Supplemental Document

Twist-free ultralight two-photon fiberscope enabling neuroimaging on freely rotating/walking mice: supplement

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Twist-free ultralight two-photon fiberscope enabling neuroimaging on freely rotating/walking mice

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Table S1. Survey of off-the-shelf single mode fiber-optic rotary joints

Vendor	Model	Loss (dB)	Rotary Variation (dB)	Rotary Variation (%)	Reference
Princetel	R Series	<2	±0.25	11	link
SPINNER	1.14	<1.5	1	20	link
MERIDIAN Lab	Single Channel	<1	0.5	11	link
MOOG	FO206	<1.5	0.5	11	link
Grand	CHG007-1	<3	1	11	link
SENRING	FO100	1.2	0.6	13	link

Table S2. List of mice involved in this work

Mouse #	Fiberscope type	Experiment type	Total recoding time	Figure contributions
1	II	Freely walking somatic imaging	3000 s	Fig. 2; Fig. 5; Fig. 6; Fig. 7
2	II	Freely walking somatic imaging	4333 s	Fig. 6; Fig. 7
3	II	Freely walking somatic imaging	3333 s	Fig. 6; Fig. 7
4	I	Freely walking somatic imaging	2500 s	Fig. 1; Fig. 4
5	I	Freely walking dendritic imaging	1333 s	Fig. 4
6	II	Head fixed somatic imaging	666 s	Fig.3
7, 8 and 9	II	Free/Tethered behavior recording	666 s, 666 s, and 666s	Fig. S3

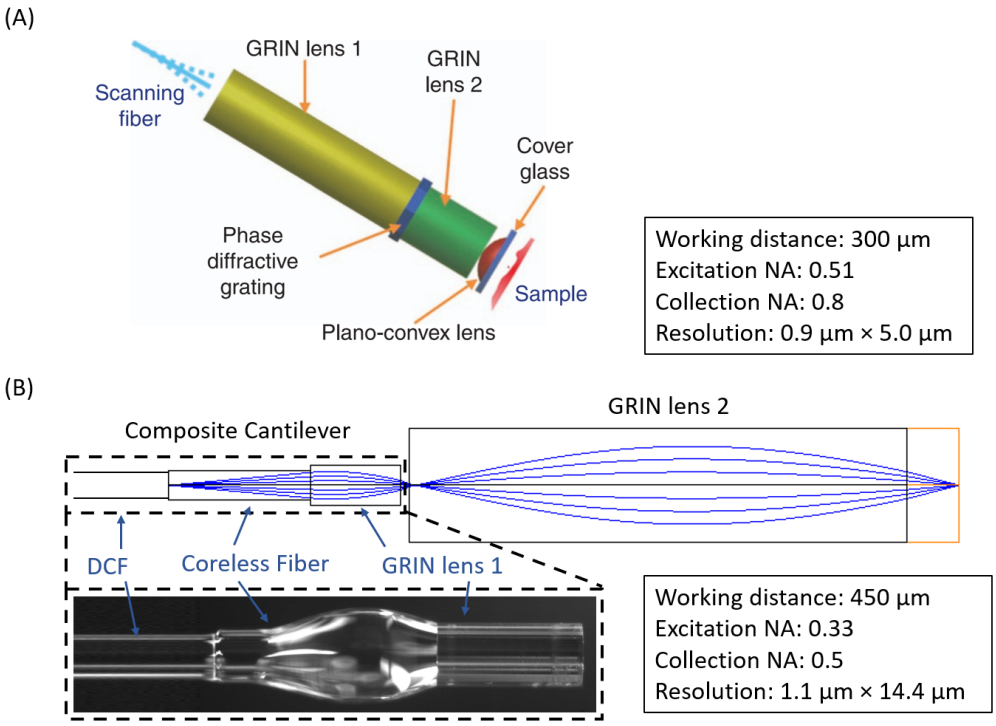

Fig. S1 Type I and II fiberscope design. (A) Design of type I fiberscope. A phase diffractive grating is sandwiched between two GRIN elements. The first GRIN element (~1/4 pitch) collimates the light from the DCF core, and the second GRIN element (<1/4 pitch) pre-focuses the light before it enters a high NA plano-convex lens for tight focusing. All the glass elements are encapsulated and fixed within a protective stainless-steel tube. (B) Design of type II fiberscope. This design is based on the composite cantilever [1], the light from the composite cantilever gets directly focused to tissue via a half-pitch GRIN lens 2 (NEM-100-25-10-860-S, GRINTECH). DCF: Double Clad Fiber.

