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Supplementary Information for

Ultrafast Optical Clearing Method for Three-dimensional Imaging with Cellular Resolution

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Captions for Movies S1 to S4

Other supplementary materials for this manuscript include the following:

Movies S1 to S4

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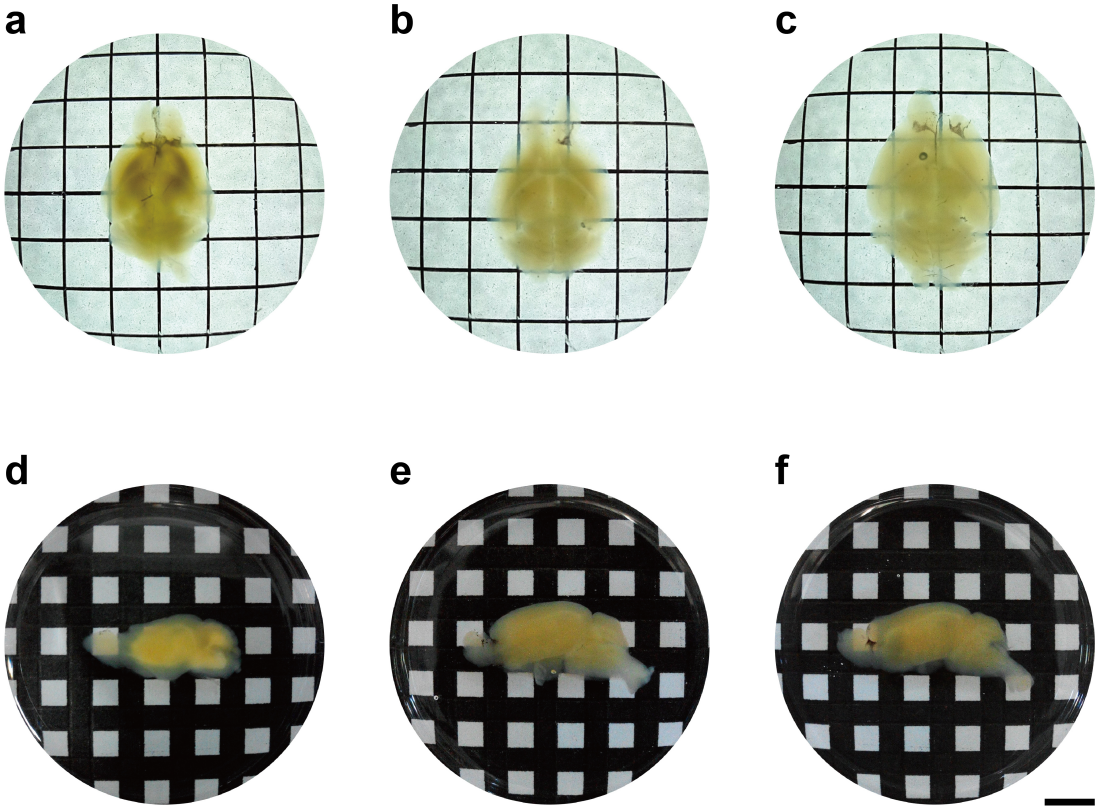

Fig. S1. Development of FOCM by reagent concentration screening. (a) Whole brain treatment with 32% urea, 58% DMSO and 1% TritonX-100 for 48 h. (b) Whole brain treatment with 35% urea, 64% DMSO and 1% TritonX-100 for 48 h. (c) Whole brain treatment with 35% urea and 65% DMSO for 48 h. (d) Hemisphere treatment with 35% sorbitol, 15%urea and 5% glycerol dissolving in DMSO. (e) Hemisphere treatment with 30% sorbitol, 20%urea and 1% glycerol dissolving in DMSO. (f) Hemisphere treatment with 30% sorbitol, 20%urea and 3% glycerol dissolving in DMSO. Scale bar represents 5 mm.

