APPLICATION NOTE

AX R MP/A1R MP+ Multiphoton Confocal Microscope

In vivo Deep Imaging of Mouse Brain using Novel Fluoropolymer Nanosheet

A research group led by Professor Tomomi Nemoto, director of the Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences, and Professor Yosuke Okamura, School of Engineering, Tokai University reported their techniques for visualization of neurons in deep regions of a living mouse brain using novel polymer thin film (nanosheet). This application note introduces the results obtained using the A1R MP+ multiphoton confocal microscope, which enables observation of living brain tissue.

Research background

In the field of neuroscience, understanding the function of neural networks that connect multiple areas of the brain is very important. For high- resolution imaging of local neural circuits in a living mouse brain using a two-photon fluorescence microscope, the "open skull method" is commonly used, in which a hole is made in a part of the skull and covered with a glass coverslip to create a cranial window for optical imaging. However, unexpected bleeding is inevitable during open skull method surgery and decreases the fluorescent signal intensity in the living mouse brain due to the light absorption of hemoglobin contained in the blood. In addition, the size of the cranial window is limited to a maximum of approximately 6 mm in diameter because the hard, flat glass coverslip presses against the brain tissue.
To solve the above problems, Prof. Nemoto and his research group developed a method utilizing a novel polymer thin film (nanosheet) for the open skull method and *in vivo* imaging of the mouse brain.

Large cranial window of living mouse brain utilizing a PEO-CYTOP nanosheet

Nanosheet refers to a thin film composed of a polymer material with a thickness of about 100 nm, and is known to have unique properties such as high flexibility, optical transparency and high adhesion.
The research group led by Prof. Nemoto have developed a PEO-CYTOP nanosheet, in which the surface of CYTOP, a highly light-transparent fluoropolymer, is chemically modified with polyethylene oxide (PEO). The addition of PEO to the adhesive surface with the brain realized the hydrophilization of the adhesive surface and the high adhesion of PEO- CYTOP nanosheets to living brain tissue.
Next, a PEO-CYTOP nanosheet was used as the sealing material of a cranial window in the open skull method instead of a conventional glass coverslip. Due to its high adhesiveness, the PEO-CYTOP nanosheet was able to suppress bleeding from the brain. By taking advantage of the flexibility of the PEO-CYTOP nanosheet, it was also possible to create a large cranial window covering the whole parietal region of a living mouse (Fig. 1).

