

Electronic Supplementary Information

Deciphering of cerebrovasculatures via ICG-assisted NIR-II fluorescence microscopy

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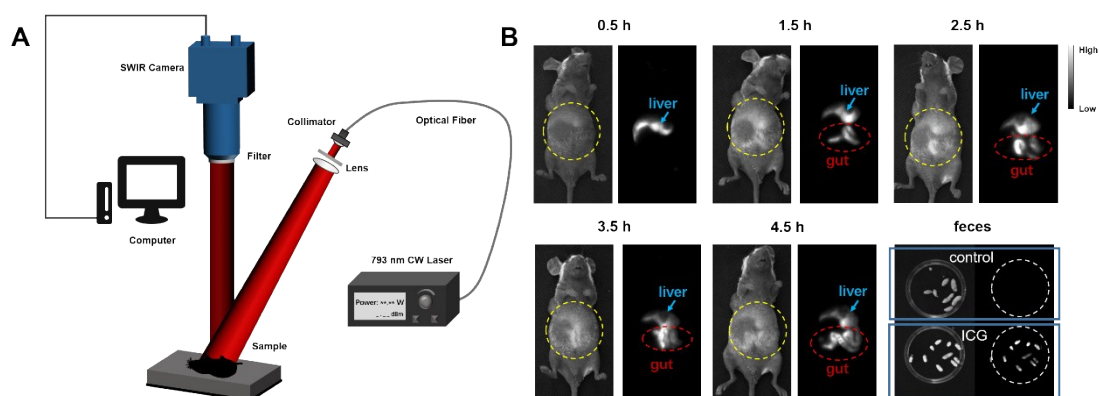


Fig. S1 (A) Schematic illustration of the setup for NIR-II fluorescence whole-body imaging system. (B) *In vivo* real-time NIR-II fluorescence imaging of the whole

intestinal tract in the mouse at various time points after IV injection of ICG (1 mg/mL, 100 μ L). The feces were collected 5-8 hours post injection. The yellow-dashed circles represent the abdomen regions. Excitation: 793 nm laser (15 mW/cm²). Optical filter: 1100 nm long-pass. Exposure time: 100 ms.

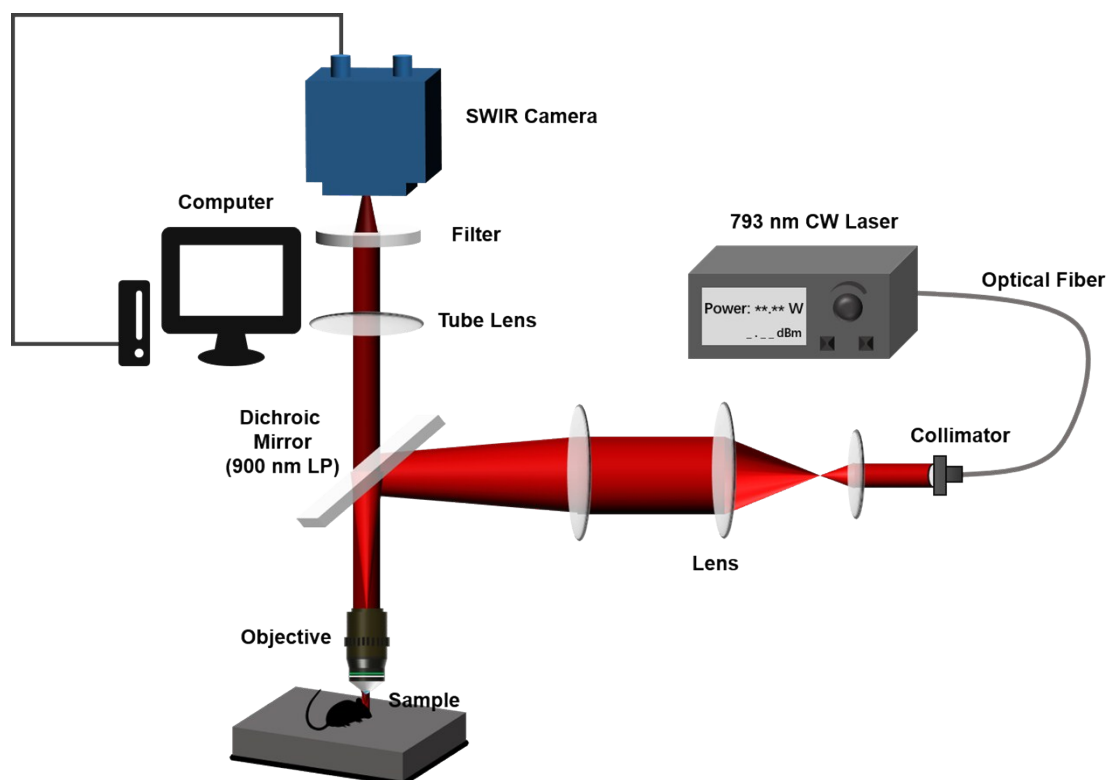


Fig. S2 Schematic illustration of the setup for NIR-II fluorescence wide-field microscopic imaging system.

