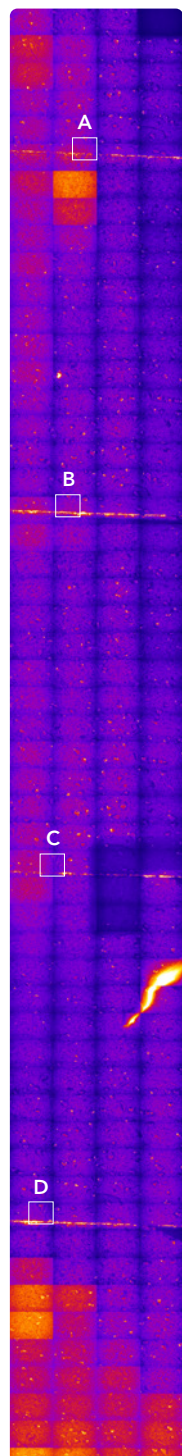


Brain tissue neurodegeneration in a new dimension: resolving single synapses with PALM array tomography



Challenge

The human brain is one of the most complex systems in biology. This has hindered the understanding of crucial molecular mechanisms that determine neuronal connections both in health and disease, particularly in a three-dimensional (3D) landscape.

When imaging brain tissue sections, resolution and sensitivity are limited by the depth of the tissue section, which makes visualization of single molecules challenging, particularly in the Z axis. Array tomography allows researchers to achieve an isotropic high-resolution in all three dimensions and obtain high sensitivity even in deep tissues by limiting light scattering. This relies on ultrathin physical tissue sectioning - as compared to optical sectioning of conventional confocal microscopy.

Additionally, array tomography can be combined with super-resolution imaging of fluorescent proteins in individual tissue sections arranged into a serial ribbon. As synapses can't be resolved with conventional light microscopes, until recently this was only possible using electron microscopy.

Results

Visualizing key synaptic markers at a single-molecule level within a tissue section allows researchers to image protein aggregates, which are linked to many life-threatening neurodegenerative disorders, including Alzheimer's or Parkinson's disease.

The experiments here show a unique combination of array tomography and PALM imaging using the Nanoimager platform to image individual brain sections. Figure 1 shows an overview scan of a 70nm-thick human brain tissue with the postsynaptic protein PSD95 labeled with the fluorophore mEOS.

Summary

The Nanoimager platform allows imaging of brain tissue at high-sensitivity and the visualization of individual synaptic proteins at high-resolution.

This type of research enable us to:

- Determine the heterogeneity of synaptic structures at a single-molecule level
- Study different pre or postsynaptic markers in health and disease
- Understand the molecular hallmarks of neurodegeneration and brain plasticity
- Identify protein aggregates contributing to neurodegenerative diseases
- Find novel neuromodulator function therapies and understand their mechanism of action

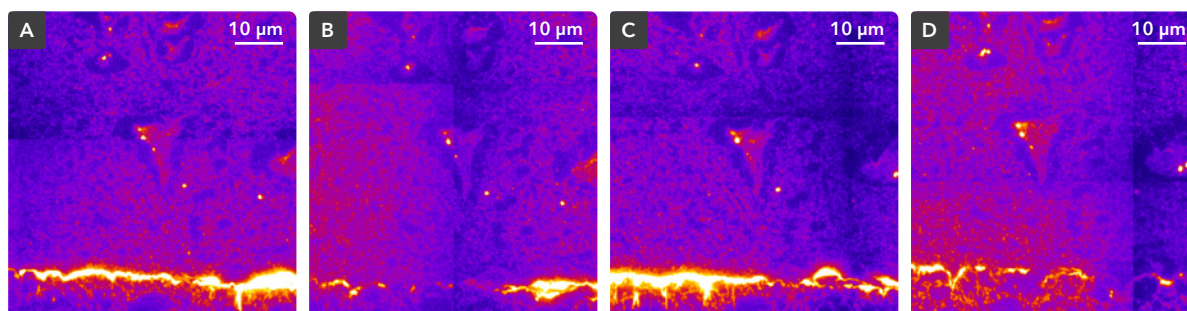
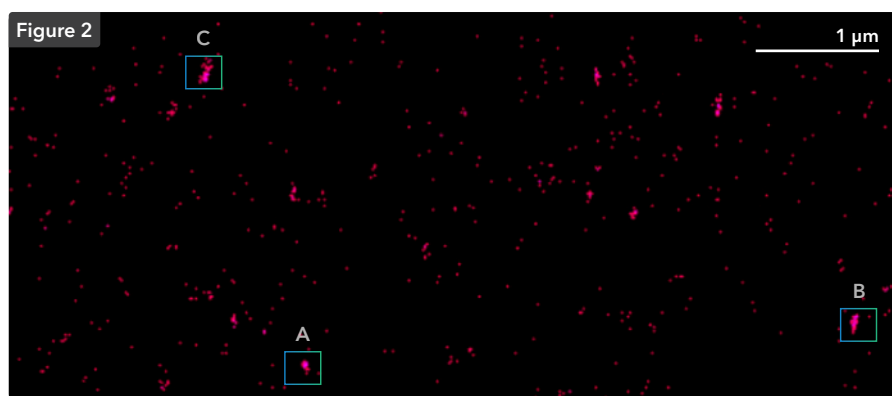