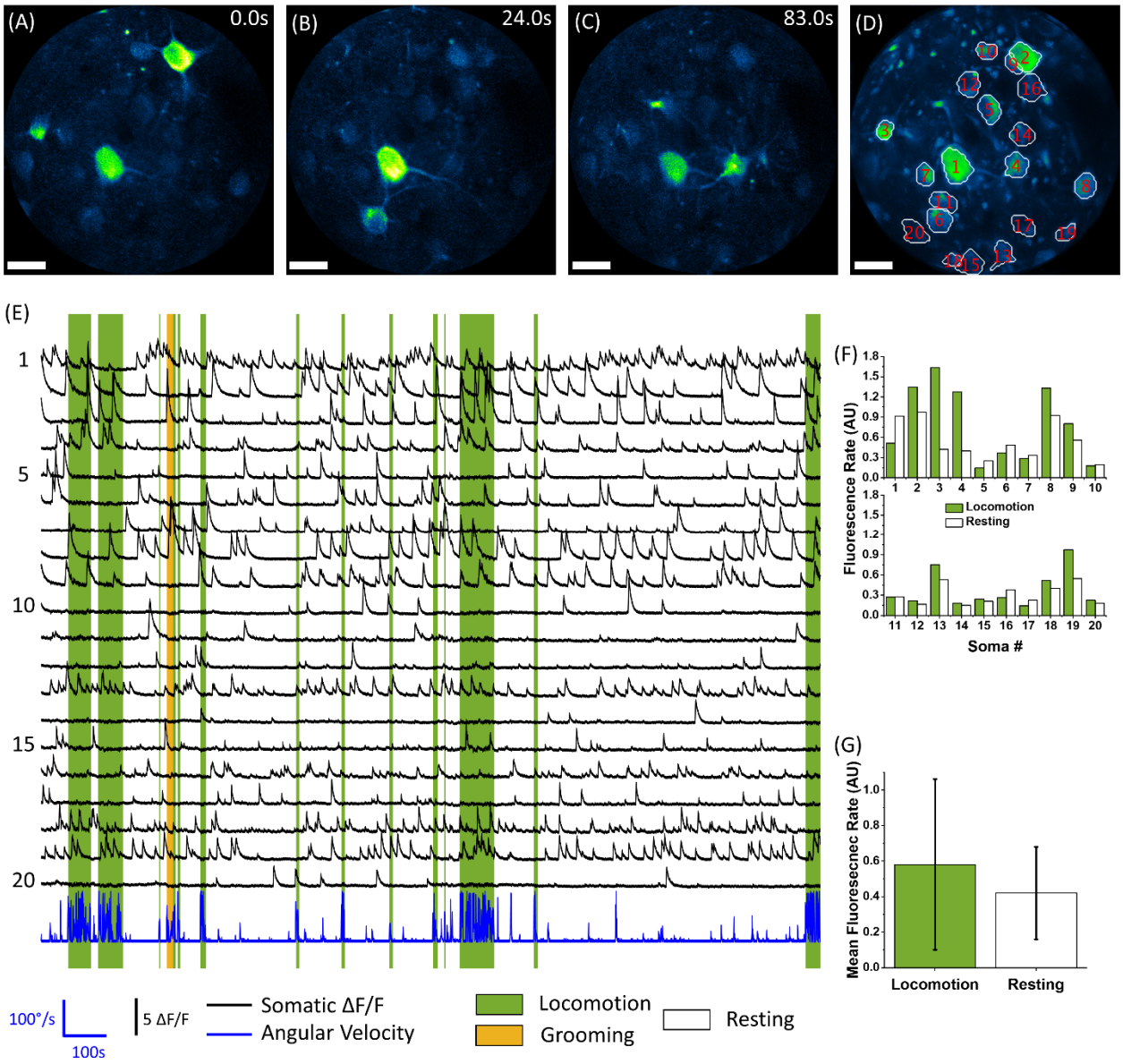

Fig. S2. Type I fiberscope somatic data processing results. (A)-(C) Representative time-series images showing different neurons and their processes activated at different times. (D) Segmentation masks of the 20 neurons overlaid on the max intensity projection of the dataset. (E) Extracted ΔF/F of the 20 neurons along with the angular velocity trace and behavior labelling. (F) Fluorescence rates of the 20 neurons during periods of locomotion and resting. (G) Mean fluorescence rates of the 20-neuron-ensamble during periods of locomotion and resting. Error bar: standard deviation of the fluorescence rates. Scale bar in (A-D): 20 µm. Somatic imaging data with simultaneous behavioral recording available in Visualization 1.

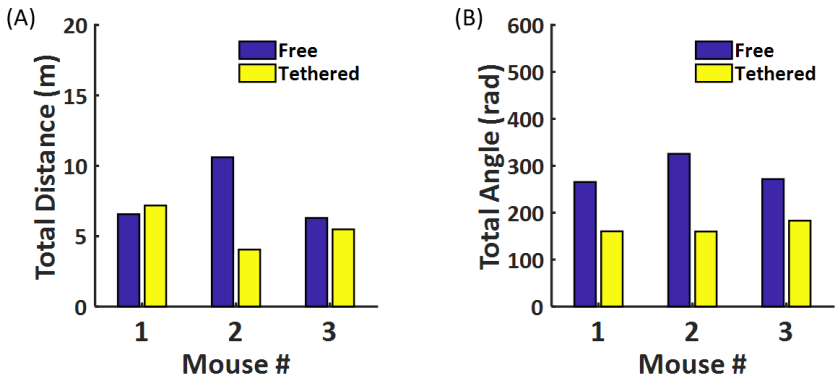

