Supplementary Information

Simultaneous Dual-Modal Multispectral Photoacoustic and Ultrasound Macroscopy for Three-Dimensional Whole-Body Imaging of Small Animals

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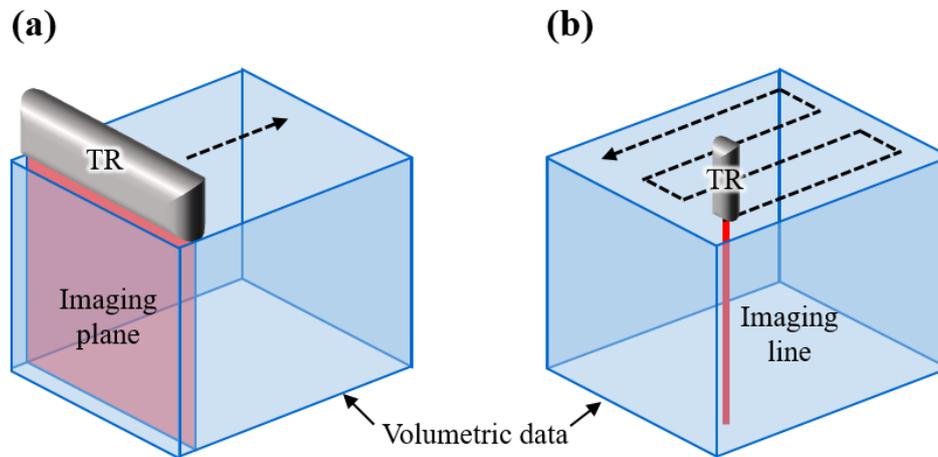† These authors contributed equally to this work.

Figure S1. Scanning methods for acquiring volumetric data. (a) Volumetric data acquisition with the elevational scanning of a linear-array transducer. (**b**) Volumetric data acquisition with the raster scanning of a spherical single-element transducer. The black dashed lines represent the scanning paths. The red plane and line depict the imaging plane and imaging line with a single laser pulse. TR, transducer.
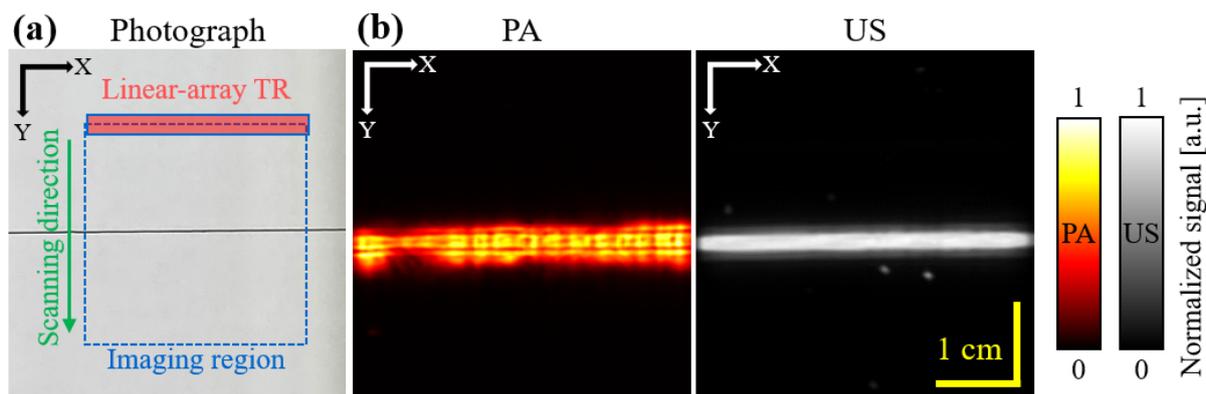
Figure S2. PA and US images from a linear-array-based system with an elevational scanning. (**a**) Photograph of the resolution phantom and schematic illustration of the scanning method. A 128-element linear-array transducer (L3-12, Alpinion Medical Systems, Republic of Korea) with a center frequency of 7.5 MHz was scanned along its elevational direction to acquire volumetric images. (**b**) PA and US MAP images of the resolution target. The data acquisition and image processing were performed using a clinical PA and US imaging system, which had been reported in our previous article (doi.org/10.1038/srep35137). PA, photoacoustic; US, ultrasound; TR, transducer; MAP, maximum amplitude projection.
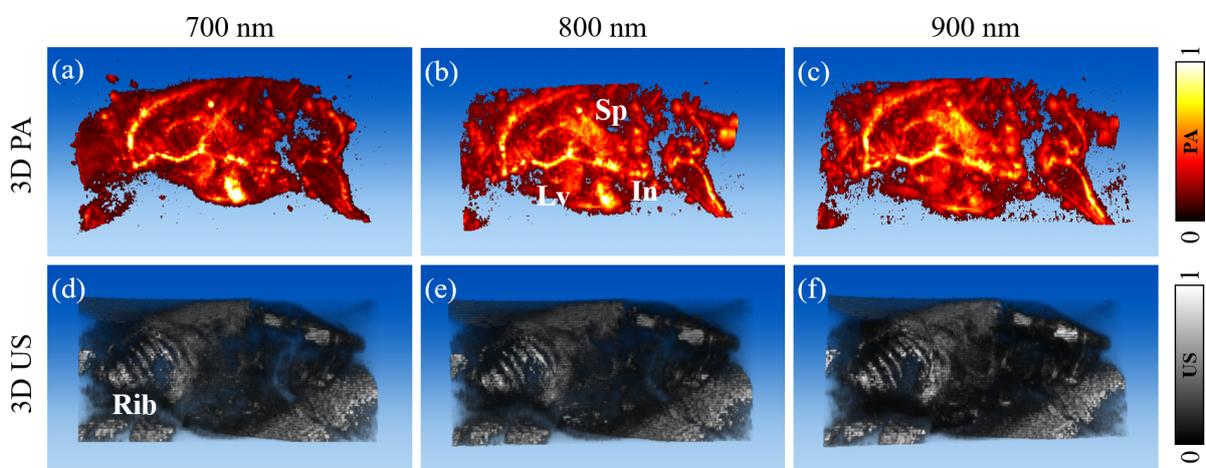