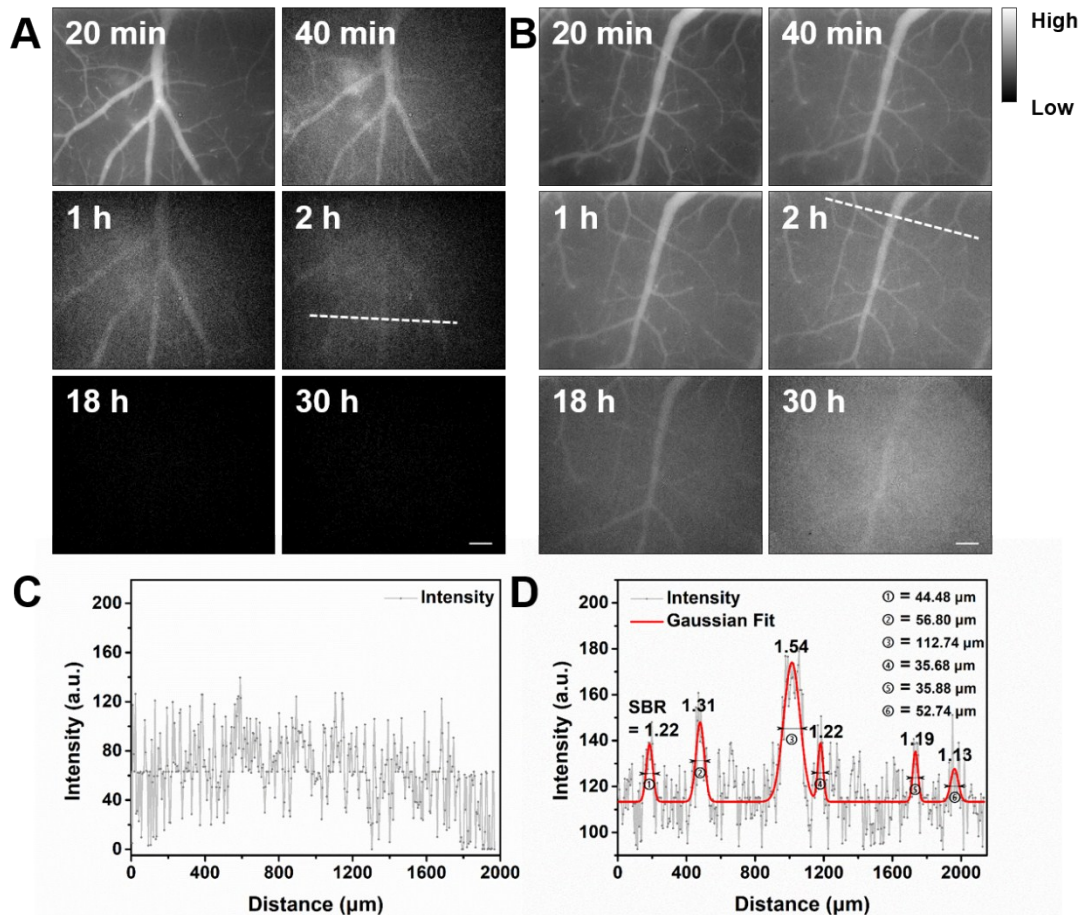


Fig. S3 NIR-II fluorescence images of cerebrovasculature in the mice at various time points post (A) intravenous injection and (B) intramuscular injection of ICG (1 mg/mL, 100 μL), under 793 nm laser irradiation. A cross-sectional fluorescence intensity profile (gray) and a Gaussian fitting curve (red) along the white-dashed lines in the recorded images at 2 h post (C) IV injection and (D) IM injection. Objective: scan lens (LSM03, Thorlabs). Optical filter: 1100 nm long-pass. Exposure time: 100 ms. Scale bar indicates 300 μm .

