

Electronic Supplementary Information

***In vivo* photoacoustic mapping of lymphatic systems with plasmon-resonant nanostars**

Chulhong Kim,^{1,3§} Hyon-Min Song,^{2§} Xin Cai,¹ Junjie Yao,¹ Alexander Wei,^{2*} and Lihong V. Wang^{1*}

¹Dr. C. Kim, X. Cai, J. Yao, and Prof. L. V. Wang
Optical Imaging Laboratory
Department of Biomedical Engineering
Washington University in St. Louis, MO
One Brookings Drive, St. Louis, MO 63130 (USA)

²H-M. Song and Prof. A. Wei
Department of Chemistry
Purdue University
560 Oval Dr., West Lafayette, IN 47907 (USA)

³Current address:
Department of Biomedical Engineering
University at Buffalo, The State University of New York
328 Bonner Hall, Buffalo, NY 14260 (USA)

§These authors contributed equally to this work.

*Address correspondence to:
Lihong V. Wang (photoacoustic imaging)
One Brookings Drive, St. Louis, MO 63130 (USA)
Email: lhwang@biomed.wustl.edu

Alexander Wei (gold nanostars)
560 Oval Dr., West Lafayette, IN 47907 (USA)
E-mail: alexwei@purdue.edu

Nanostar synthesis

NST samples A and B were prepared by seeded growth using 13-nm core-shell $\text{Fe}_3\text{O}_4@Au$ particles and 8-nm Au particle, respectively. The NST growth solution was prepared by the sequential mixing of 0.2 M CTAB (5 mL), 4 mM AgNO_3 (0.9 mL), 7.8 mM HAuCl_4 (5 mL), and 0.08 M ascorbic acid (0.3 mL), using a vortex mixer in between each addition. The freshly prepared mixture was then treated with a dispersion of nanoparticle seeds in 2.5 mM sodium citrate (1 mg in 250 μL). NST growth was complete within 15 minutes after seed addition, and separated from the reaction mixture by centrifugation at 7300 g for 15 min. The CTAB-stabilized NSTs were twice resuspended in 1% polystyrenesulfonate followed by centrifugation to remove excess CTAB, then resuspended in 1% bovine serum albumin (BSA) to produce NSTs in their final, biocompatible form.

Solid gold nanostars (Sample B)

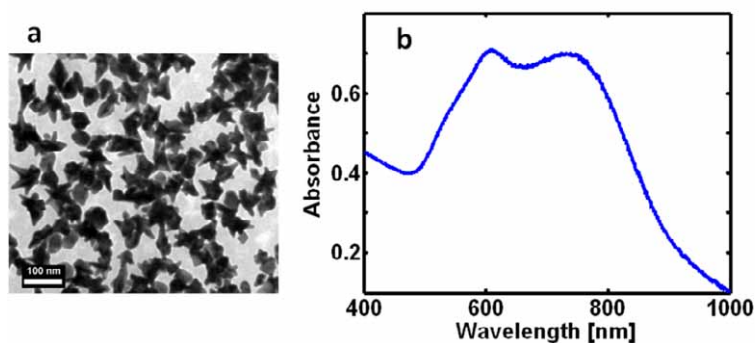


Figure S1. (A) Transmission electron microscopy image of solid gold nanostars. (B) Optical extinction spectrum of diluted sample B. We note that a significant fraction of these particles are not star-shaped, and contribute primarily toward extinction at visible wavelengths (<750 nm).

Measuring NST concentration and limit of detection

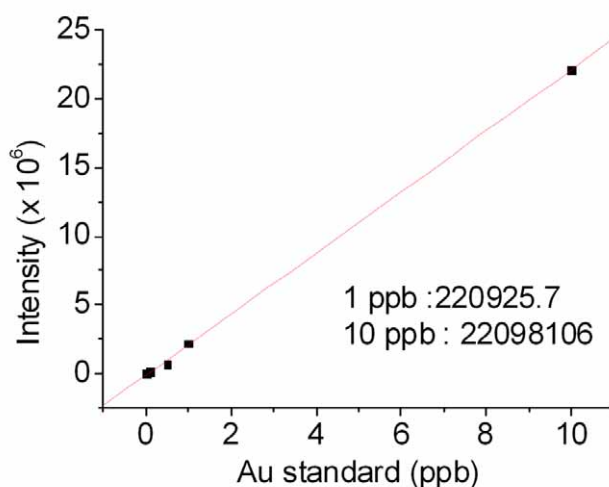


Figure S2. Calibration plot of Au standards (0.1–10 ppb) for ICP-MS measurements.

The Au content of NSTs with and without magnetic cores was determined using ICP-MS. All measurements were calibrated against a set of serial dilutions (0.1, 0.5, 1, 10 ppb) starting from a commercial Au standard for ICP-MS (Fluka, 1 mg Au/mL in hydrochloric acid). A linear fit was obtained (Fig. S2), and used to calculate the Au content in digested NST samples ($N=3$). These were prepared by first combining 10 μL of a concentrated NST solution (Sample A: O.D = 13.1 at 870 nm; Sample B: O.D. = 15.0 at 780 nm) with 1 mL of aqua regia ($\text{HNO}_3:\text{HCl} = 1:3$), then diluting a 10- μL aliquot of the digested NST with 5 mL of 2% HNO_3 (ultrapure grade). Content of Au is reported in ppm ($\mu\text{g Au/g sample}$).

