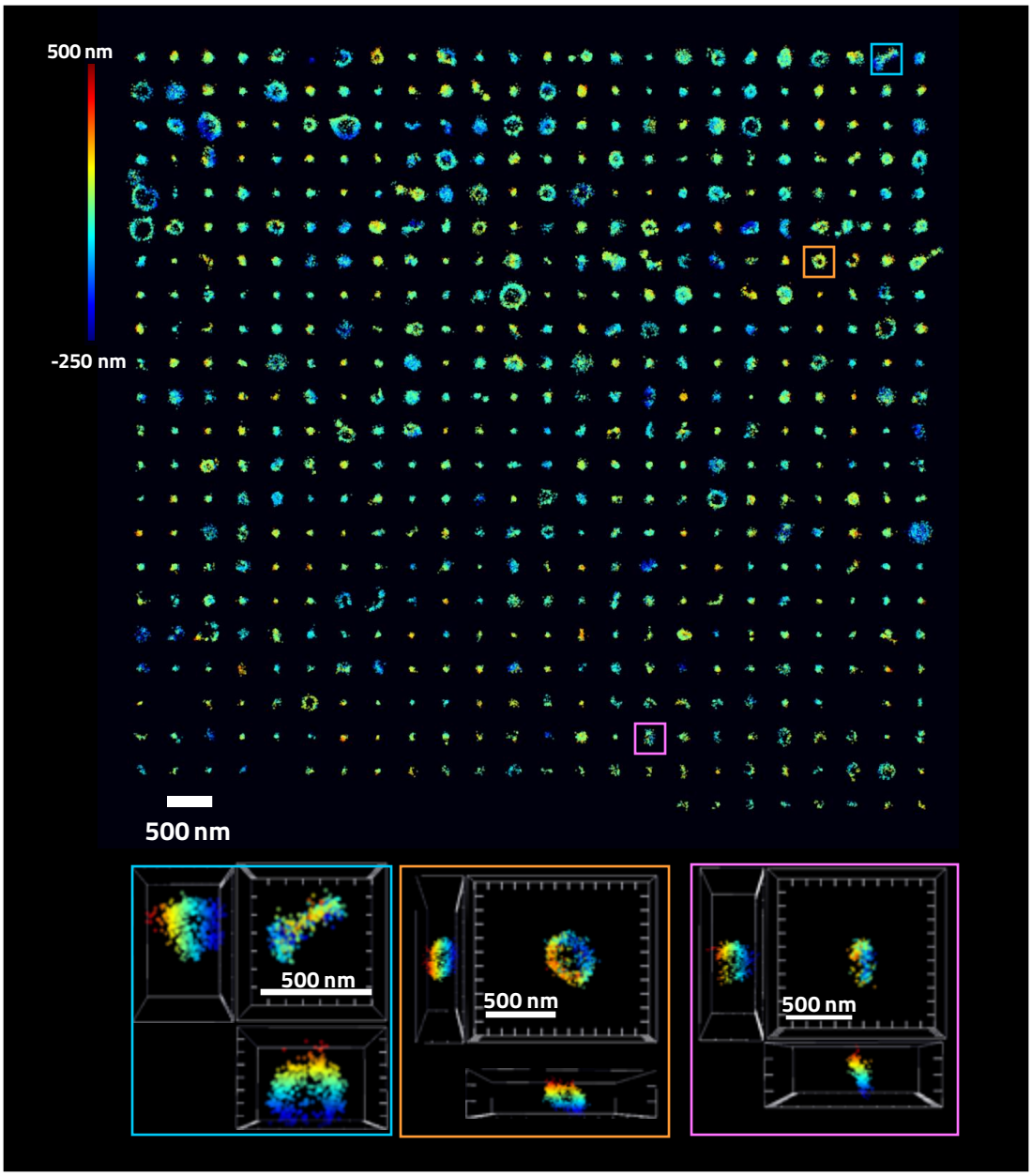

Figure 3 Extracellular vesicles in 3D. **TOP**: a collage of the imaged EVs, showcasing the rich diversity of the sample. **Bottom**: closeup of three EVs with the XY plane and the YZ and XZ section shown.

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Quantify and Colocalize your EV biomarkers:

Spectral Demixing technology enables simultaneous multicolor imaging using a single laser. This approach saves researchers time by allowing multicolor imaging in a single acquisition, while also eliminating the need for channel alignment and chromatic aberration correction.

