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.