Sample A (gold NSTs with Fe_3O_4 cores): 1035 ppm Au (RSD 1.89%)

Sample B (pure gold NSTs): 836 ppm Au (RSD 0.42%)

The irregular structure and polydispersity of the NSTs preclude an exact particle count, based on estimates derived from assumed particle volumes. Nevertheless, upper and lower limits on particle concentrations can be obtained by estimating the volume of a single NST as 100- or 50-nm Au spheres, with a specific density of 18.88 ($\sim 10^{-8}$ – 10^{-9} $\mu\text{g Au}$ per particle), then applying this toward the ICP-MS data. This yields a concentration range of 10^{11} – 10^{12} NSTs/mL (1.6–16 pM) for sample A in its undiluted state. Therefore, 1 ppm Au corresponds to a NST concentration in the femtomolar range (10^9 – 10^{10} NSTs/mL).

Deep-reflection mode photoacoustic tomography (PAT) system

A tunable Ti:sapphire laser (LT-2211A, LOTIS TII), pumped by a Q-switched Nd:YAG laser (LS-2137, LOTIS), generated 6-ns laser pulses at a repetition rate of 10 Hz. An optical wavelength of 767 nm was used for most of the experiments, but the wavelength was tuned from 754 to 806 nm for spectroscopic measurements. A light fluence of *ca.* 7 mJ/cm² was illuminated on skin, well below the ANSI limit of 27 mJ/cm². PA pressure waves were detected by a 5-MHz spherically focused ultrasound transducer (V308, Panametrics-NDT). The axial and lateral resolutions of this system are ~150 and 560 μm, respectively. 1D depth-sensitive images were obtained by measuring the PA signals' arrival time, followed by a single laser pulse irradiation. 3D PA images were formed by two directional mechanical scanning. The acquired volumetric data were processed in either of the following two forms: (1) a maximum amplitude projection (MAP) and (2) a volumetric image formed by Volview software (Kitware).

All animal experiments were performed according to the guidelines of Washington University in St. Louis on the care and use of laboratory animals. Sprague-Dawley rats weighing ~250 g (*N*=3 for *in vivo* imaging, *N*=2 for *ex vivo* imaging) were either anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (8 mg/kg), or maintained under full anesthesia using a vaporized isoflurane system. At the end of the *in vivo* imaging study, the animals were euthanized with an overdose of pentobarbital, and the lymph nodes were harvested for *ex vivo* analysis.

Photoacoustic sensitivity calculation

1. The dimensions of one resolution voxel are 150 and 560 μm along the axial and lateral directions, respectively. Therefore, the volume is calculated by $150 \times \pi \times 280^2 \mu\text{m}^3 = 3.7 \times 10^{-11} \text{ m}^3$ ($3.7 \times 10^{-5} \text{ mL}$).
2. The number of Au NSTs per voxel at the detection limit (1 ppm Au) is estimated to be $(10^9\text{--}10^{10} \text{ NSTs/mL}) \times (3.7 \times 10^{-5} \text{ mL})$, or $10^4\text{--}10^5 \text{ NSTs}$ (see ICP-MS analysis, above).

Depth resolved B-mode photoacoustic images of sentinel lymph nodes in a rat *in vivo*

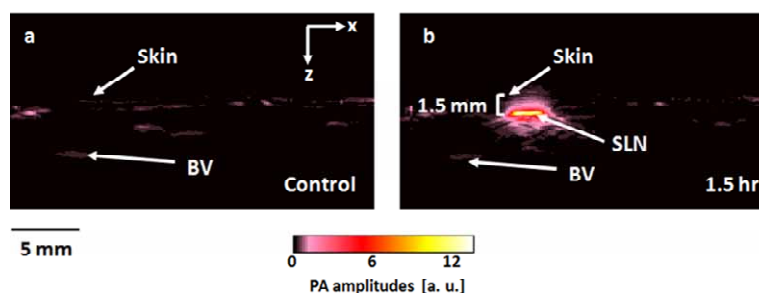


Figure S3. *In vivo* depth resolved B-mode photoacoustic (PA) images of rat sentinel lymph nodes (SLN), enhanced using NSTs (sample A). (a) Control PA image acquired before NST injection, displaying only blood vessels (BV). (b) PA image acquired post-injection ($t = 1.5$ h), revealing the SLN. The SLN is located 1.5 mm below the skin surface.

Optical-resolution photoacoustic microscopy (OR-PAM) system

The experimental setup of the OR-PAM is detailed in Ref 12. Briefly, in OR-PAM, a wavelength-tunable dye laser, pumped by a Nd:YLF laser, produces 7-ns laser pulses with a repetition rate of up to 5 kHz. The laser is reshaped by a pinhole and then focused by an objective into a nearly diffraction limited spot. An acoustic-optical beam splitter, consisting of two right-angle prisms and a thin layer of silicone oil, is positioned under the objective lens to separate optical illumination and acoustic detection. A 75-MHz ultrasonic transducer is used as a detector. Like the deep-reflection mode PAT system, through time-resolved ultrasonic detection and 2-D raster scanning along the transverse plane, 3-D information of tissues is formed, and can be viewed in maximum amplitude projection (MAP) image.