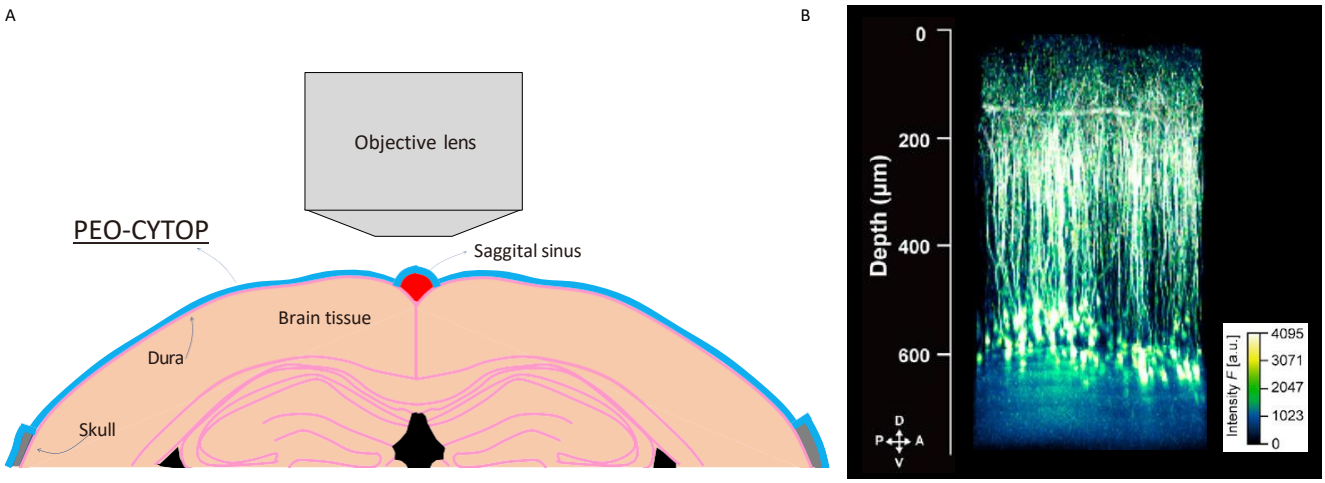

Fig. 1 A: Scheme of large cranial window using a PEO-CYTOP nanosheet **B**: *In vivo* two-photon imaging of mouse brain using a PEO-CYTOP nanosheet

In vivo two-photon imaging of deep region using a PEO-CYTOP nanosheet

The research group led by Prof. Nemoto verified the cranial window using PEO-CYTOP nanosheet by *in vivo* imaging in a mouse. In order to perform morphological imaging of neurons, a Thy1-EYFP transgenic mouse that expresses the EYFP fluorescent protein in pyramidal cells in the fifth layer of the cerebral cortex was used.

Fig. 2A shows a cranial window using PEO-CYTOP nanosheets. Through this cranial window, *in vivo* imaging using a two-photon fluorescence microscope was conducted, and imaging over a wide area of neurons in a deep region was performed. As a result, the curved shape of the brain surface was visualized through the cranial window using the PEO-CYTOP nanosheet, confirming that it was not pressing against the brain surface (Fig. 2C). The axons, dendrites (Fig. 2D, E), and cell bodies (Fig. 2F) of neurons were also visualized in a vast field of view. As a notable result of deep-area imaging, the cranial window using a PEO-CYTOP nanosheet also able to visualize the cell bodies of neurons in layer 6 of the cerebral cortex (approximately 700 µm). These results indicate that *in vivo* two- photon imaging with a vast field of view and in deep region of the mouse brain can be performed by using PEO-CYTOP nanosheets as the sealing material of a cranial window.