Fig. S3. Behavior of three mice untethered (free) vs. tethered (OEC on). (A) Total distance traversed of three mice over 333 seconds when untethered (free) and tethered (OEC on). (B) Total angular movements of the same three mice over 333 seconds when untethered (free) and tethered (OEC on). The behavior of three mice (with cranial window and head restraining bar) was recorded tether-free on the first day and tethered (with OEC on) on the second day. The three mice showed a 29% reduction in distance traversed and a 41% reduction in angular movement on average when tethered.

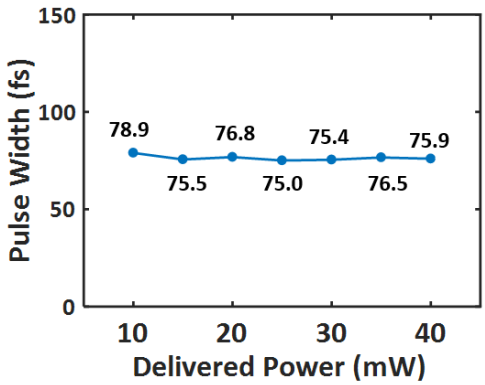

Fig. S4. GRISM-delivered pulse width vs. delivered power. The GRISM delivered consistent ~76fs pulses over the 10-40 mW power range.

Reference

1. W. Liang, H.-C. Park, K. Li, A. Li, D. Chen, H. Guan, Y. Yue, Y.-T. A. Gau, D. E. Bergles, M.-J. Li, and X. D. Li, "Throughput-speed Product Augmentation for Scanning Fiber-optic Two-photon Endomicroscopy," IEEE Transactions on Medical Imaging **39**, 3779-3787 (2020).