Figure S3. 3D rendering of PA and US images of mice *in vivo*. 3D PA images with the excitation wavelengths of (**a**) 700, (**b**) 800, and (**c**) 900 nm. (**d**–**f**) 3D rendering of the corresponding US images. PA, photoacoustic; US, ultrasound; Sp, spleen; Lv, liver; In, intestine; Lib, ribcage.
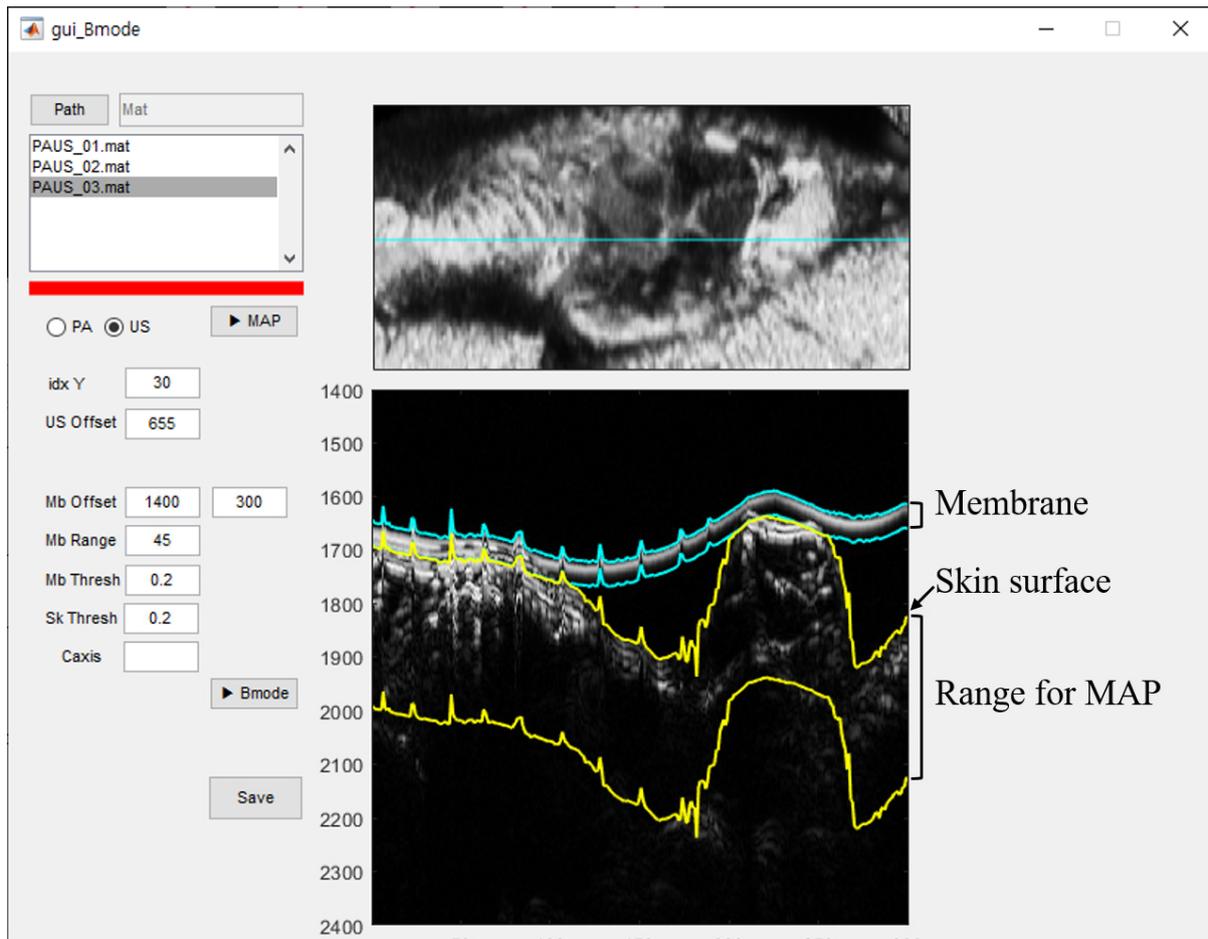
Figure S4. Matlab based skin detection software with graphical user interfaces. The blue and yellow lines denote the membrane and animal-body, respectively.

Movie. S1. The 3D visualization of PA images with an optical wavelength of 700 nm.

Movie. S2. The 3D visualization of PA images with an optical wavelength of 800 nm.

Movie. S3. The 3D visualization of PA images with an optical wavelength of 900 nm.