Spectrally and Polarization-Dependent Scattering of Gold Nanobipyramids for Exogenous Contrast in Optical Coherence Tomography

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1. Preparation of GNBPs

The gold nanobipyramids (GNBPs) were synthesized as we previously reported. Briefly, the gold seeds were prepared by mixing 4 mL of 0.5 mM HAuCl4, 4 mL of 95 mM cetyltrimethylammonium chloride (CTAC) and 72 μL of 250 mM HNO3, followed by rapidly injecting 100 μL of 50 mM ice-cold NaBH4 solution containing 50 mM NaOH under vigorous stirring. Then, 16 μL of 1 M citric acid was added to the solution. The vial containing this seed solution was then placed in a 80 °C water bath for 1 h. The growth solutions of GNBP1200, GNBP1274 and GNBP1394 were prepared by dissolving 49, 44 and 38.5 mg CTAB respectively in 400 mL of 140 mM CTAC solution, followed by adding 4 mL of 25 mM HAuCl4, 1.2 mL of 10 nM silver nitrate, and 4 mL of 0.4 M ethanol solution of 8-hydroxyquinoline. Then 2 mL of the aged seed solution was injected into the growth solution, which was gently stirred for 10 s and then placed in a water bath at 45 °C for 15 min. After that, 3 mL of 0.4 M ethanol solution of 8-hydroxyquinoline was added to the growth solution, which was kept in the 45 °C water bath for another 1.5 h. After the reaction, the growth solution was transferred to 250 mL Nalgene centrifuge bottles, which were centrifuged at 30,000 g for 45 min. The supernatant was discarded, and the obtained pellet was washed by double distilled water twice. The GNB dispersion was incubated with 0.5 mg/mL solution of mPEG-SH 5000 overnight at 4 °C to PEGylate the nanoparticles. The NIR spectra of the GNBPs were measured by UV-Vis-NIR spectrometer (Jasco Model V670). The surface morphology and dimensions of the GNBPs were characterized by transmission electron microscopy (FEI Tecnai G2 F20 X-TWIN).

2. OCT Systems

This work utilized two wavelength-swept PS-OCT systems, one for in vitro imaging and a second portable system for small animal imaging in vivo. Both PS-OCT systems have a central wavelength at 1310 nm. For in vitro work, we used a custom-built bench-top PS-OCT system with a sweep range of 110 nm, leading to an approximate axial resolution of 10 μm. The repetition rate of the source was 50 kHz. The incident power on the sample was 40.3 mW with a system sensitivity of 110 dB. The polarization of light in the sample arm was alternated between two orthogonal polarization states on the Poincare sphere in adjacent A-lines. Light backscattered by the sample and coupled into the sample fiber interferes with
light from the reference arm in a polarization diverse receiver. For in vivo work, a portable PS-OCT system built using a commercial Santec (Hackensack, NJ) swept-source with a central wavelength of 1310 nm, sweep range of 100 nm, and 50 kHz repetition rate was used for in vivo imaging in the animal facility. The incident power on the sample was 16.1 mW with a system sensitivity of 104 dB. All images using either system were acquired with a LSM03 lens (ThorLabs, Newton, NJ), which provides an approximate spot size of 20 µm.

3. Image Processing

Employing Stokes formalism, degree of polarization (DOP) was averaged over the input polarization states \( p \) and the 3 spectral bands \( n \):

\[
DOP = \frac{1}{2N} \sum_{p=1}^{N} \sum_{n=1}^{3} \frac{\langle |I_{p,n}| \rangle}{\langle |I_{p,n}| \rangle}.
\]

Here, a Stokes vector is composed of the four components I, Q, U, and V, and \( \langle . \rangle \) indicates lateral filtering with a Gaussian filter spanning 6 A-lines. DOP is always in the range of 1 (completely polarized) to 0 (completely depolarized). Afterwards, DOP and intensity values were combined in a custom hue (DOP) saturation (intensity) color scheme shown in multiple figures.

The spectral contrast (SC) images were obtained by performing the spectral reconstruction of the OCT image using a dual band method we reported previously. In brief, we divided the OCT interferogram into two equal sub-bands with matching axial resolutions. We used Hanning windows for cropping the spectral bands to avoid excessive side-lobes. In each B-scan a spectrally neutral sample, 1% intralipid for in vitro and high DOP valued tissue for in vivo, was used to correct for any power imbalance between the two spectral bands due to source imbalance. After corrections, spectral contrast was then computed by subtracting the two bands and then normalizing the result by the linear average intensity of the medium used for balancing. For display, spectral contrast and intensity were combined into a hue (spectral contrast -1 to 1) and saturation (intensity, dB) color scheme for display throughout the manuscript.

Enface projections of in vivo imaging data were constructed by masking the volumetric spectral contrast data acquired during PS-OCT imaging with DOP and signal-to-noise (dB) cut-offs to avoid areas of low DOP due to noise dominated pixels. Pixels with a DOP value greater than 0.7 and 0.65 were masked to isolate lymphatic vessels in the mouse leg and ear respectively. Pixels below 20 dB were masked in both anatomical sites. After pixels with low signal and high DOP were masked, a mean enface projection was computed using the remaining pixels and displayed. Due to a higher concentration of GNBP particles entering the lymph vessels in the ear, only the top half of the vessels were used for computing the projections to avoid contributions from spectral shadowing. Angiography enface images were computed by taking a mean projection over the full width of the tissue after thresholding to ignore areas of low signal.