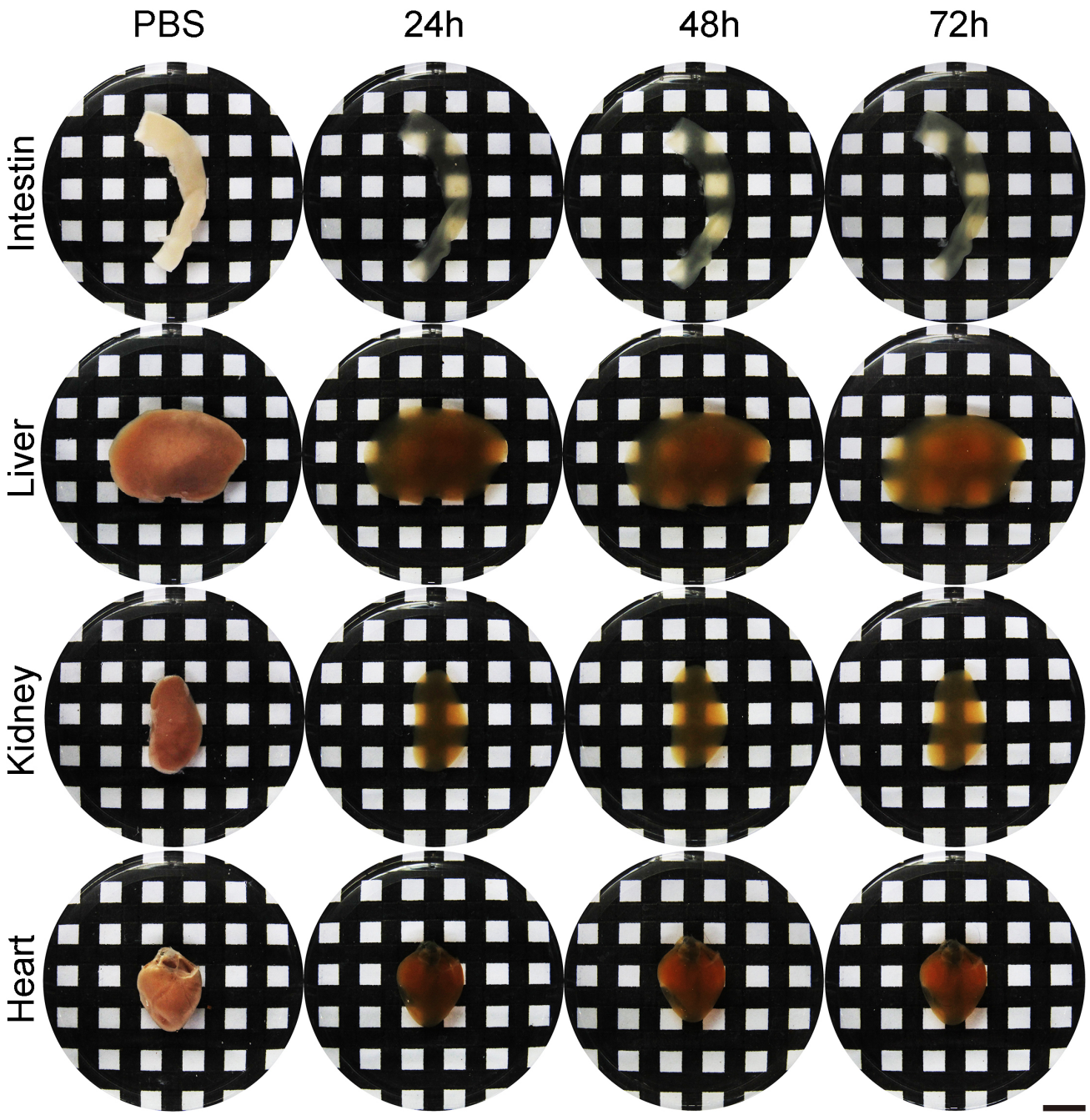

Fig. S2. Rapid organs clearing with FOCM. Rapid clearing of organs including intestine, liver, kidney and heart within 72 hours in an adult C57BL/6 mouse (9 weeks old). Scale bar represents 5 mm.

