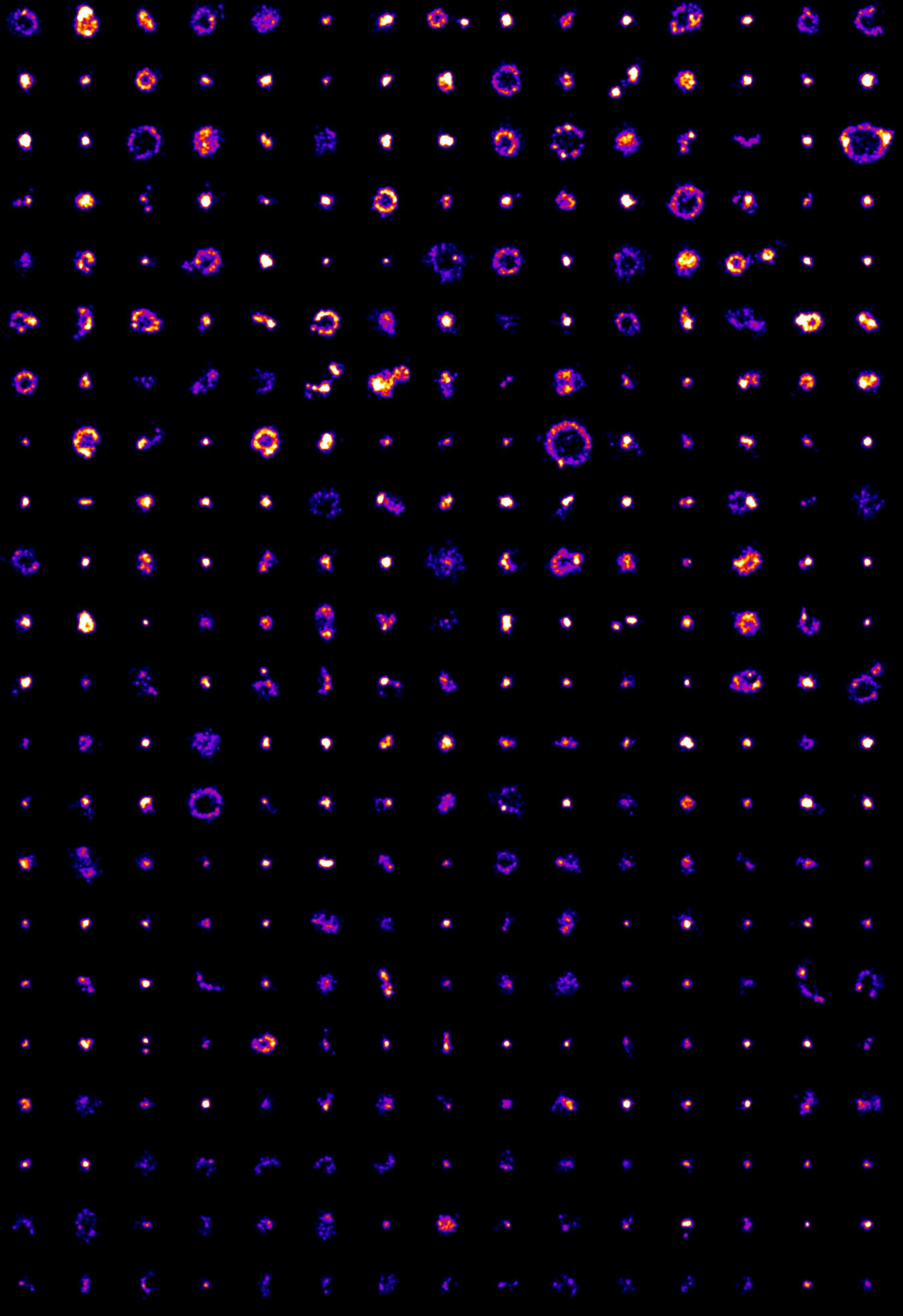




Light up your research

From Sample Preparation to Data Analysis: A Comprehensive Workflow for Imaging EVs with SMLM



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Extracellular vesicles (EVs) are small, membrane-bound particles that are secreted by all cell types. They vary in size, typically ranging from 20 to 5000 nm, and carry a diverse array of biomolecules, including proteins, lipids, and nucleic acids. Hence, EVs play a key role in cell-to-cell communication by transferring their cargo to target cells.

