# Supplementary Material for: "Five-Wavelength Optical-Resolution Photoacoustic Microscopy of Blood and Lymphatic Vessels"

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## 1. Simulated focal spots at the focal plane

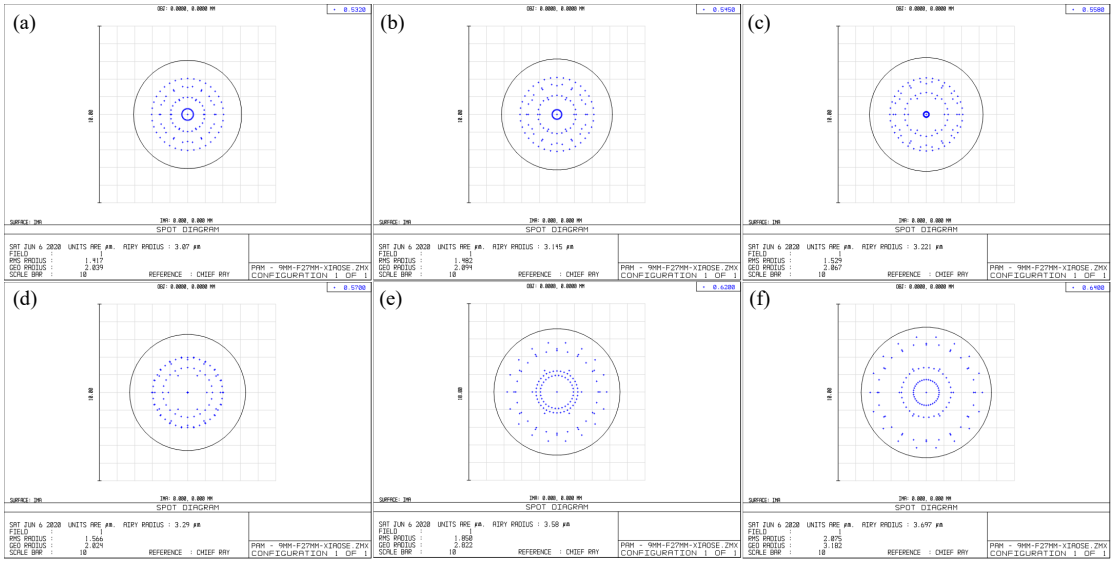


**Fig S1** (a)~(f) are the simulated focal spots at the focal plane of 532 nm. The airy radii are 3.070, 3.145, 3.221, 3.290, 3.580, and 3.697 μm for the 532, 545, 558, 570, 620 and 640-nm wavelengths.

To very that all wavelengths are focused on the same focal plane, here we simulate the focal spots of 532, 545, 558, 570, 620 and 640-nm wavelengths at the focal plane of 532 nm. The airy radii are 3.070, 3.145, 3.221, 3.290, 3.580, and 3.697 μm for these wavelengths, respectively, matching well with the results shown as Figs. 2(f, g, h, i, j) in the main text.

## 2. Flow parameter calibration

To calibrate $A$, $\tau_\alpha$, and $b$, we use a syringe pump to set the blood flow at different speeds. The blood phantom is anticoagulated bovine blood filled in a transparent rubber tube. Via setting the flow speed $v$ from 0 to 23 mm/s, we measure the fluence and PA signals of 532 nm and 545 nm and fit them to an exponential decay model (the Eq.2 in the main body). We can determine that $b$ is 0.25± 0.06 (SD) with $R^2 = 0.942$. Different time delays $\delta t$ are used to determine $A$ and $\tau_\alpha$. We use different $\delta t' = x\delta t$, maintain other parameters (e.g. surface fluence) unchanged, and set the flow speed to 0. Then we can simplify the Eq.(2) as $m = Ae^{-\tau_\alpha \delta t}$ and $n = Ae^{-x\tau_\alpha \delta t}$ for

time delays of $\delta t$ and $\delta t'$. Thus we can obtain, $A = (\frac{n}{m^x})^{\frac{1}{1-x}}$, $\tau_\alpha = \frac{1}{(1-x)\delta t}\ln(\frac{n}{m})$. $A$ and $\tau_\alpha$ were calibrated as 0.09±0.04 (SD) and 9.68±0.85 (SD).

### 3. Flow rate calculation

The blood vessel flow can be assumed as laminar. After acquiring the flow speeds and the corresponding lumen diameter of blood vessels, the average volume blood flow rate $F$ can be defined as:

$$F = vA = \frac{\pi}{8}v(0)d^2, \quad \textbf{(S1)}$$

where $v$ is the average blood flow, $A$ is the cross-sectional area of the vessel lumen, $v(0)$ is the blood flow along the central axis of the vessel, and d is the lumen diameter.