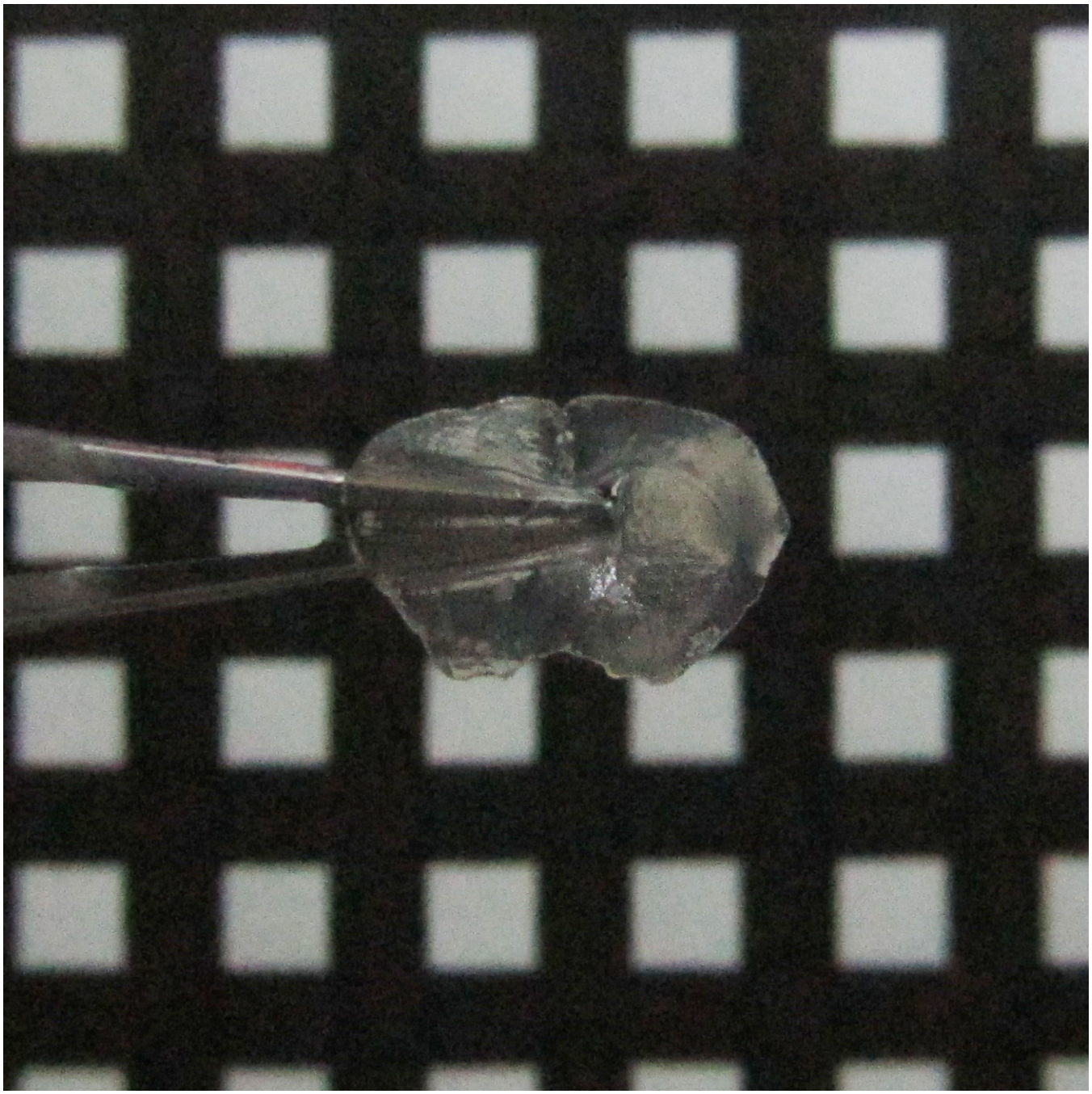

Fig. S3. The 200-μm-thick brain slice cleared by FOCM became firm enough to be moved by tweezers without any distortion.