SMLM can give insights on the size, composition and morphology of single vesicles and the entire population, in one quick, simple high-throughput measurement.

Why Single Molecule Localization Microscopy?

The main techniques for EV characterization include flow cytometry (FC) for surface marker profiling, electron microscopy (TEM and cryo-EM) for morphology and sizing, and nanoparticle tracking analysis (NTA) for size and concentration¹. Flow cytometry enables EV phenotyping by detecting surface and luminal markers but has limited resolution for detection and accurate characterization of small vesicles, while electron microscopy provides high-resolution structural details but lacks molecular profiling capabilities.

Single molecule localization microscopy (SMLM) is an emerging technique that bridges the gap between flow cytometry and electron microscopy by allowing simultaneous assessment of EV phenotype and morphology at the nanoscale. With super-resolution capability, SMLM can visualize individual molecules on EV surfaces with high spatial precision, enabling detailed mapping of specific markers while capturing structural information. This unique ability makes SMLM a valuable tool for comprehensive EV characterization, revealing both molecular profiles and structural features in ways previously not achievable with either FC or EM alone.

Abbelight offers a comprehensive workflow, from sample preparation to advanced data analysis, for gathering insights on EV samples with SMLM.

SAMPLE PREPARATION

Purifying extracellular vesicles (EVs) is challenging due to their similarity in size and density with other particles, their low concentrations, and their diversity in biological fluids. Isolation methods like ultracentrifugation and precipitation often struggle to balance purity with yield².

To address these challenges, Abbelight has developed the Smart EVs Kit, a solution for selectively immobilizing and staining EVs from both purified and unpurified samples (such as plasma or cell culture supernatant).

Abbelight's Smart EVs kit contains:

- Washing buffer
- Passivated biotinylated glass coverslips
- Sticky 6-channel microfluidic flow chamber
- Avidin solution
- Biotinylated capture antibodies (against CD9, CD63 and CD81) or biotinylated lectins
- Blocking solution
- Fluorescently labeled antibodies (against CD9, CD63 and CD81)

The kit comes in two sizes: **5 coverslips** (30 samples), **1 coverslip** (5 samples).



Figure 1 Abbelight's Smart EVs Kit

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This Smart EVs Kit is based on avidin-biotin interaction, allowing for customization with various antibodies for targeted EV capture and staining.

Sample preparation consists in:

1. Preparing the immunocapture surface
2. Incubating the EV sample
3. Staining the sample with fluorescent antibodies

The Smart EVs kit simplifies sample preparation with its ready-made solutions and user-friendly protocol, ensuring an effortless and efficient process.

IMAGING

The prepared EV sample (fluorescent EVs from HEK293 cells (CD63-EGFP), HansaBioMed Life Sciences) is stained for two markers, CD63 and CD81 (with respectively CF680 and AF647), then covered with Abbelight's Smart kit buffer and imaged in simultaneous multicolor mode with SMLM using Abbelight's SAFe MN360 platform (Figure 4).

Single Molecule Localization Microscopy is a super-resolution imaging technique that allows visualization of structures at the nanoscale. It works by exciting a small, random subset of fluorescent molecules in a sample, so their signals don't overlap. Each molecule's position is then pinpointed with high precision, and this process is stochastically repeated thousands of times for different molecules in the sample.

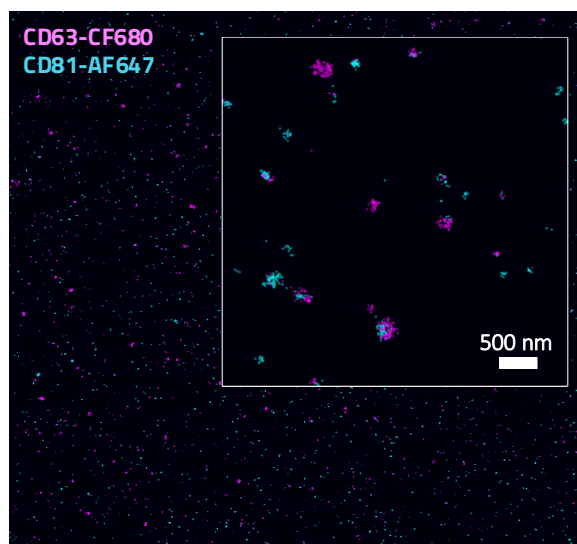


Figure 3 Super-resolution image of an EV sample prepared with Abbelight's Smart EVs kit and stained for CD63 (purple) and CD81 (cyan). SMLM enables researchers to observe the significant heterogeneity of EV samples, in terms of both size and composition.

