Modeling disease through single-molecule *in vivo* labeling in brain tissues

Summary

The Nanoimager platform supports imaging of brain tissue at high sensitivity and the visualization of synaptic proteins at a single-molecule level after *in vivo* labeling.

This type of research enables researchers to:

- Study multiple synaptic markers in tissue sections of disease models
- Determine synaptic structures and numbers in healthy and disease-affected brain tissue
- Understand the molecular mechanisms and progression of tissue neurodegeneration
- Identify molecular events contributing to neuronal disorders and improve early diagnosis

Challenge

The human brain is a highly complex and densely packed tissue containing an estimated 86 billion neurons and a similar number of supporting cells, including astrocytes and microglia. Neuronal connections between dendritic spines and axon terminals make up over 100 trillion synapses governing crucial brain functions. Neuronal synapses are around 20-40 nm in size, making their intricate details 'invisible' to conventional light microscopy techniques.

In dense tissue environments, *in vivo* labeling can pose an additional challenge, especially when preparing samples for single-molecule super-resolution imaging. Different labeling approaches can be used for neuronal tissue *in vivo* imaging, where depth often affects light scattering. The Nanoimager provides a platform compatible with different imaging modes, facilitating the resolution of synaptic proteins at single-molecule level in brain tissue using different labeling modes.

Results

A tissue overview scan image was acquired to assess tissue integrity and identify regions or the structure of interest, in this case the hippocampus, prior to single-molecule imaging (Figure 1).

Combining automation features with super-resolution imaging allows the quick and efficient identification of ROIs to then gather localizations from thousands of synaptic proteins. It also facilitates acquisition of data on single molecule distribution, co-localization, or postsynaptic density profiles.

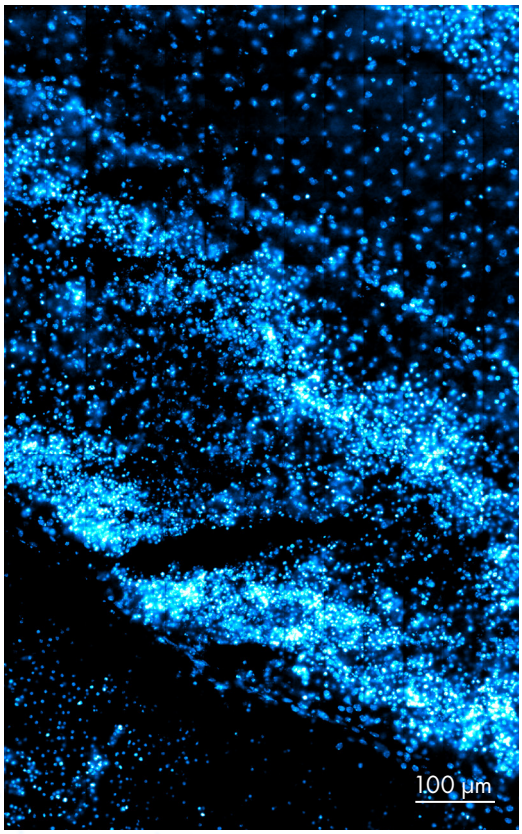

FIGURE 1

Overview scan of a mouse brain tissue section. Image of a 10 µm-thick mouse brain tissue. Nuclei were labeled with DAPI and the overview scan feature was used to identify the structure of interest, the hippocampus. Sample prepared by Robert Hinshaw, Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Boston.

In a separate experiment, comparison of the photo-activable protein PSD95-mEos2 (blue) and the labeled PSD95-HaloTag (magenta) localizations was used to validate the efficiency of HaloTag labeling *in vivo*. Figure 2 shows an example 6 µm-thick mouse brain tissue section in which the postsynaptic density component PSD95 was labeled with two separate fluorescent tags, mEos2 and Halo. The corresponding HaloTag®-PA ligand was then used to label the PSD95-Halo fusion, prior to photo-activation during PALM imaging. The co-localization of the signal proves that the use of *in vivo* HaloTag dye labels the same structures as the transgenically expressed proteins and is, therefore, a viable method for labelling neuronal tissue.

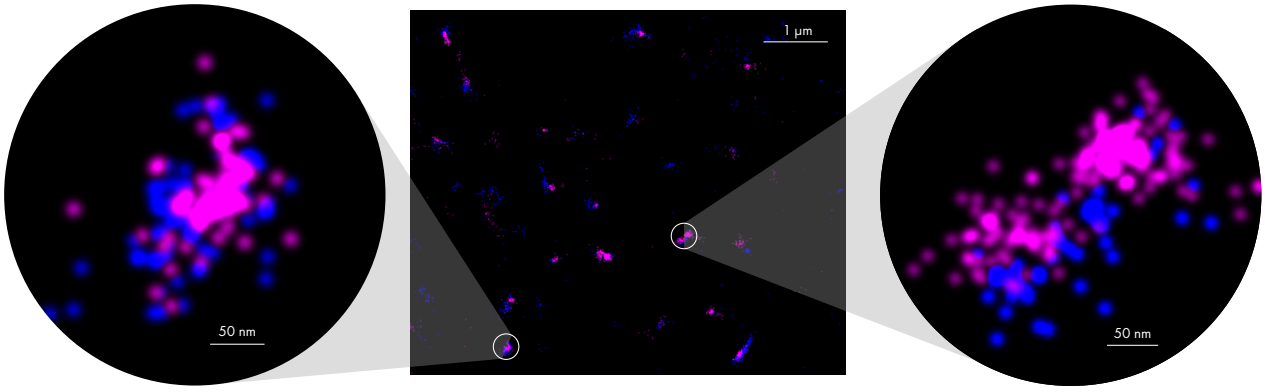

FIGURE 2

Whole mouse brain tissue labeled with two fluorophores to evaluate *in vivo* labeling efficiency. PALM image of a 6 µm-thick brain tissue section of a PSD95-mEos2/PSD95-Halo mouse (blue and magenta, respectively) labeled with HaloTag-PA Janelia Fluor® 646 dye. Two color PALM imaging was used to assess the labeling efficiency of postsynaptic protein using HaloTag *in vivo* (zoomed in regions). Sample prepared by Edita Bulovaite (Seth Grant's group) Centre for Clinical Brain Sciences, University of Edinburgh, UK.

Solution With The Nanoimager

The Nanoimager provides a solution for imaging brain tissues with high-resolution, by applying PALM imaging or *in vivo* HaloTag labeling to better characterize synaptic structures at a molecular level, and better understand brain function in health and disease. The platform provides individual real-time localizations and additional automation features that can help with multiplexing capabilities, as a tissue section can be put through iterative cycles of immuno-labelling and imaging to obtain high order number of synaptic markers. The Nanoimager enables imaging of 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.

To learn more about the microscope and ONI, please **visit www.oni.bio**

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