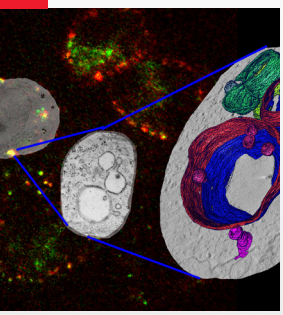
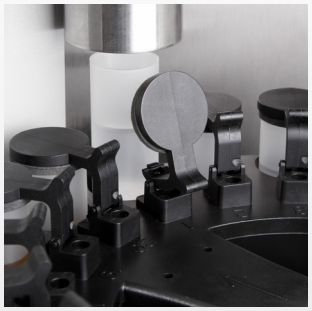
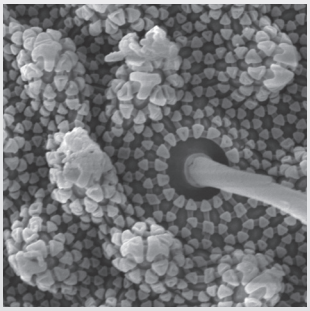
Leica
MICROSYSTEMS



Material
Research





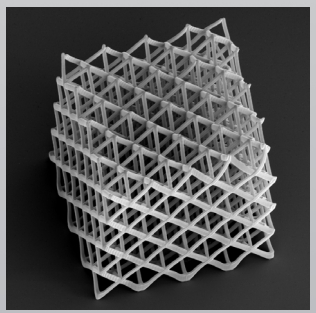
Life Science
Research

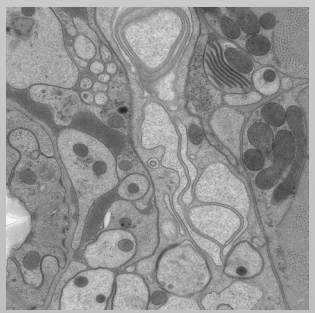
Application Note

Visualization of membrane dynamics with millisecond temporal resolution

related instruments Leica EM ICE, Leica EM AFS2

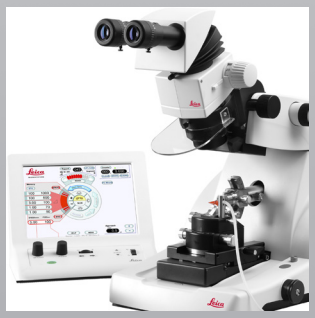
Medical
Research





Industrial
Manufacturing

Natural
Resources



Visualization of membrane dynamics with millisecond temporal resolution

Electrical stimulation of neurons combined with high-pressure freezing allows physiological activation of synaptic activity and precise control over the time frame of the induced synaptic activity.

Mouse hippocampal neurons were stimulated by electrical impulse in the high pressure chamber by applying a single, continuous light pulse of 7 ms. This light pulse discharges the electricity stored in the middle plate/PCB (Fig.1) triggering an action potential throughout the sample. The sample was frozen at the end of the light pulse. This experiment successfully captured fusing vesicles in mouse hippocampal neurons (Fig. 2).

The hippocampal neurons were cultured on sapphire disks. Details on how to prepare the hippocampal culture and the sapphire disk for this type of experiment can be found in the publication Watanabe et.al, 2016:Front. Synaptic Neurosci. http://journal.frontiersin.org/article/10.3389/fnsyn.2016.00024/full).

The 6 mm sapphire disk with the cultured neurons was placed in the recess of the middle plate (PCB). A spacer ring (100 µm thickness) was carefully placed on the sapphire disk so that cells are not damaged when the assembly with another sapphire disk is completed. To ensure stability of the assembly a cover ring was placed on top of the second sapphire. It is critical all parts to be non-conductive in this assembly (Fig.3).

Once the sample is frozen it is transferred to precooled acetone (-90°C) in the automated freeze substitution unit (Leica EM AFS2). The freeze-substitution was carried out in a mixture of 1% osmium tetroxide, 1% glutaraldehyde and 1% milliQ water in anhydrous acetone. Samples were kept 5-30 hours at -90ºC, 5ºC/hour to -20ºC, 12-16 hours at -20ºC, 10ºC/hour to 20ºC. Once the freeze-substitution was completed, specimens were embedded in Epon/Araldite resin (Detailed description of the procedure can be found in Watanabe et.al, 2016:Front. Synaptic Neurosci. ttp://journal.frontiersin.org/article/10.3389/fnsyn.2016.00024/full).