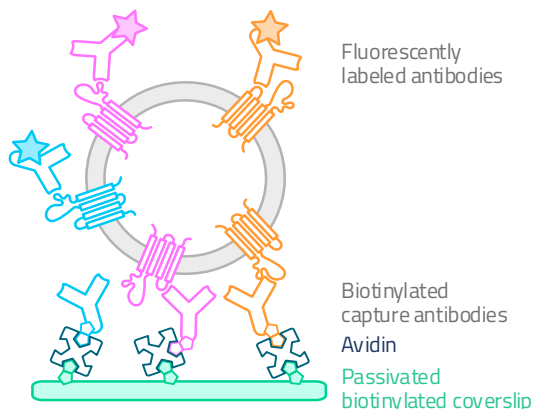


Figure 2 Working principle of the Smart EVs Kit: EVs are immobilized on an immunocapture surface and stained with fluorescent antibodies. The capture surface is based on an avidin-biotin bridge.

By combining all these positions, a detailed, high-resolution image is reconstructed, revealing details far smaller than those visible with conventional light microscopy.

The imaging workflow is made user-friendly using Abbelight's NEO acquisition software. Moreover, Abbelight's patented ASTER technology, featuring an ultra-wide field of view, enables researchers to capture super-resolution images of thousands of EVs in less than 5 minutes.



Figure 4 Abbelight's SAFe MN360 platform (left) attached to an inverted microscope (right).

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DATA ANALYSIS

Once the SMLM acquisition has been recorded with Abbelight's NEO acquisition software, the SMLM localization data can be processed using Abbelight's NEO analysis software for visualization and detailed analysis, both software are part of the package NEO Software Suite.

NEO analysis automatically segments each EV using the DBSCAN algorithm³, enabling single-vesicle analysis (**Figure 5**). This streamlined workflow facilitates rapid evaluation of key properties such as size, biomarker composition, and morphology. By analyzing individual data, the software generates population-level distributions from thousands of vesicles, delivering comprehensive insights efficiently and automatically.

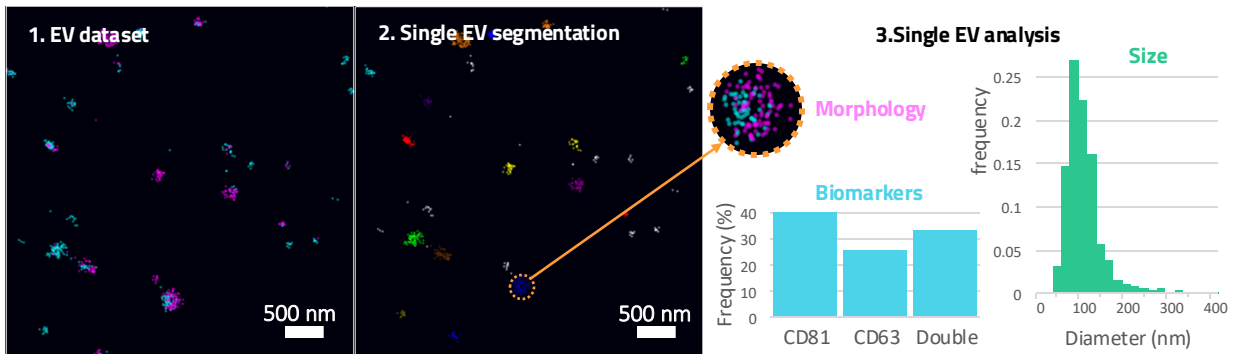


Figure 5 Single EV analysis workflow in SMLM: The EV dataset is loaded into Abbelight's NEO analysis software, where individual EVs are segmented using the DBSCAN algorithm. Key properties such as EV size, composition, and morphology are calculated for each vesicle, and population-level statistics are then generated.

Abbelight's NEO Software Suite offers advanced tools for rendering and visualizing EV datasets, enabling users to create publication-ready figures with ease while gaining deeper insights into their EV samples.