### 4. Compensation for Grueneisen relaxation effect in $sO_2$ imaging

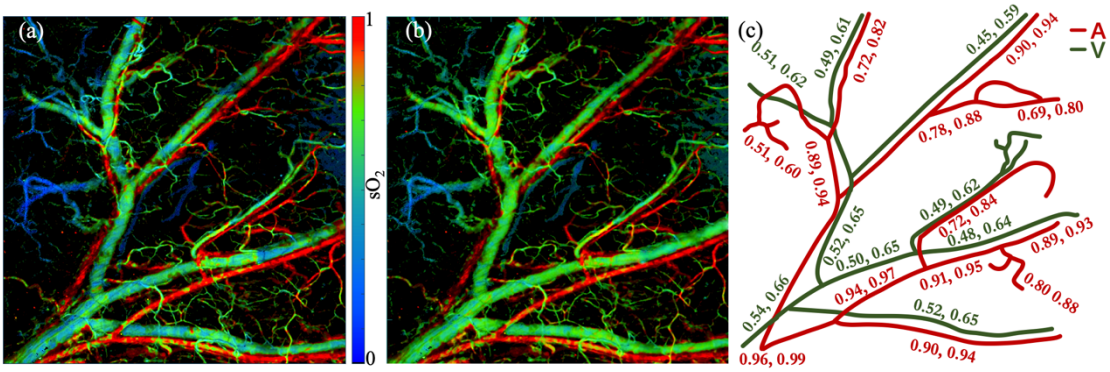


**Fig S2.** (a) $sO_2$ image of the mouse ear without compensation for the Grueneisen relaxation effect. (b) $sO_2$ image of the mouse ear with compensation for the Grueneisen relaxation effect. (c) Averaged $sO_2$ values in vessel segments. The first value is without compensation for the Grueneisen relaxation effect, and the second one is with compensation. A: artery, V: vein. The imaging area is 2.5×2.5 $mm^2$.

We compensate for the Grueneisen relaxation effect to improve the accuracy of $sO_2$ imaging. The detailed methematic compensation model is shown as formula (3) in the main text. Figs S2(a, b) are the $sO_2$ results without and with compensation for the Grueneisen relaxation effect. The detailed $sO_2$ comparison is shown in the Figs S2(c), and the first value is without compensation for the Grueneisen relaxation effect, and the second one is with compensation. The compensation for the Grueneisen relaxation improves the averaged $sO_2$ values by 4% ~17% in the arteries, and by 21% ~24% in the veins.

