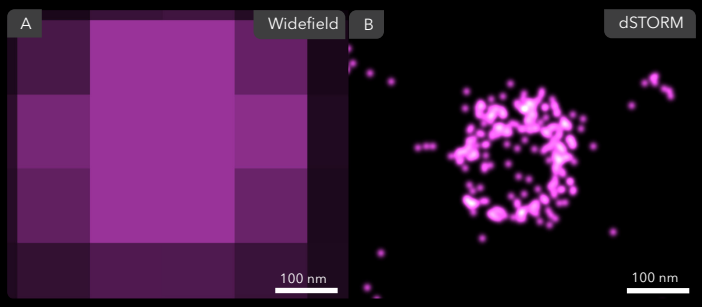
Revealing the structure of clathrin-coated pits with DNA-PAINT

Introduction

Clathrin coated pits (CCPs) are spherical, cage-like structures located on the plasma membrane that are critical mediators of molecular uptake and intracellular transport[1]. These structures play a key role in clathrin-mediated endocytosis (CME) by budding off the cell membrane with their receptor cargo. They are formed by the assembly of major coat proteins, structurally composed of three clathrin-heavy chains and tightly associated clathrin-light chains and additionally associate with numerous accessory proteins to promote and regulate endocytosis e.g. by acting as scaffolds or recruiting cargo[2].



Summary

The Nanoimager enables single-molecule imaging at sub-20 nm resolution using a variety of SMLM techniques:

Using this approach it is possible to

- Characterize the nano-architecture of organelles and other cellular structures
- Reveal the spatial distributions of single-molecules
- Assess protein co-localization
- Understand phenotypic changes in disease
- Visualize multiple markers by multiplexing probes through microfluidics

Figure 1 (left) | Single CCP within fixed COS-7 cells was imaged through standard widefield microscopy in TIRF mode (left) and dSTORM (right). The CCP was labelled with a rabbit anti-clathrin heavy chain primary antibody and an AlexaFluor™ 647 anti-rabbit secondary antibody.

Challenge

To better understand the process of CME, it is important to visualize the structure of pits and assess their molecular interactions at all stages of CCP formation. However, as the pits are typically between 150-200 nm in diameter, they are below the resolvable limit of conventional light microscopy and while the structure of CCPs can be clearly resolved by electron microscopy (EM), it is technically challenging to assess CCP interactions with accessory proteins through this method[3].

Single-molecule localization microscopy (SMLM) techniques have been used to image CCPs and their structures in super-resolution[4], which have advantages over electron microscopy through their ability to image multiple markers simultaneously. In contrast to conventional epifluorescence imaging, SMLM techniques can break the diffraction limit to image CCPs with greater than 20 nm resolution and gain deeper structural insights (Figure 1).

Results

Here, we have imaged CCPs using the SMLM technique of DNA-PAINT on the Nanoimager. In this example, CCPs were labelled with antibodies against clathrin heavy chain[5], and detected with commercially available secondary antibodies conjugated with short single-stranded DNA "docking" strands (Massive-sdAb 2-Plex kit by Massive Photonics). Complementary DNA "imager" strands containing fluorescent dyes were added which transiently bind to their complementary DNA docking strand, allowing for the position of individual fluorescent molecules to be localized over a large number of frames (Figure 2, B). The powerful resolution achieved by this technique means that single CCPs can be analysed with sub-20 nm localization precision (Figure 2, C).

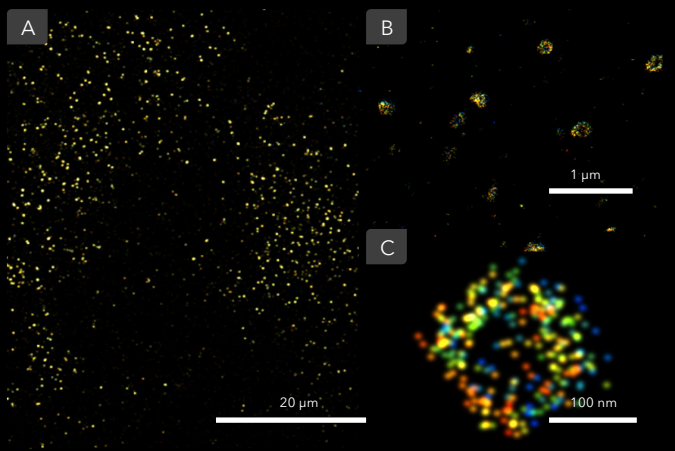


Figure 2 | Clathrin coated pits labelled with a rabbit anti-clathrin heavy chain primary antibody and detected with the Massive Photonics "massive sdAb-2-plex" kit for DNA-PAINT imaging in fixed COS-7 cells. Localizations are colored by time of detection.

