The high-performance miniature two-photon microscopy enabled long-term and multi-site brain imaging in free-moving animals.

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Recent developments in miniaturized microscopes have furthered the quest to visualize brain activities and structural dynamics in freely moving animals engaged in self-determined behaviors. Recently, we have published a fast, high-resolution, miniaturized two-photon microscope (**FHIRM-TPM**) weighing 2.15g and capable of imaging commonly used biosensors at high spatiotemporal resolution [1].

Here we have designed an upgrade of our previous miniature two-photon microscope, named **FHIRM-TPM 2.0**. By optimizing the whole optics with new customized high-NA miniature objective and scan lens, the **FHIRM-TPM 2.0** has larger field-of-view, deeper working distance with chronic glass window compensation. Moreover, we have integrated a miniature Z-scanning module in the headpiece of the microscope in order to do the real-time focusing and 3D imaging.

With **FHIRM-TPM 2.0**, we simultaneously recorded more than 100 neurons` activity in the V1 cortex of the mouse. Combined with a GRIN lens, we acquired single-dendrite-resolution imaging of neurons in the hippocampus when the mouse was freely moving. Furthermore, by mounting two headpieces in one rat`s head, we first time achieved dual-site neuronal activity imaging (V1 cortex and prefrontal cortex) in free-moving rats.

We anticipate that this new version of FHIRM-TPM 2.0 will provide a new generation of miniaturized, high-performance imaging tools for biologists in general and neuroscientists in particular to image structural and functional dynamics in freely-behaving animals over multiple spatial and temporal scales.

1. W. Zong, R. Wu, M. Li, Y. Hu, Y. Li, J. Li, H. Rong, H. Wu, Y. Xu, Y. Lu, H. Jia, M. Fan, Z. Zhou, Y. Zhang, A. Wang, L. Chen, and H. Cheng, "Fast high-resolution miniature two-photon microscopy for brain imaging in freely behaving mice," Nat Methods (2017).