Novel fluoropolymer nanosheet "PEO-CYTOP" realizing *in vivo* two-photon deep and wide-field imaging of living mouse brain

Taiga Takahashi[1,2,3], Hong Zhang[4], Ryosuke Kawakami[5], Kenji Yarinome[6], Yosuke Okamura[4,6] and Tomomi Nemoto[1,2,3,7]

([1] Research Institute for Electronic Science, Hokkaido University, Hokkaido, Japan [2] Graduate School of Information Science and Technology Hokkaido University, Hokkaido, Japan [3] Exploratory Research Center on Life and Living Systems; National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan [4] Department of Applied Chemistry, School of Engineering; Micro/Nano Technology Center, Tokai University, Kanagawa, Japan [5] Graduate School of Medicine Ehime University, Ehime, Japan [6] Graduate School of Engineering, Tokai University, Kanagawa, Japan [7] School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Okazaki, Japan) E-mail: tttaiga_at_nips.ac.jp

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In the field of neuroscience, *in vivo* two-photon microscopy has become widely used to reveal the anatomical and functional connectivity based on abundant neurons. For deep imaging of the living mouse brain with a broad field of view, "open skull method" is usually required for making transparent observation windows by removing the skull bone and sealing the hole with a glass coverslip. However, this operation frequently caused bleeding and injured the brain tissue, resulting in limitation of the observable area. On the other hands, polymer thin films, also known as nanosheets, have been proposed as novel materials for surgical applications to suppress bleeding and inflammation in living animals[1]. Recently, fluoropolymer CYTOP nanosheet had been developed for the use of bioimaging[2][3].

Here, we proposed a novel observation technique that utilizes newly developed nanosheet "PEO-CYTOP" as a sealing material for the surface of living mouse brains [T. Takahashi, *et al.*, *in submitted*]. PEO-CYTOP is composed of the amorphous fluoropolymer CYTOP layer (thickness: ~130 nm) and polydimethylsiloxane layer (thickness: ~5 nm), the surface of which was hydrophilized by attaching polyethene oxide. This nanosheet showed excellent water-retention effect and strong adhesiveness, which was suitable to suppress bleeding from the brain surface. Moreover, the optical disturbance by PEO-CYTOP nanosheet was negligible, since the thickness of PEO-CYTOP (~135 nm) was smaller than conventional glass coverslip (~170 μm) and the wavelength of the visible light.

By taking advantages of its adhesiveness, transparency and flexibility, we successfully achieved *in vivo* deep imaging in living mouse brains with a broad field of view. We made a sizeable cranial window that was tightly sealed with PEO-CYTOP nanosheet, covering most of the surface of the parietal region. Through this window, wide-field time-lapse *in vivo* Ca^{2+} imaging was archived. Also, we achieved *in vivo* deep imaging of the prefrontal cortex via the cranial window of PEO-CYTOP nanosheet. This improvement will contribute to the elucidation of neural functions resulting from long-time interactions among multiple regions.

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