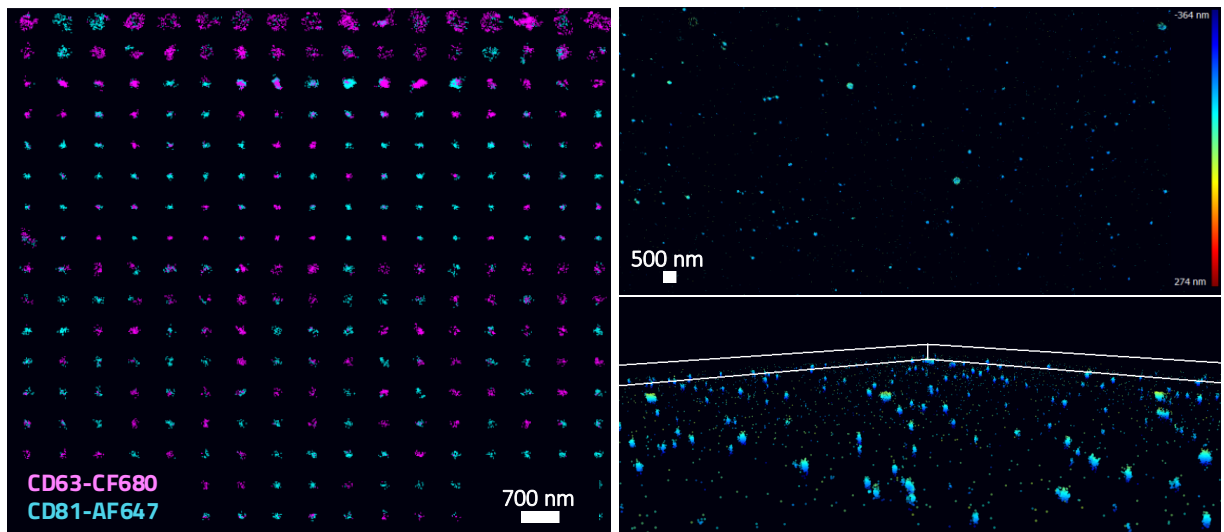


Figure 6 Left: EV dataset arranged in a grid; this type of visualization offers an intuitive overview. Right: Abbelight's SAFE platforms offer Ultimate 3D imaging, allowing researchers to investigate the 3D morphology of their EV sample.

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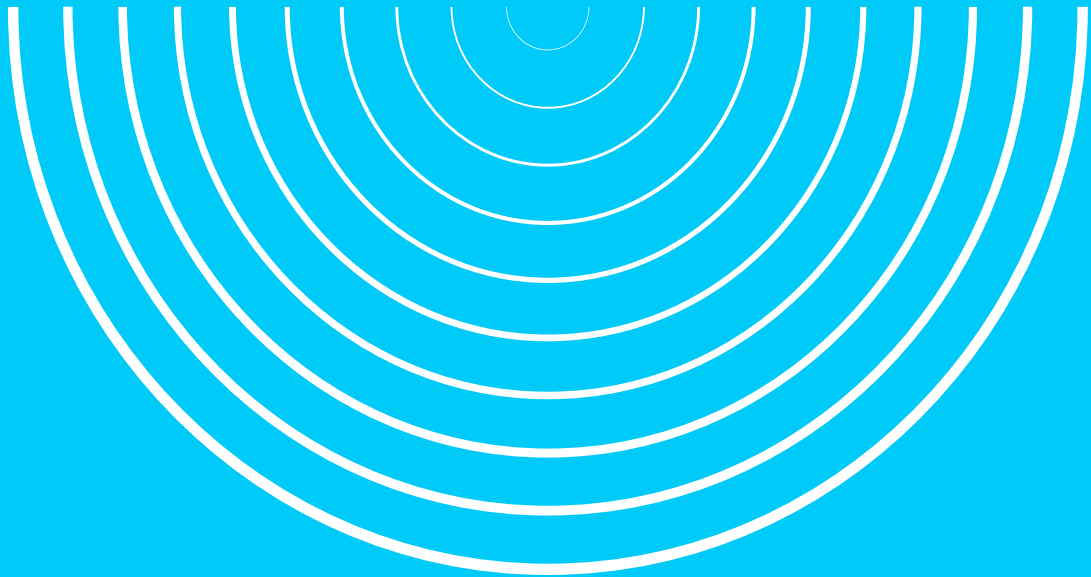


Founded in 2016, Abbelight is a fast-growing company specialized in imaging solutions focusing on microscopy and unique single molecule detection (super-resolution).

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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