Capturing organelle contact sites assembly to unveil disease onset in live cells

Summary

The Nanoimager allows real-time visualization and quantification of changes at a single-molecule level.

This type of research supports investigation of:

- MAMs molecular content, assembly, distribution and behavior
- Dynamic changes in the association/dissociation of contact-associated proteins
- Membrane contact site formation changes in response to stress conditions
- Early diagnostic tools development for neurodegenerative diseases
- Mechanisms underlying early onset disease and design of novel disease prevention approaches

Challenge

Membrane contact sites between cellular organelles such as those between the ER and mitochondria, known as MAMs (Mitochondria-associated ER membranes), play crucial roles in intracellular signaling and metabolic pathways. Abnormal changes in organelle contact site formation have strong links to a number of diseases, including Alzheimer's, Parkinson's and motor neuron disease.

Investigating membrane contact sites is typically limited to fixed-cell imaging with electron microscopy, which does not provide insights into their real-time or dynamic behavior. Proteins that are involved in the formation of MAMs are also found along the entirety of the organelle membrane, making specific labelling of the contact sites challenging. The use of live single-particle tracking is a solution for real-time investigation of contact sites. With single-molecule sensitivity, the Nanoimager provides an accessible and versatile imaging platform for a comprehensive characterization of organelle contact sites in live cells.

Results

The Nanoimager enables the visualization of two fluorophores at the same time, allowing simultaneous detection of contact-associated proteins present on the ER and mitochondrial membranes. In this experiment (Figure 1), the movement of single PDZD8 proteins (in red), a molecular tethering protein connecting the ER and the mitochondria, were tracked to investigate potential interactions with proteins of the mitochondrial network in real-time (in green).

The fluidity of the ER-mitochondria interaction was visualized and assessed through dynamic changes in the contact-associated PDZD8 proteins as they moved in close proximity to the target mitochondria.

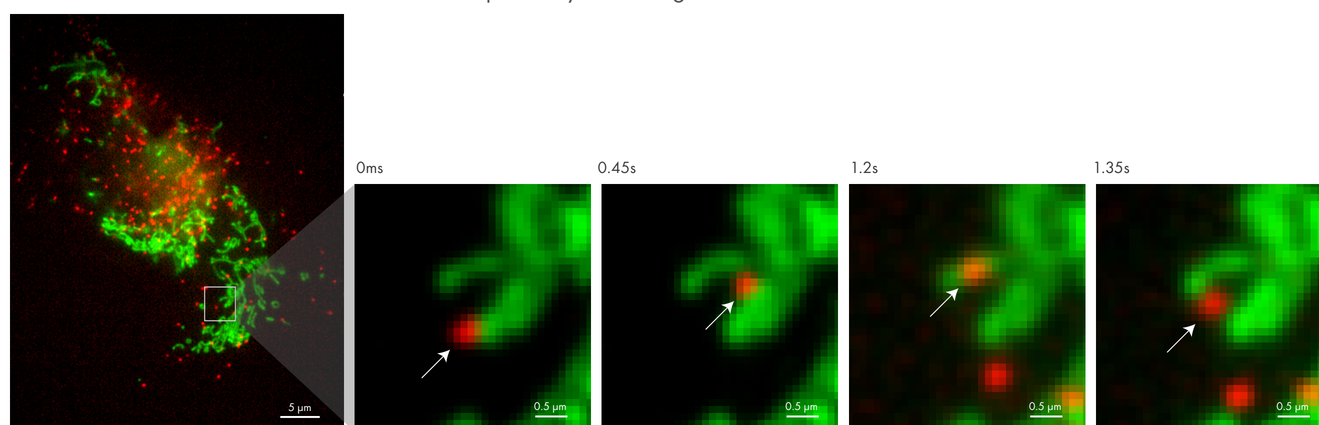
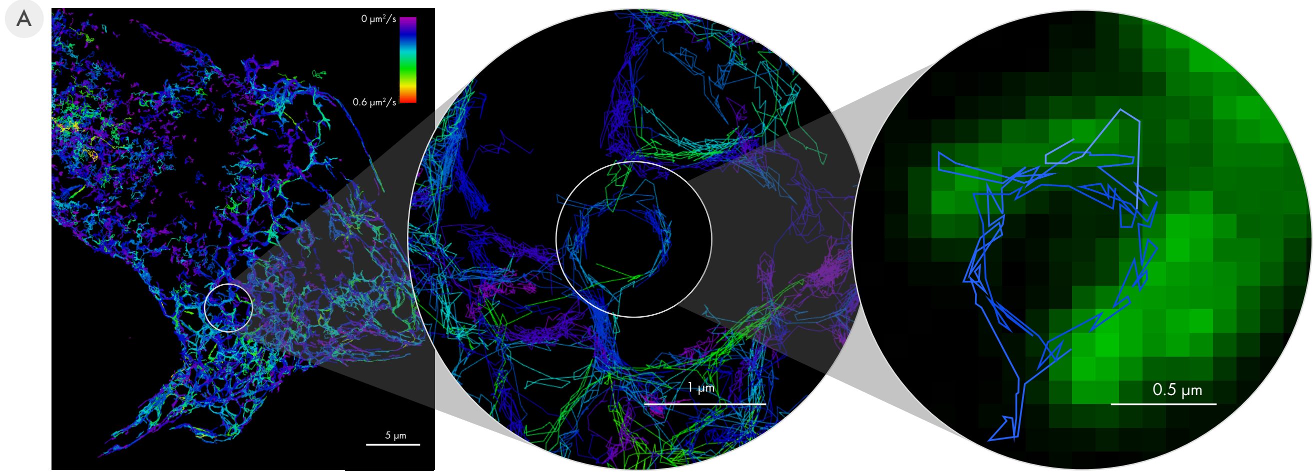


