## **Supplementary Information**

## Ultrasound-aided Multi-parametric Photoacoustic Microscopy of the Mouse Brain

Bo Ning<sup>1</sup>, Naidi Sun<sup>1</sup>, Rui Cao<sup>1</sup>, Ruimin Chen<sup>2</sup>, K. Kirk Shung<sup>2</sup>, John A. Hossack<sup>1</sup>, Jin-Moo Lee<sup>3</sup>, Qifa Zhou<sup>2,\*</sup>, and Song Hu<sup>1,\*</sup>

<sup>1</sup>Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908
<sup>2</sup>Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089
<sup>3</sup>Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110

\* Corresponding authors: Song Hu (photoacoustic microscopy) <u>songhu@virginia.edu</u> Qifa Zhou (ultrasonic transducer)<u>qifazhou@usc.edu</u>



Supplementary Figure S1 | A movie showing the 3D surface contour of the plastic ball extracted by SAM.



Supplementary Figure S2 | A movie showing the 3D rendering of the ball surface imaged by PAM with

contour scan.



**Supplementary Figure S3** | A movie showing the 3D rendering of the ball surface imaged by PAM without contour scan.



**Supplementary Figure S4** | A movie showing the 3D surface contour of the mouse skull extracted by SAM.



**Supplementary Figure S5** | A movie showing the 3D rendering of the mouse brain simultaneously imaged by SAM (gray) and PAM (hot) with contour scan.



**Supplementary Figure S6** | A movie showing the 3D rendering of the mouse brain simultaneously imaged by SAM (gray) and PAM (hot) without contour scan.



**Supplementary Figure S7** | Validation of PAM-based C<sub>Hb</sub> quantification using defibrinated bovine blood *in vitro*.



**Supplementary Figure S8** | Orientation of the brain vasculature determined by the ultrasound-aided contour PAM. The angle ( $\alpha$ ) is formed by the vessel axis and the transverse plane.