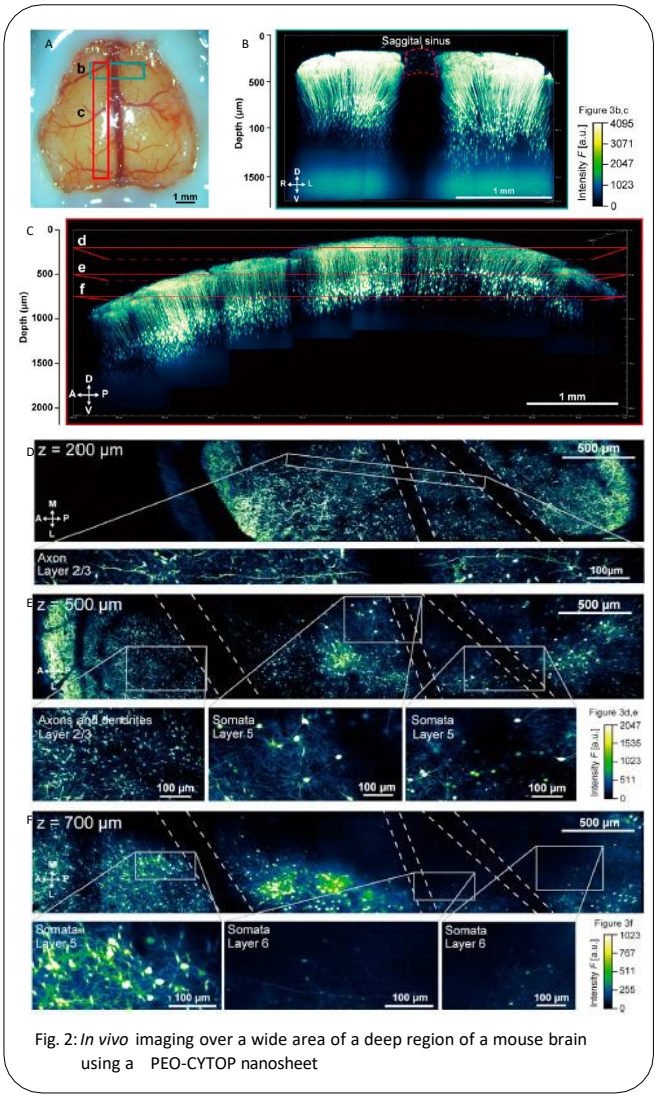

Fig. 2: *In vivo* imaging over a wide area of a deep region of a mouse brain using a PEO-CYTOP nanosheet

Long-term imaging using a PEO-CYTOP nanosheet

Finally, the research group led by Prof. Nemoto evaluated whether PEO- CYTOP nanosheets could be used after removal of the dura mater in the brain for a long-term period. In this evaluation, PEO-CYTOP nanosheets were adhered to the brain surface after removing the skull and dura mater. To protect the brain and enable long-term observation, a glass coverslip was fixed to the PEO-CYTOP nanosheet. As a result, it was confirmed that there was no bleeding even after 14 weeks after surgery. Furthermore, the same depth of neurons and axons could be observed when comparing the results at 1 day and 14 weeks after surgery. Therefore, the PEO-CYTOP nanosheets functioned as a sealing material, even after the removal of the dura.

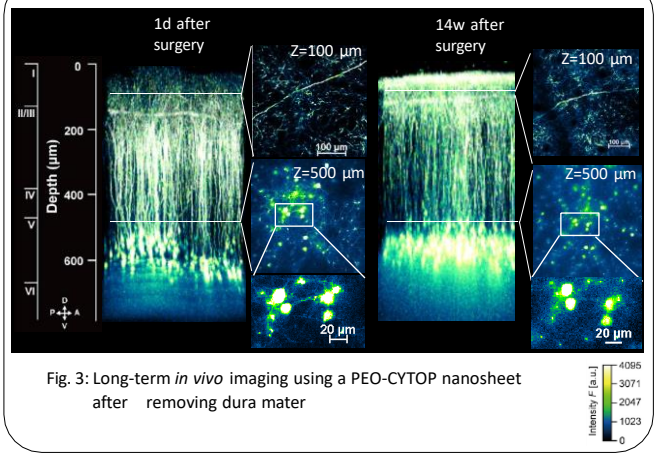

Fig. 3: Long-term *in vivo* imaging using a PEO-CYTOP nanosheet after removing dura mater

Summary

PEO-CYTOP nanosheets can be used as the sealing material of a cranial window in the open skull method, realizing *in vivo* deep brain imaging over a vast field of view.

This new technology improves surgical techniques essential for the observation of living mouse brains, and enables expansion of the optically observable area. Therefore, it is expected to contribute to the investigation of functional interrelationships among multiple brain regions.

In addition, by applying PEO-CYTOP nanosheets to in vivo imaging of the mouse brain, there is an advantage in observing whole brain regions of disease model animals and drug-administered animals in pharmacokinetic studies at the discovery stage of new drugs.

This imaging technology can meet the needs of observing the activities of all neurons over a longer time and over a wider area.

Reference

Taiga Takahashi, Hong Zhang, Ryosuke Kawakami, Kenji Yarinome, Masakazu Agetsuma, Junichi Nabekura, Kohei Otomo,Yosuke Okamura, Tomomi Nemoto PEO-CYTOP Fluoropolymer Nanosheets as a Novel Open-Skull Window for Imaging of the Living Mouse Brain, iScience 23, 101579, October 23, 2020

Product Information

AX R MP Multiphoton Confocal Microscope

Achieves deep imaging with a large field of view, high speed and high resolution. Ensures a large space for flexible sample placement.

- Large FOV: 22 mm field of view
- High speed:max. 720 fps (2048 x 16 pixels)/ resonant
- High resolution: max. 8K pixels/Galvano, max. 2K pixels/resonant