FIGURE 1
ER and MAMs (Mitochondria-associated ER membranes) protein PDZD8 (HaloTag®-PA, red) were imaged 'walking' along the ER in fibroblast cells in close vicinity of mitochondria (mitochondrial protein fused to GFP, green). Sample provided by Dr. Xufeng Wu, NIH, Bethesda, USA.

Further analysis on the diffusion of the PDZD8 molecules can reveal the differences between those closely or not at all associated with the mitochondria, providing evidence of organelle interactions at MAM sites. Traces of moving PDZD8 proteins captured in a 5 min video reveal the ER network shape. Figure 2 presents the trace of a protein from the data in Figure 1 (arrows) overlaid with fluorescently-labeled mitochondria (green) (Figure 2A, right panel). A diffusion coefficient distribution for the entire field of view shows a broad spectrum of molecular behaviors, with a high population diffusing at approximately 0.08μm^2/sec (B).



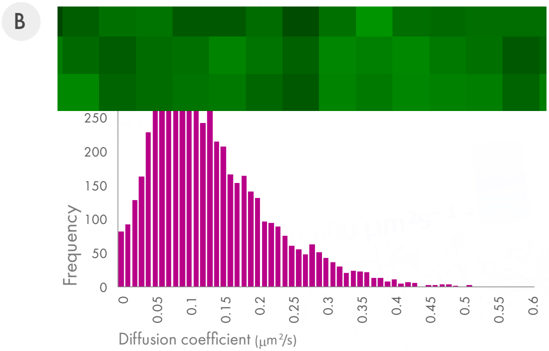


FIGURE 2
Tracking of the MAM protein PDZD8 in fibroblast cells. (A) Individual protein traces were colored according to their diffusion coefficients. Panel on the right shows a selected trace overlaid with a widefield fluorescence signal from mitochondria (green). (B) Diffusion coefficient graph of the PDZD8 proteins tracked in the analyzed cell.

Solution With The Nanoimager

Understanding the mechanisms underlying membrane contact sites formation and dysfunction between the ER as well as other organelles enhances development of better, more accurate and timely treatments for life-changing diseases. The Nanoimager provides a comprehensive solution to develop diagnostic tools and better understand organelle contact sites as a potential therapeutic target for neurodegenerative disease onset, including Alzheimer's, Parkinson's and motor neuron disease.

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

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