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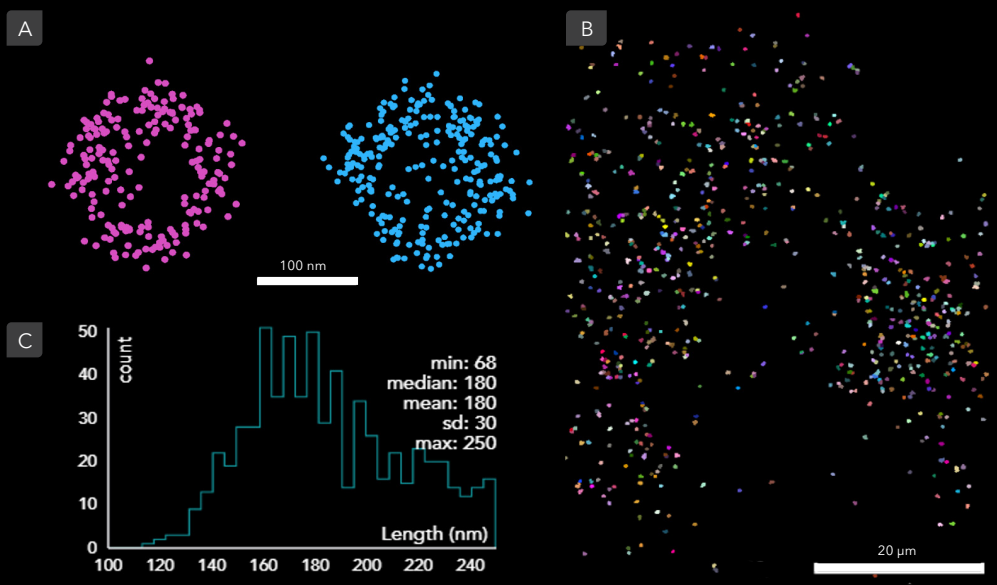

Figure 3 | Clustering analysis was applied to the DNA-PAINT images to extract sizing information of the CCPs. (A) Single clusters representing single CCPs showing the ring-like structures and (B) All CCP clusters detected with fixed COS7 cells. (C) Histogram plot showing size ranges of CCPs within the cells

After image acquisition, cluster analysis was performed on the super-resolution images to extract quantitative information and characterize individual CCPs based on parameters such as size and shape, using the integrated software tools of the Nanoimager. Clustering analysis works by identifying the localizations corresponding to the clathrin heavy chain signal on the CCPs surface. All localizations within a defined radius are grouped to represent single CCPs (Figure 3, A). This is then applied to the entire image to identify all CCP clusters within the cell (Figure 3, B) and generate a size distribution (Figure 3, C). The mean size of CCP clusters in this data of 180nm +/- 30nm is comparable to previously published dSTORM analysis of CCPs, which itself was benchmarked to data collected using electron microscopy (180nm +/- 40nm)[4].

This quantitative analysis is important for understanding the different stages of CCP formation within cells (for example by constraining based on size or shape features of the clusters) or population dynamics of CCPs (for example, quantifying the number of CCP clusters of various sub-categories in experimental versus control conditions), which will give further insight into their role in receptor-mediated endocytosis.

Solution with the Nanoimager

The Nanoimager offers a user-friendly, desktop-compatible solution for the precise and detailed characterization of clathrin coated pits and other subcellular structures of interest via a range of SMLM techniques such as PALM, dSTORM and DNA-PAINT. With microfluidics integration with the Nanoimager, it is possible to image multiple targets with different indexing DNA strands for multiplexed imaging of a sample for as many targets as the user desires.

The multitude of imaging modalities combined with the in-built analysis software of the Nanoimager provides a comprehensive workflow for full characterization of structures at the single-molecule level. www.oni.bio

References

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