

Miniature Two-Photon Microscopy for Freely Moving Animals

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Abstract

Here we present the FHIRM-TPM 3.0, a 2.6 g miniature two-photon microscope capable of multicolor deep-brain imaging in freely behaving mice. The system was integrated with a broadband anti-resonant hollow-core fiber featuring low transmission loss, minimal dispersion from 700-1060 nm, and high tolerance of laser power. By correcting chromatic and spherical aberrations and optimizing the fluorescence collection aperture, we achieved cortical neuronal imaging at depths exceeding 820 μm and, using a GRIN lens, hippocampal Ca^{2+} imaging at single dendritic spine resolution. Moreover, we engineered three interchangeable parfocal objectives, allowing for a tenfold scalable field-of-view up to $1 \times 0.8 \text{ mm}^2$, with lateral resolutions ranging from 0.68 to 1.46 μm . By multicolor imaging at excitation wavelengths of 780 nm, 920 nm and 1030 nm, we investigated mitochondrial and cytosolic Ca^{2+} activities relative to the deposition of amyloid plaques in the cortex of awake APP/PS1 transgenic mice. Thus, the FHIRM-TPM 3.0 provides a versatile imaging system suitable for diverse brain imaging scenarios.

Keywords :

Two-photon microscopy, Miniature, Freely behaving mice, Neuroimaging, Neuroscience

Short biography

Runlong Wu, Ph.D., is a professor and doctoral advisor at Beijing Information Science and Technology University. He received his Ph.D. in Engineering from Peking University.

Dr. Wu's research focuses on the development of advanced in vivo imaging technologies. His team has developed a series of miniaturized two-photon microscopes and was among the first to achieve stable, high-resolution imaging of neurons and synapses in the brains of freely behaving mice. These efforts helped establish a new paradigm for imaging in unrestrained animals. His contributions were recognized among China's Top 10 Scientific Advances of 2017 and contributed to the selection of "Imaging in freely behaving animals" as *Nature Methods*' 2018 Method of the Year.

He has co-authored over 20 publications in leading journals such as *Nature Methods*, *Nature Cell Biology*, and *Nature Biotechnology*. Four of these have been designated ESI Highly Cited Papers (top 1%), with total citations exceeding 1,000.