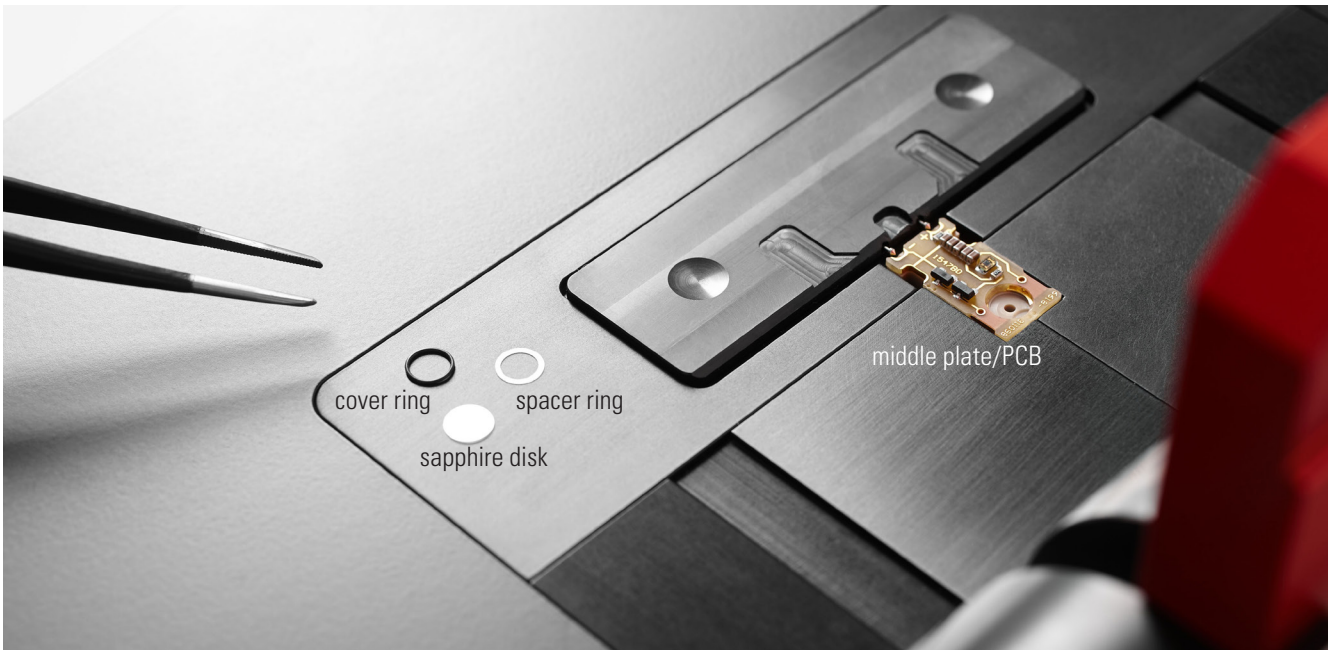
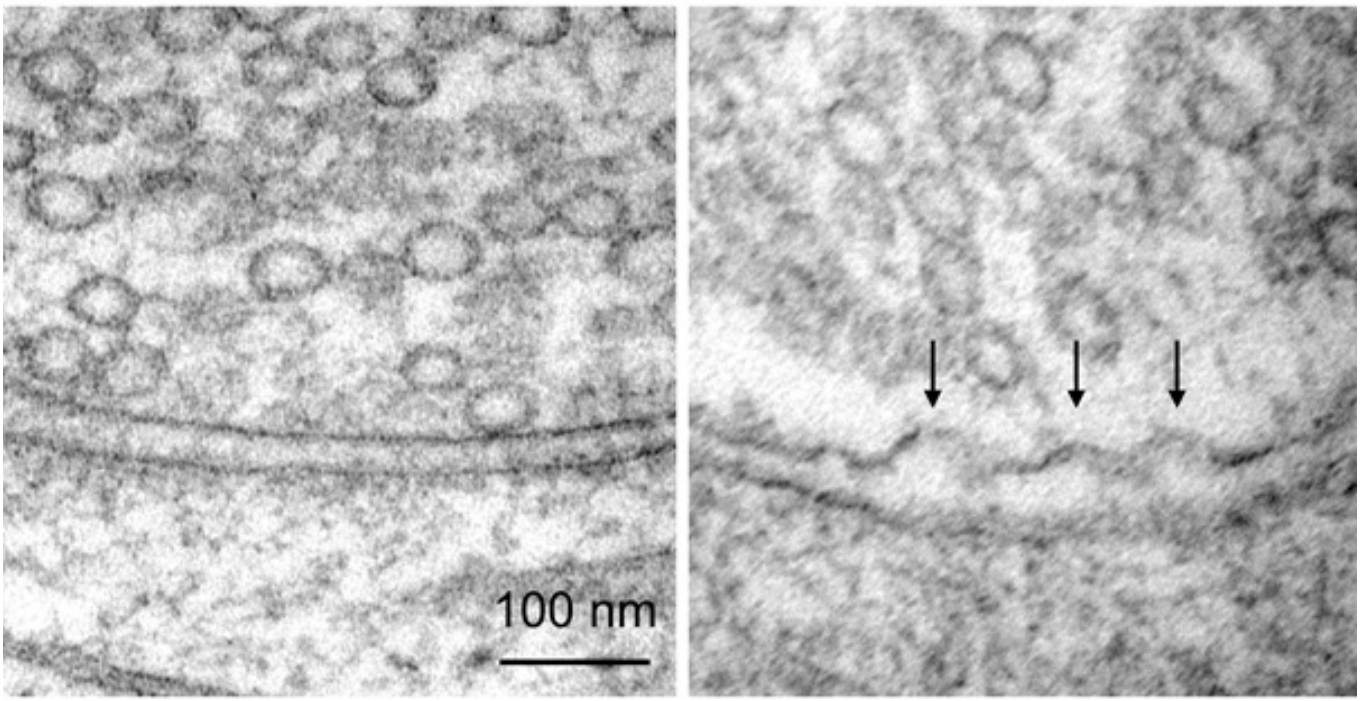


