**Supplemental materials**

**Derivation of temperature modulation amplitude**

The fluid temperature in response to sinusoidal heating varies according to

, (1)

where  is the temperature variation from the average temperature, tis the “slow” time instead of the “fast” PA flight time, is the amplitude of the temperature variation at x, f0 is the heating modulation frequency, and  is a constant.

In Eq. ,  is determined by thermal conduction, and the sinusoidal term is determined by the heating and the thermal convection. To explicitly express , we track the flow of a voxel that passes through the heating spot (position *x* = 0) at time *t* = 0, i.e.,  
 . (2)

Substituting Eq. into Eq. (1), we obtain the temperature variation of this voxel:  
 . (3)  
whose initial temperature change is . Tracking the flow of the voxel allows us to remove the convection term. Once the voxel flows away from the heating spot, its temperature change is mainly determined by thermal conduction.

Blood in a vessel can be considered as a thin and long cylinder that has a constant temperature at the boundaries. In this case, the temperature gradient in the radial direction is much greater than that in the axial direction. The radial thermal conduction is thus the main cause of temperature decay. A general solution of this model can be expressed by (1)  
 , (4)  
where  is the thermal diffusivity,  is a constant coefficient. Combining Eq. ,(3), and , we can derive an approximated expression for the amplitude: .

**Detailed description of experimental setup**

An Nd:YAG pump laser and a Ti:sapphire laser generate short pulses at 800-nm wavelength, with a 10-Hz pulse repetition rate. The trigger signal from the pump laser synchronizes both a field-programmable gate array (FPGA) (PCI-7830R, National Instruments) and a custom-made 64-channel data acquisition system (DAQ). The FPGA provides an amplitude-modulation signal for a modulator that generates a 7.5-MHz sinusoidal waveform to drive the heating ultrasound transducer. The effect of the amplitude-modulation signal is twofold: First, it turns off the modulator during PA signal acquisition to avoid interference, and second, it applies a 0.0625-Hz sinusoidal amplitude modulation to the 7.5-MHz waveform. The modulated signal is amplified and sent to a custom-made heating ultrasound transducer. PA signals are detected by a 512-element full-ring ultrasound transducer array with a 5-MHz central frequency and 50-mm ring diameter (2, 3). Each element in the array is cylindrically focused, and the combined foci of all elements generates a central imaging region of 20 mm diameter and 1 mm thickness. The heating and receiving transducers are aligned in elevation to ensure that the focus of heating is in the imaging plane. The 64-channel DAQ is multiplexed to receive signals from all 512 elements, and the PA images are reconstructed using backprojection (4). The PA imaging has a frame rate of 0.625 Hz. A silicon tube (inner diameter: 1.5 mm) filled with whole bovine blood is placed on the focus of the ultrasound transducer and is parallel with the PA imaging plane.

**References**

1. D. Poulikakos, Conduction heat transfer, Englewood Cliffs, NJ: Prentice Hall (1994).

2. J. Gamelin, A. Maurudis, A. Aguirre, F. Huang, P. Guo, L. V. Wang and Q. Zhu, "A real-time photoacoustic tomography system for small animals," Opt. Express 17(13), 10489-10498 (2009)

3. J. Xia, Z. Guo, K. Maslov, A. Aguirre, Q. Zhu, C. Percival and L. V. Wang, "Three-dimensional photoacoustic tomography based on the focal-line concept," J. Biomed. Opt. 16(9), 090505 (2011)

4. M. Xu and L. V. Wang, "Universal back-projection algorithm for photoacoustic computed tomography," Physical Review E 71(1), 016706 (2005)