

## Description of Additional Supplementary Files

**File:** Supplementary Movie 1

**Description:** Real-time ex vivo spectroscopic PAUS imaging for needle guidance with nanoparticle injection at a 50-Hz frame rate showing three stages: needle insertion into chicken breast, GNR injection, and needle pullout. For each stage, PA images are acquired at a single wavelength (775 nm), followed by a 10-wavelength sweep ranging from 700 to 875 nm. The laser sequence (right) is shown synchronized with the real-time image (left).

**File:** Supplementary Movie 2

**Description:** Real-time in vivo mouse spectroscopic PAUS imaging for needle guidance with nanoparticle injection at a 50-Hz frame rate. PA images are acquired at a single wavelength (775 nm), followed by a 10-wavelength sweep ranging from 700 to 875 nm. The laser sequence (right) is shown synchronized with the real-time image (left).

**File:** Supplementary Software 1

**Description:** PAUS beamforming

Beamforming.m (main program): The main program for performing image reconstruction. The final beamformed PA image has a dimension of 512 (axial) × 128 (lateral) pixels.

**File:** Supplementary Software 2

**Description:** PAUS beamforming

parameterAll.mat: Parameters required for image formation. A structure called "param" contains all parameters for system, data and reconstruction.

**File:** Supplementary Software 3

**Description:** PAUS beamforming

LUTGenerator.m: Subroutine to generate the look-up table for image reconstruction. After execution, the code can automatically save a table file called "PADASFastTable.mat".

**File:** Supplementary Software 4

**Description:** Fluence estimation

parameter2DsearchGPU.m (main program): To obtain the effective attenuation coefficient, absorption coefficient, reduced scattering coefficient and free path length.

**File:** Supplementary Software 5

**Description:** Fluence estimation

multiWavParam.m: Parameters required for fluence compensation. A 'param' contains all parameters for system, data and reconstruction.

**File:** Supplementary Software 6

**Description:** Motion correction

PM\_realtime.m (main program): The main program for inter-wavelength motion estimation and compensation.

**File:** Supplementary Software 7

**Description:** Motion correction

PM2DabsRT\_mex.mexw64: MEX-based core function performing PatchMatch US speckle tracking.  
Box 355061 1705 NE Pacific St. Seattle, WA 98195-5061  
206.685.2002 fax 206.685.3300 biochr@u.washington.edu depts.washington.edu/bioep. 5

**File:** Supplementary Data 1

**Description:** rfData.mat: RF raw channel data representing PA signals from 4 optical absorbers. The dimension is 2048 (axial number of samples) × 128 (receive channels) × 20 (number of fibers) × 30 (number of frames) samples.

**File:** Supplementary Data 2

**Description:** multiWavData.mat: Beamformed experimental image data called 'totalImg' are processed. The dimension is 1024 (axial)  $\times$  128 (lateral)  $\times$  20 (number of fibers)  $\times$  1 (number of frames)  $\times$  10 (number of wavelengths) samples.

**File:** Supplementary Data 3

**Description:** PAUS\_10frame\_motion.mat: in vivo mouse PAUS beamformed data during nanoparticle injection, including 10 different wavelengths (the variable "pamode\_all") and interleaved B modes (the variable "bmode\_all"). Both data sets have a dimension of 513 (axial)  $\times$  128 (lateral)  $\times$  10 (number of wavelengths) samples. The corresponding lateral and axial extents (the variables "SL\_pos" and "range", respectively) required for motion estimation are also included in this file.