Fig. 1. Middle plate/PCB, ready to be charged in the loading station of Leica EM ICE



Mouse Hippocampal Neurons, Dr. Shigeki Watanabe, Johns Hopkins University

Fig.2. Micrographs showing the unstimulated control (left) and the stimulated mouse hippocampal neurons (right). A single pulse of 7 ms is applied and specimen frozen at the end of the pulse.

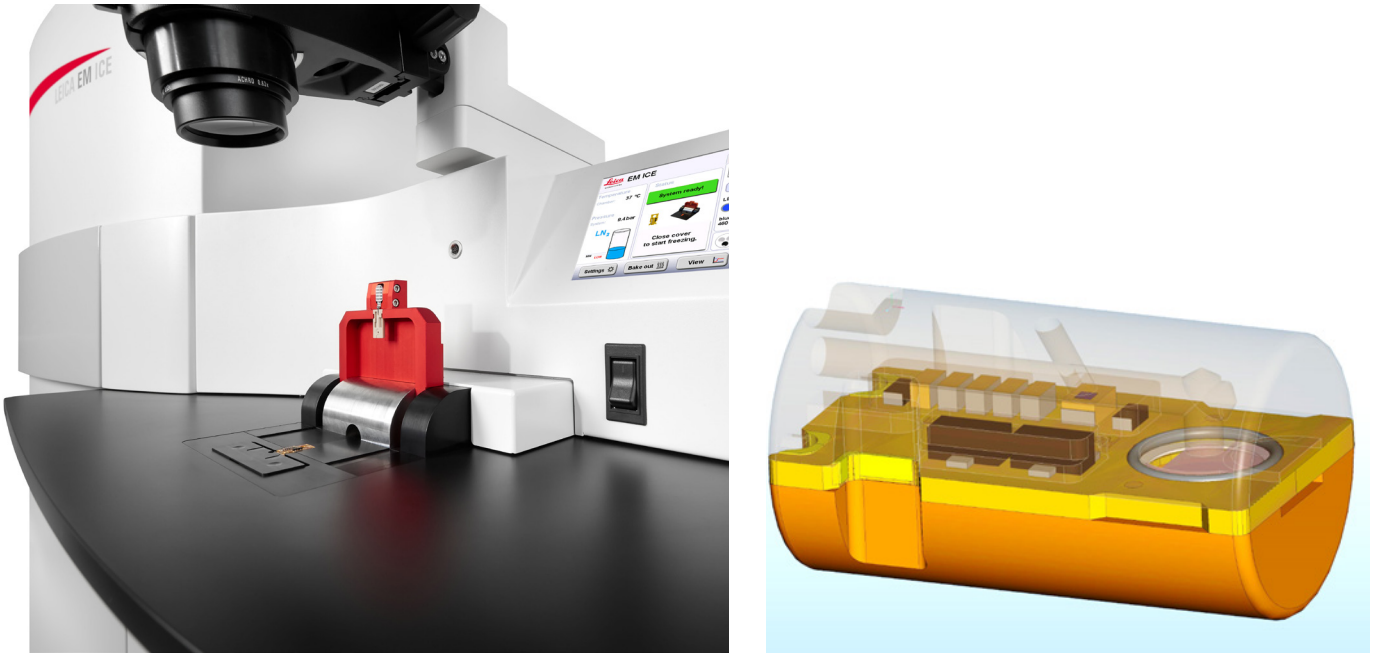


Fig.3. Assembly of two half cylinders and a middle plate/PCB for electrical stimulation in the Leica EM ICE

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RELATED PRODUCTS

Leica EM ICE

Leica EM AFS2

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