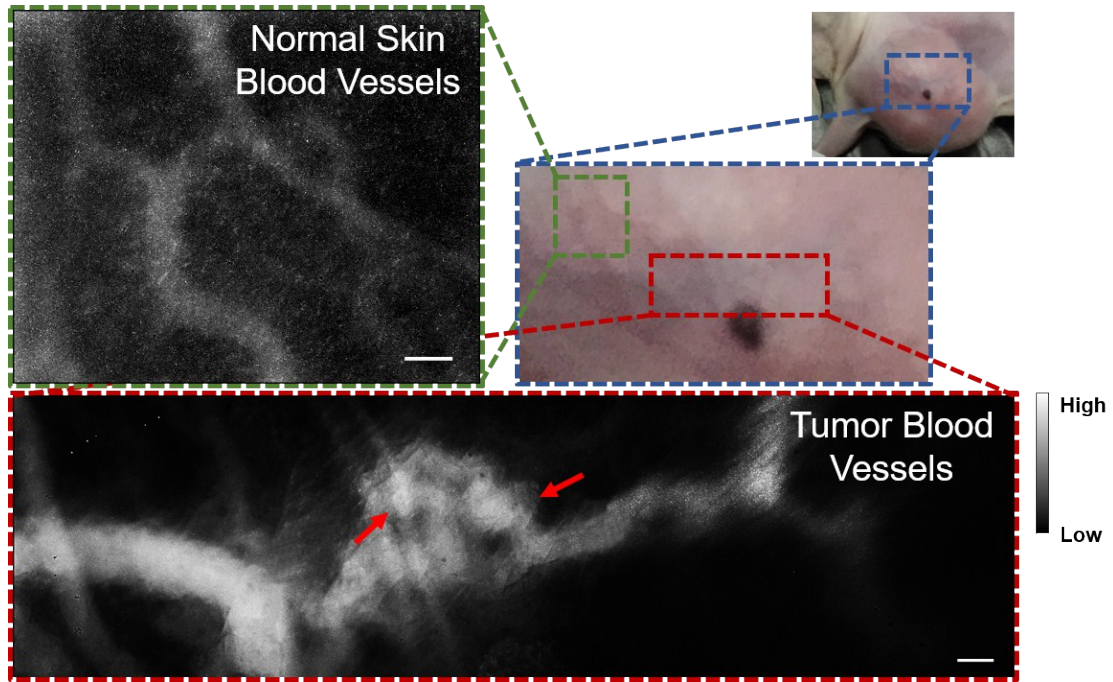


Fig. S4 *In vivo* NIR-II fluorescence microscopic imaging of the blood vessels in the subcutaneous bladder tumor of mouse, which was intramuscularly injected with ICG (1 mg/mL, 100 μ L). Excitation: 793 nm laser. Objective: scan lens (LSM03, Thorlabs). Optical filter: 1100 nm long-pass. Exposure time: 300 ms. Scale bar indicates 300 μ m.

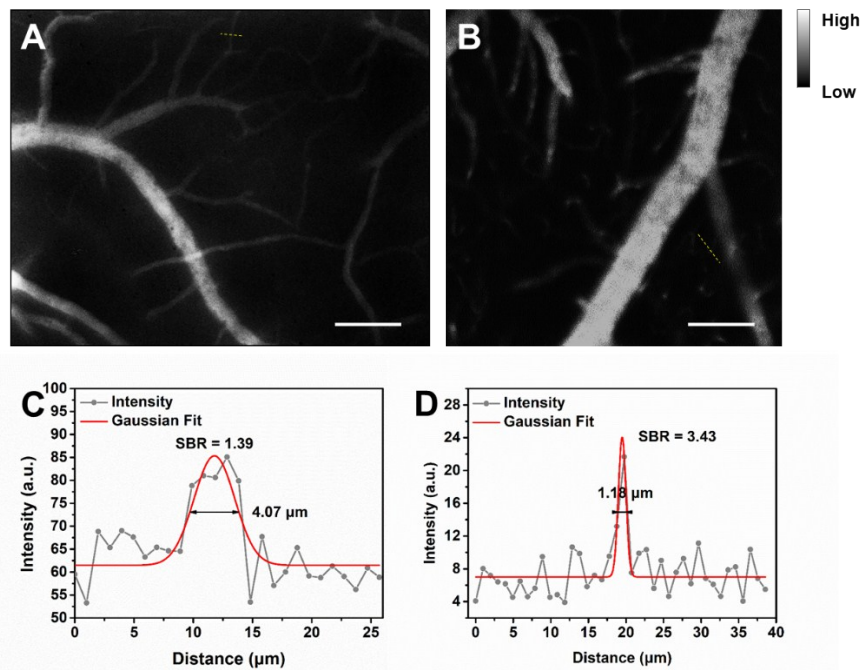


Fig. S5 *In vivo* imaging of brain vasculature in a mouse **(A)** at the depth of 150 μm via NIR-II fluorescence wide-field microscopy and **(B)** at the depth of 160 μm via NIR-II fluorescence confocal microscopy after intramuscular injection of ICG (1 mg/mL, 100 μL). Scale bar indicates 100 μm . A cross-sectional fluorescence intensity profile (gray) and a Gaussian fitting curve (red) along the yellow-dashed lines in the NIR-II fluorescence **(C)** wide-field micro-images and **(D)** confocal micro-images.

	ICG in serum
QY >900 nm	4.2 %
QY >1000 nm	0.83 %
QY >1100 nm	0.18 %
QY >1200 nm	0.05 %

Tab. S1. Fluorescence QYs of ICG in serum beyond 900 nm, 1000 nm, 1100 nm and 1200 nm.

Video S1. Cerebral vessels at different depths below skull in a mouse. Objective: 25 \times . Exposure time: 100 ms.