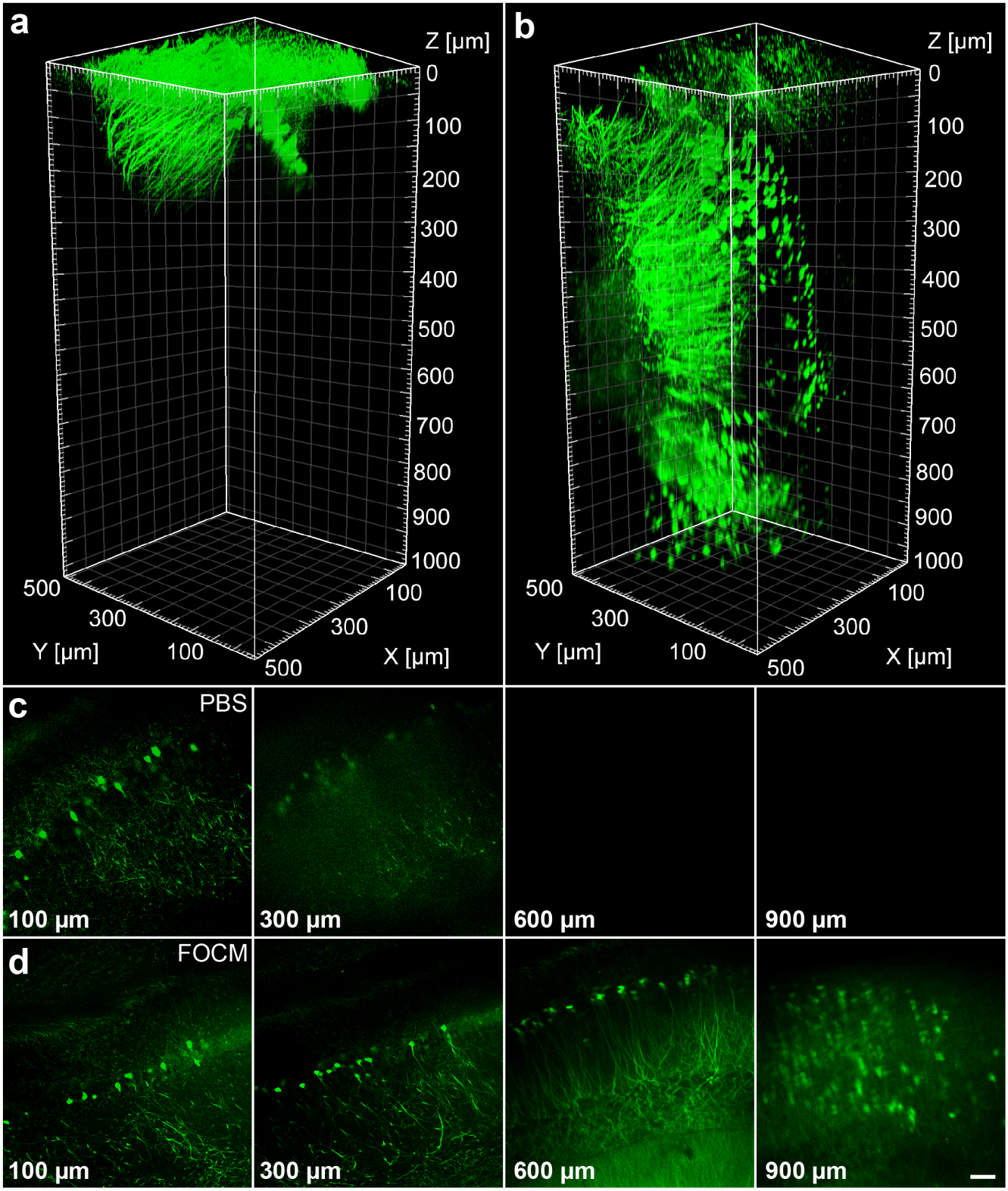

Fig. S4. FOCM improved imaging depth of two-photon excitation microscopy. (a) The reconstruction with two-photon excitation microscopy before FOCM clearing. (b) The reconstruction after FOCM clearing. (c) Selected single imaging with two-photon microscopy at the depth of 100 μm, 300 μm, 600 μm, 900 μm in Z-axis before clearing. (d) Selected imaging with two-photon microscopy after clearing. Scale bar represents 50 μm.

