ONI Application note

Neuroscience

Neurons: revealing synapse architecture at a single-molecule level

Challenge

Brain tissue degeneration is caused by disorders that destroy neurons and currently affects millions of people worldwide. The mechanisms underlying disease onset remain largely unresolved and current efforts are focusing on understanding the key molecular hallmarks of disease at a single-molecule level, with the aim to improve early diagnosis.

Synapses are the centre of crucial connections between neuronal dendrites and axon terminals. They are under 200nm in size, with the synaptic cleft being as small as 20-40nm, rendering them 'invisible' to conventional light microscopy techniques. Super-resolution microscopy is required to better understand the molecular diversity and architecture of synapses, as well as to study the pre- and postsynaptic proteins involved in neurotransmission.

Results

Visualization of synaptic proteins at the nanoscale level allows researchers to study the specific localization and dynamics of proteins prone to aggregate, which can heavily alter the release and detection of neurotransmitters at the synaptic gap and impact brain function.

The Nanoimager can overcome the limitations of conventional fluorescence microscopy and facilitate synaptic architecture analysis at single-molecule resolution (Figure 1). It enables imaging of proteins with 3 different colors, making it possible to reveal the protein composition of the synapses in cells with a 20nm resolution. It can tell apart pre- and postsynaptic proteins and quantify distances between them, which is crucial in the study of synaptic structure.

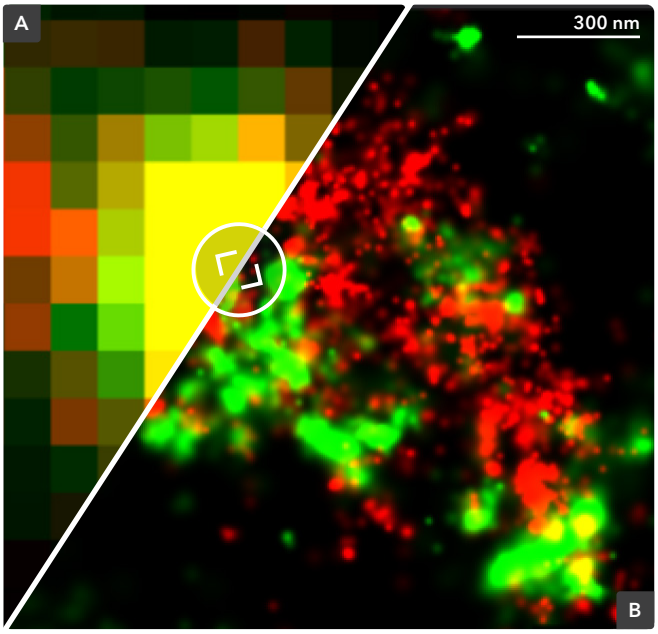
Summary

The Nanoimager platform supports super-resolution imaging of cultured neuronal cells and the precise localization of different synaptic proteins.

This type of research enables us to:

aneurodegenerative, psychiatric or neurodevelopmental disorders

- Determine the heterogeneity of synaptic structures at a single-molecule level
- Follow the localization of neurotransmitter vesicles at the synaptic gap
- Better understand the molecular hallmarks of brain plasticity in health and disease
- Identify targets for novel neuromodulation therapies and study their mechanism of action



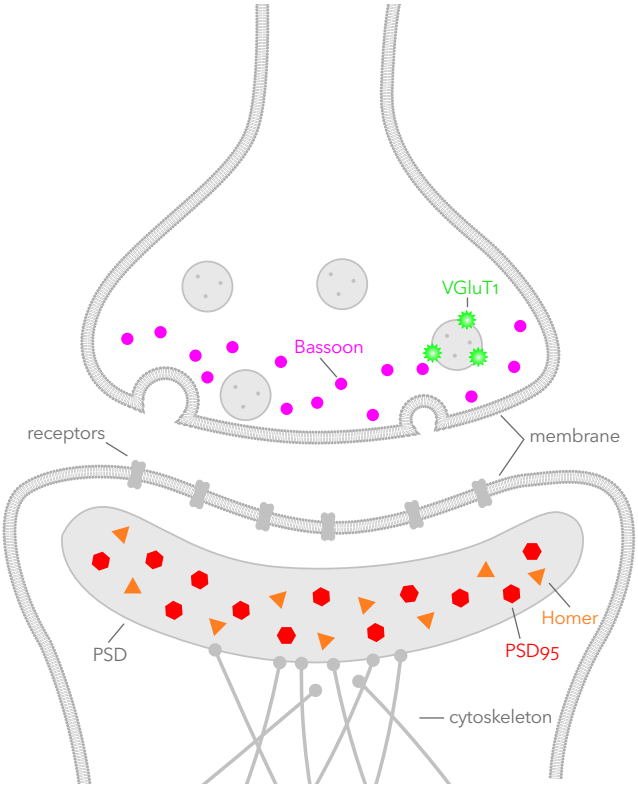


Figure 1 | dSTORM image of pre- and postsynaptic proteins in rat cortical neurons. Image of a single synapse acquired with widefield (A) and super-resolution dSTORM (B) imaging showing the major scaffolding protein of the postsynaptic density PSD95 (in red) and VGluT1, a presynaptic vesicle protein (in green).

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Rat primary cortical neurons were cultured for 14 days, allowing synapse formation on a dish. Neuronal synapses were then visualized in 3 color dSTORM imaging using two presynaptic proteins Bassoon (synaptic active zone, in magenta) and VGluT1 (synaptic vesicles, in green); and the postsynaptic protein Homer1 (postsynaptic density, in orange). This revealed the presence of neurotransmitter vesicles in between pre- and postsynaptic protein clusters (Figure 2). In this particular example, the measurements with the integrated software also showed that the distance between the pre- and postsynaptic proteins Bassoon and Homer was approximately 150nm.