Figure 1 | Overview scan of an ultrathin human brain tissue section labeled with mEOS-tagged PSD95, a postsynaptic protein. This was used to identify a unique structure in each of the sections (triangular cell body), which served as a reference point for PALM array tomography imaging. Sample prepared by Dr. M. Horrocks, School of Chemistry and Dementia Research Institute, University of Edinburgh.



Next, we generated super-resolution imaging data to be able to determine the heterogeneity of synaptic structures within the tissue at a single-molecule level. After identifying a unique structure as the reference point in each of the sections (Figure 1, panels A, B, C and D), each of the positions was imaged using PALM. This allowed researchers to visualize synaptic protein clusters and revealed PSD95 clusters as either punctate or fibrillar in structure (Figure 2).

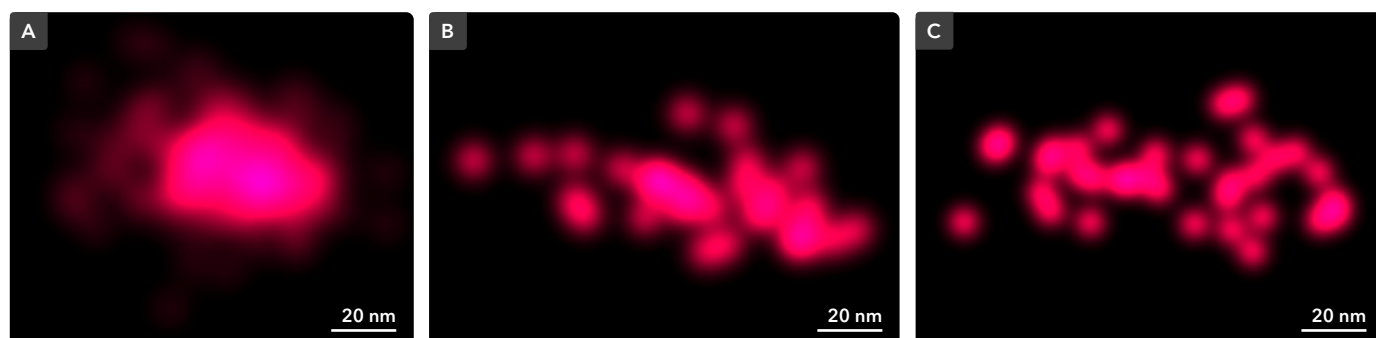


Figure 2 | PALM imaging of human brain tissue labeled with mEOS-tagged PSD95. mEos was photo-converted (from green to red emission) using UV light. Samples were pulsed with UV for 0.5sec followed by a 150 frame acquisition with 561 nm laser and repeated in 75 cycle for each tissue section. Sample prepared by *Dr. M. Horrocks, School of Chemistry and Dementia Research Institute, University of Edinburgh.*



Solution With The Nanoimager

The Nanoimager provides a solution for imaging brain tissues with high-resolution, by applying PALM array tomography to better characterize neurodegenerative diseases and resolve synaptic structures at a molecular level. It provides individual real-time localizations and additional automation features that can help with multiplexing capabilities. Tissue sections can be put through iterative cycles of immuno-labelling to obtain high order number of synaptic marker images.

After realignment, the data can be rendered into a highly detailed 3D image to visualize neuronal dendrites and axons, while uniquely resolving individual synapses. By imaging one field across serial sections and tiling fields together, one can obtain a large field of view of a brain tissue, something that has only been possible in 2D to date (Broadhead et al. Sci Rep 2016).

To learn more about the microscope features, its different applications and ONi visit www.oni.bio.