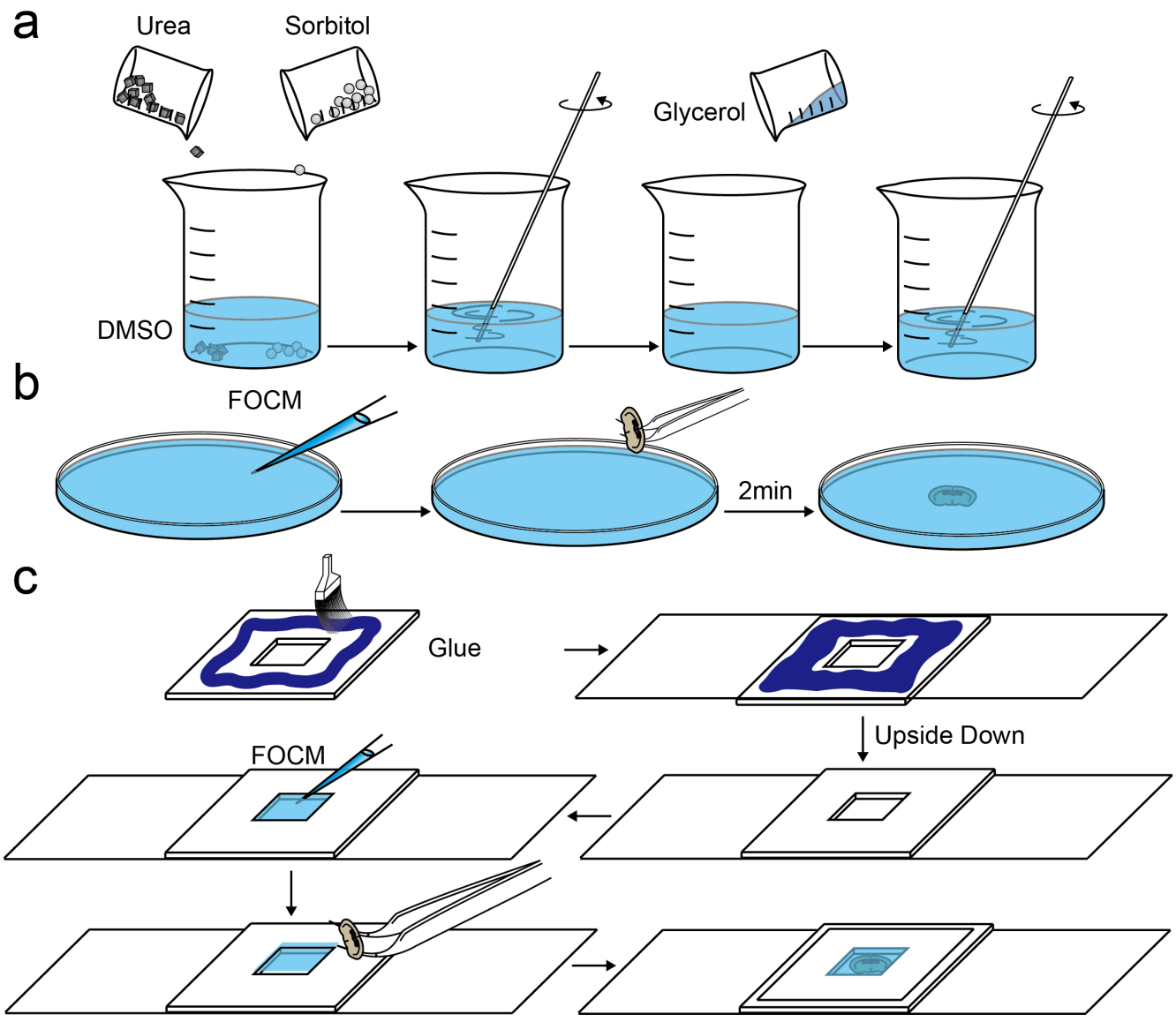

Fig. S5. Schematic diagram of sample clearing process with FOCM. (a) Reagents preparation of FOCM. (b) Biological sample clearing process. (c) Sample mounting procedure before optical imaging.

Movie S1. FOCM realized a fast optical clearing within 2 minutes.

Movie S2. 3D visualization of C57BL/6 mouse brain slices immunostained for astrocyte.

Movie S3. 3D visualization of C57BL/6 mouse brain slices immunostained for microglia.

Movie S4. 3D visualization of Thy1-GFP-M mouse brain slices immunostained for the blood vessel (red) and astrocytes (silver).