Figure 2

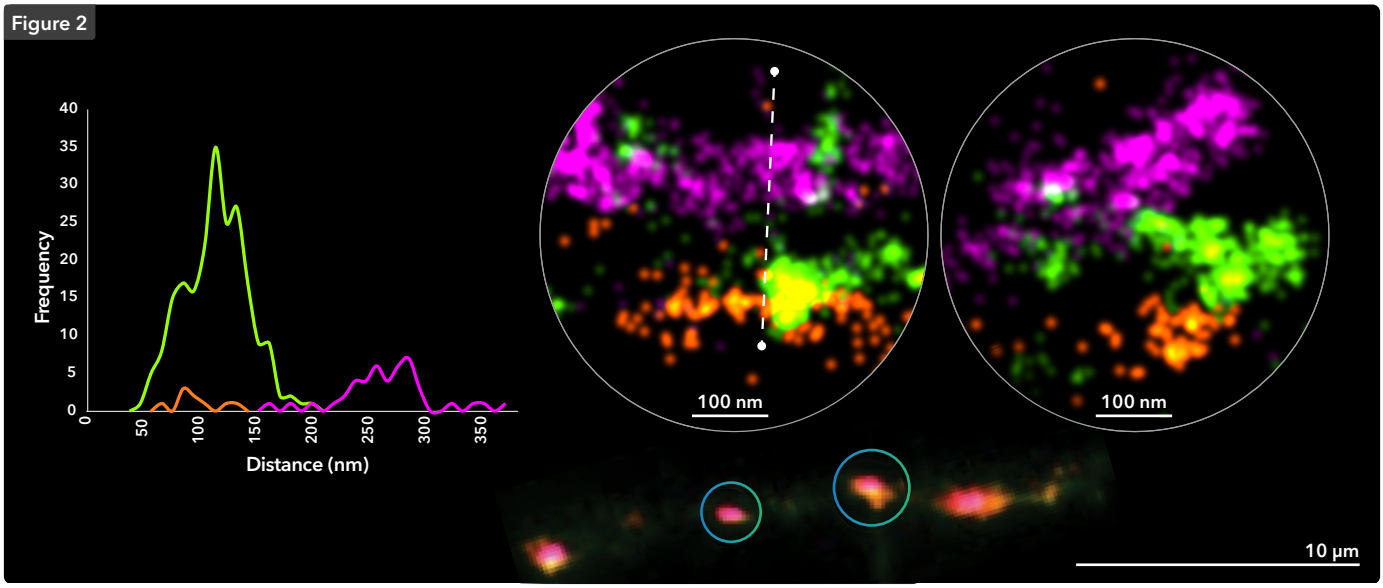
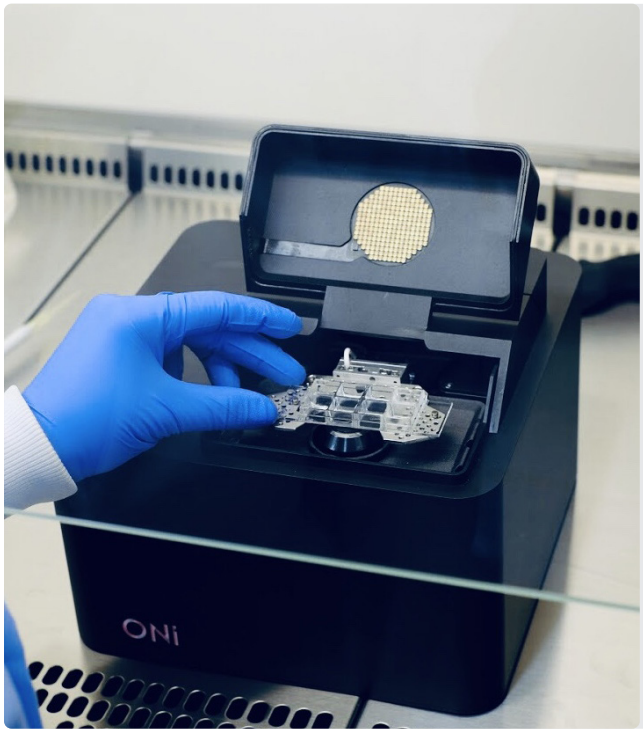


Figure 2 | 3-color super-resolution image of neuronal synapses in rat primary cortical neurons cultured for 14 days. Image of a neuronal process containing several synapses, imaged with dSTORM imaging using fluorescently-labeled antibodies against the presynaptic proteins Bassoon (magenta), synaptic vesicle marker VGlut1 (green) and the postsynaptic protein Homer1 (orange). Graph shows a line scan quantification of a region of interest showing the localization of the 3 synaptic markers. Sample prepared by the ONI Application Development team.



Solution With The Nanoimager

The Nanoimager provides a platform to study synaptic structures and resolve their molecular composition at the nanoscale by using different super-resolution modes. It offers individual real-time localizations and automation features that can help obtain information on multiple synaptic markers. The Nanoimager enables imaging with up to 4 separate fluorophores (2 simultaneously), allowing individual synaptic proteins localizations registered in one channel to be assigned to cellular markers of certain brain structures in the second channel. It can also help gather data on vesicle distribution within synapses, co-localization of protein clusters, or postsynaptic density (PSD) profiles to better characterize onset of neurodegenerative diseases.

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

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