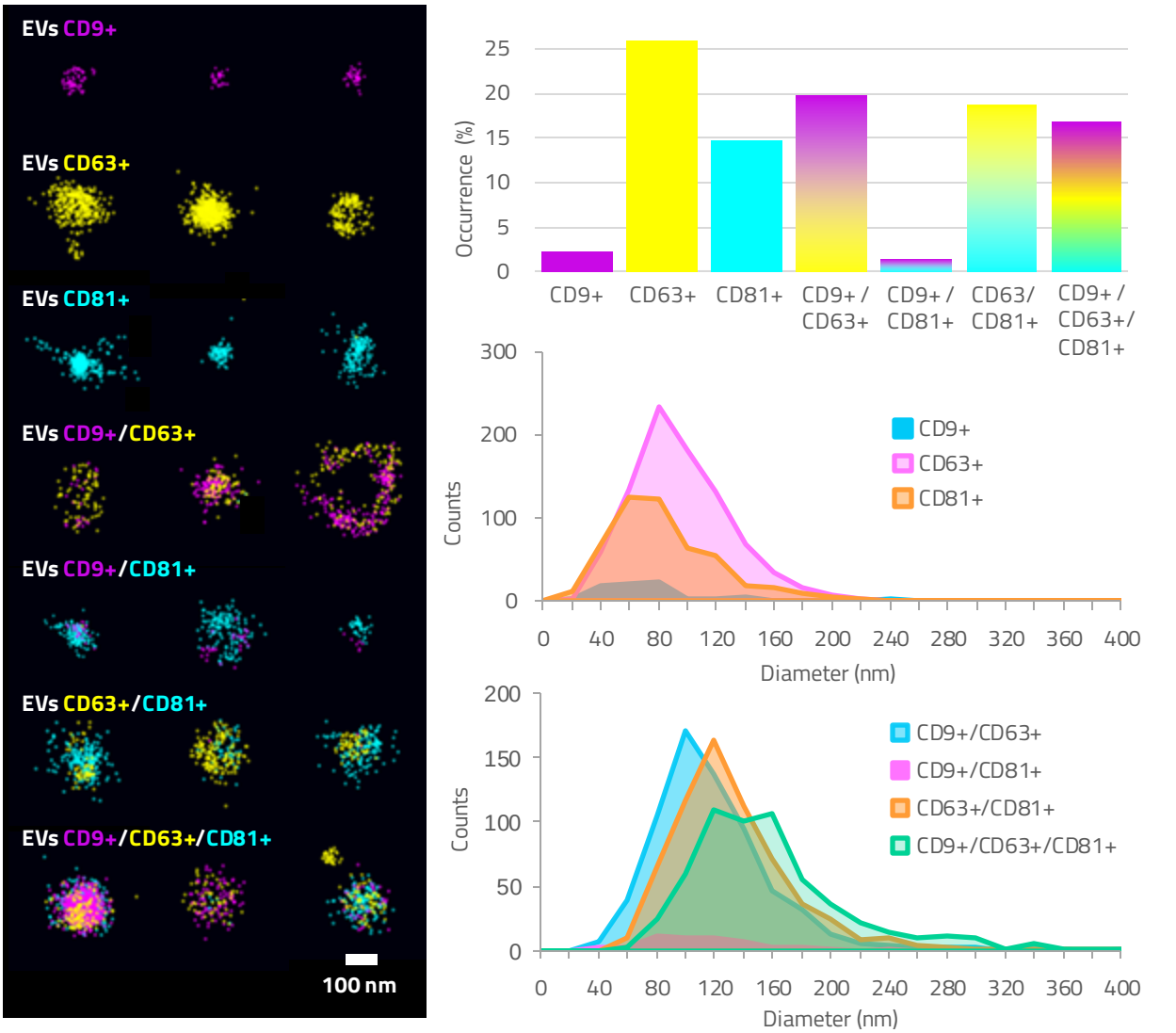

Figure 4 Colocalization of three biomarkers in extracellular vesicles. Left: a selection of EVs that are single, double or triple positive to CD9, CD63 or CD81. Top right: bar chart illustrating the abundance of EV subpopulations. Middle and bottom right: size distribution histograms for each EV subpopulation.

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Abbelight

Founded in 2016, Abbelight is a fast-growing company specialized in imaging solutions focusing on microscopy and unique single molecule detection (super-resolution).

The portfolio integrates a constantly evolving know-how on chemistry, optics and computer science to offer a complete solution, from sample preparation to data management, including an optimal bio-imaging platform that can be adapted to all researchers', biotech labs' and medical facilities' needs.

Abbelight is a French company developed by four passionate researchers who aim to help improve human health in various areas such as bacteriology, extracellular vesicles, neurosciences, structural biology...

Today, Abbelight employs over 60 people who are all driven by the goal of providing the best solutions and support to our customers all around the world.

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