**5. Functional vascular imaging of early-stage tumor**

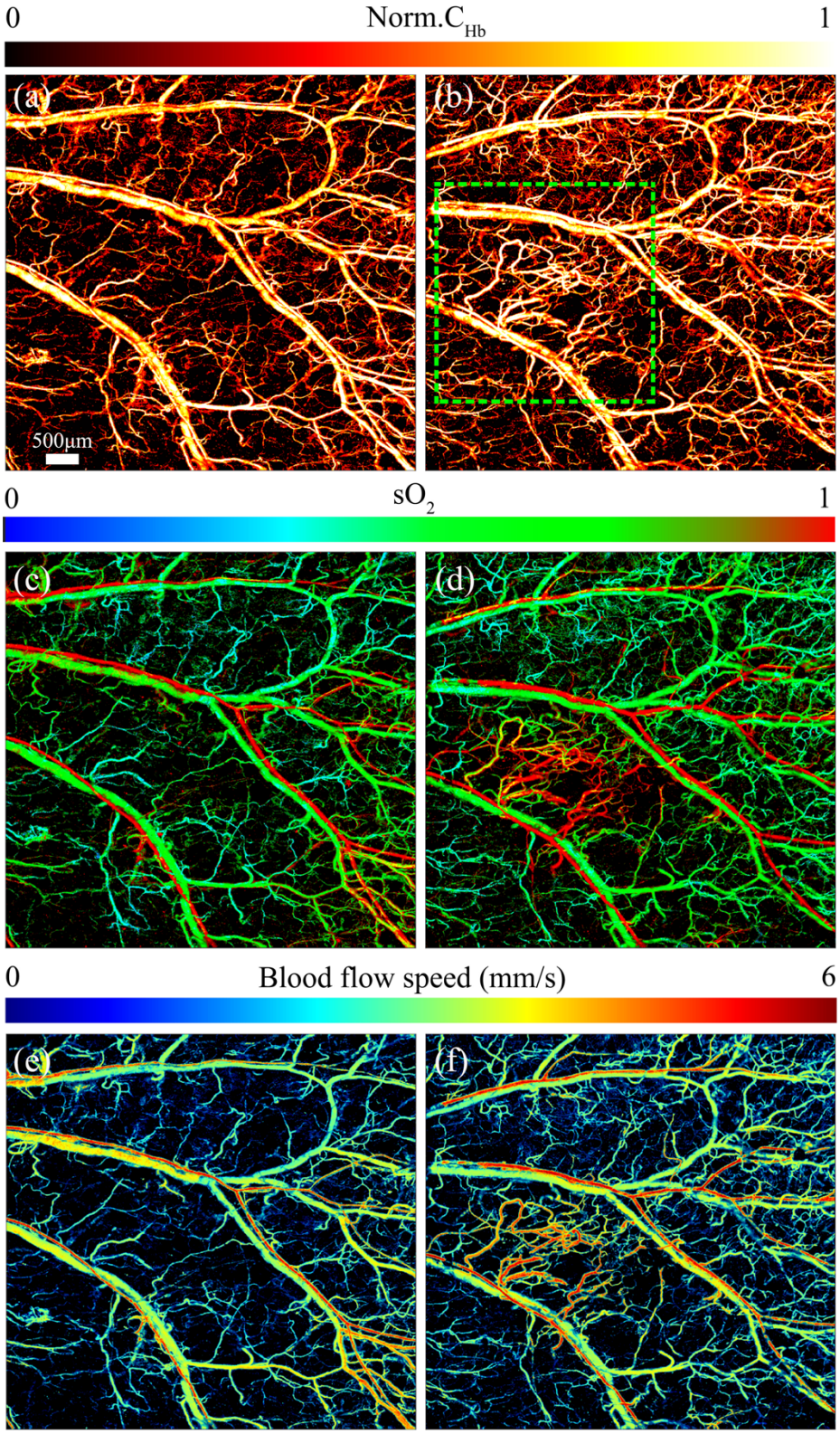


**Fig S3.** Functional vascular imaging of an early-stage tumor. (a), (c), and (e) are the hemoglobin concentration, oxygen saturation, and blood flow speed before tumor implementation. (b), (d), and (f) are the hemoglobin concentration, oxygen saturation, and blood flow speed after 5 days tumor growth. The green dashed-box in (b) shows the angiogenesis in the early-stage tumor.

**6. Quantification of vascular depth, diameter, and tortuosity in an early-stage tumor**

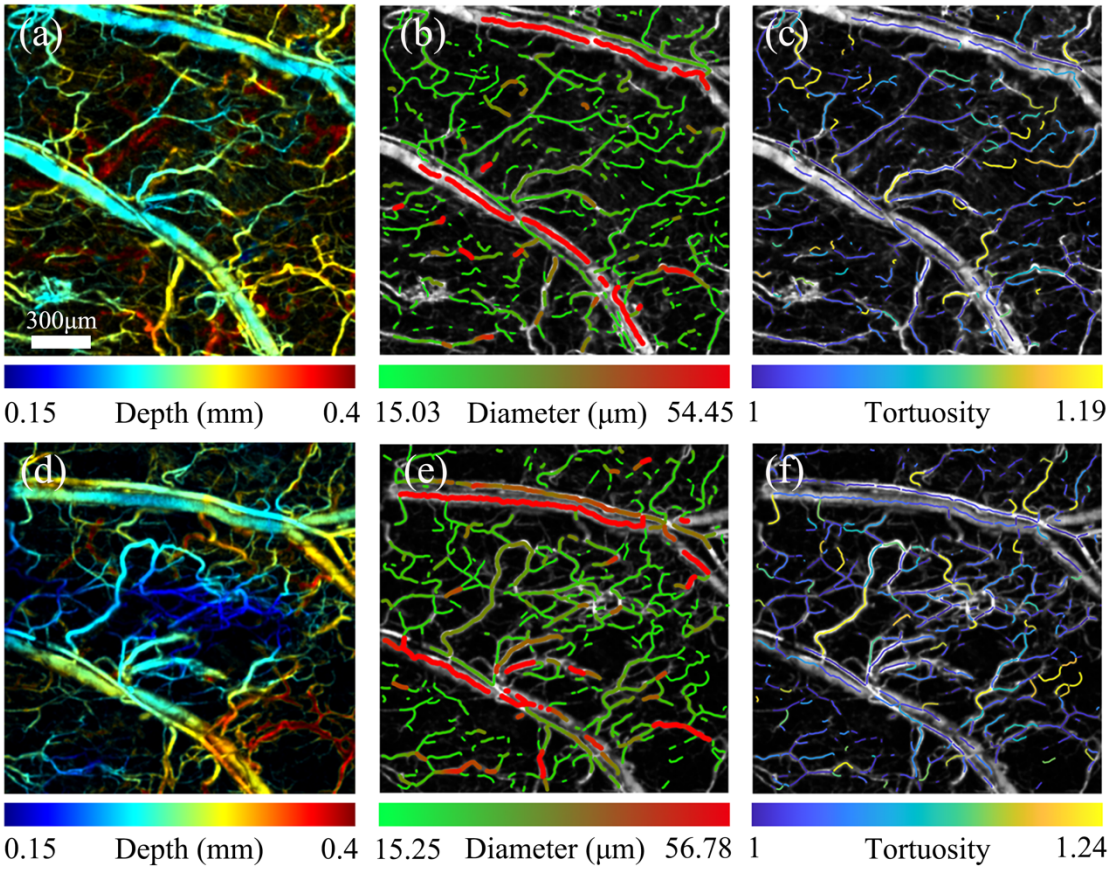


**Fig S4.** Vascular depth, diameter, and tortuosity of in the tumor region in the green dashed-box in Supplementary Figure 3(b). (a), (b), (c) are the baseline images, and (d), (e), (f) are the early-stage tumor images.

We define the vessel tortuosity as $\frac{l}{l'}$, where $l$ is the vessel path length, and $l'$ is the linear distance between the two vessel ends.