4. In vitro capillary tubes

Square glass capillary tubes (0.5 mm inner edge length and 0.1 mm wall thickness, Glass Dynamics, L.L.C., Millville, NJ) were used to characterize the spectral contrast signals of GNBP particles in vitro. PS-OCT B-scan images of capillary tubes containing an intralipid (1% w/v) control and serial diluted GNBP particles were obtained (5 pM, 10 pM, 50 pM, 100 pM, 500 pM, and 1 nM). Capillary tubes were placed on a microscope stage and imaged using LSM03 scanning lens. For in vitro imaging, 50 B-scans were averaged to produce the final image. Capillary tubes were placed at a slight angle relative to the imaging OCT beam to reduce specular reflections from the capillary tube surface. For Supplemental
Figure S2, Hgb concentration from normal human blood was held at a constant 10 g/dL and mixed with GNBPs (5 pM, 10 pM, 50 pM, 100 pM, 500 pM, and 950 nM).

5. Animal Imaging

All animal work was conducted in compliance with the guidelines of Institutional Animal Care and Use Committee (IACUC) for MGH and Shriner’s Hospital. Protocols were approved by the committee. Male nude (nu/nu-) mice (MGH Animal facility) were used for all in vivo experiments. During all imaging experiments, the mice were anesthetized by ketamine/xylazine (100/10mg/kg) injection. The mouse’s leg and ear were secured to a mount by double-sided tape to minimize tissue motion. Before and after injection of GNBPs, 11 PS-OCT volumes were recorded (1184 B-scans/volume and 1024 A-lines/B-scan) and averaged to produce the final volume.

To characterize the spectral contrast signals of GNBP in vivo in a mouse leg, we subcutaneously injected 2 µL of 6 nM PEGylated GNBPs into the foot of a healthy nude mouse. A higher concentration (6 nM) for in vivo imaging was used as we found it produced a more reliable signal when visualizing lymphatic vessels in vivo. A comparison between 6 nM and 1 nM GNBP concentrations is shown in Figure S4. Previous in vivo lymphatic studies have also used this concentration. Prior to injection into the hind limb, a layer of skin was surgically removed to expose the saphenous vein for imaging as previously described. The injections were conducted using a syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) with a 30-gauge needle. Before and after injection an OCT scan was performed along the leg and saphenous vein.

For ex vivo popliteal lymph node imaging, we performed blunt dissection to expose the popliteal lymph nodes of mice after they underwent imaging of lymphatic vessels in the leg. After dissection and removal, we acquired a single PS-OCT volume of the resected lymph nodes. Control popliteal lymph nodes were acquired from mice which had no previous injection of GNBPs. Average enface projections were computed using the intensity, DOP, and spectral contrast.

To characterize cellular uptake of GNBPs within the popliteal lymph node, we extracted lymph nodes after 6 nM in vivo injection of GNBP1394 (Figure S5). Cells were H&E stained and imaged using an Olympus FV1000-MPE microscope and 100x (UPLSAPO) objective. The sample was illuminated with a 405 nm laser in transmission mode to detect absorption. The 405 nm wavelength is near the transverse GNBP resonance (Figure S1). Cellular uptake of GNBPs was expected and observed in previously published in vitro studies using GNBPs.

To characterize multiplexing of GNBP1200 and GNBP1394 in vivo in a mouse ear, we intradermally injected 1 µL of 6 nM PEGylated GNBPs into the ear pinna of a healthy nude mouse at two different locations. Volumetric PS-OCT imaging was performed (11 averaged volumes, 1184 Bscans/volume and 1024 A-lines/B-scan) on a 5x5 mm area before any GNBP injections and then once after each particle was injected. The injections were conducted using a syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) with a 30-gauge needle.

6. Electromagnetic Simulations of the Gold Nanobipyramids

We simulated the electromagnetic response of a gold nanobipyramid (GNB) with the finite element method. The GNB is placed in a spherical dielectric environment with a perfect matched layer shell. The simulation environment is water (refractive index \(n_e = 1.32\)). The dielectric function of gold is from the reference. Linearly polarized plane waves are launched to excite the optical modes and the finite element method package solves for the stationary solutions in the frequency domain. The longitudinal or
transverse mode can be selectively excited by aligning the plane wave polarization with the GNBP longitudinal or transverse axis. The solved electric field is used to evaluate extinction cross-section spectra. The single particle optical cross sections are evaluated through the following process. The time averaged Poynting vector (energy flux density) is given by:

\[ \langle S \rangle = \frac{1}{2} \text{Re} \left[ E(r, \omega) \times H^*(r, \omega) \right] \]

where a bold italic letter indicates it is a vector with x, y and z components. The energy flux density of scattered field through a given surface with a normal unit vector \( n \) is given by:

\[ \langle S_{scat} \rangle = \langle S_{scat} \rangle \cdot n = \sum_{i=x,y,z} n_i \langle S_{i,scat} \rangle \]

We can evaluate the absorption, scattering and extinction cross sections by:

\[ \sigma_{abs} = \frac{1}{\langle S_0 \rangle} \iiint_V QdV \]

\[ \sigma_{scat} = \frac{1}{\langle S_0 \rangle} \iint_S \langle S_{scat} \rangle dS \]

\[ \sigma_{ext} = \sigma_{abs} + \sigma_{scat} \]

Supplemental Figures.
Figure S1. Simulation of (A) GNBP extinction, scattering, and absorption cross section when excited by a plane wave linearly polarized along the bipyramid (B) longitudinal and (C) transverse axis. GNBP longitudinal resonance peak is 1392 nm. (D) Strong differential in scattering is observed near the longitudinal resonance, consistent with previously studied high-aspect ratio GNRs.¹

Figure S2. Dilution series of GNBP₁₂₀₀ (5 pM, 10 pM, 50 pM, 100 pM, 500 pM, and 950 nM) mixed with human whole blood (10 g/dL Hgb), and intralipid control. (A) Intensity, (B) DOP, and (C) spectral contrast. (D) Quantification of spectral contrast and DOP within ROIs. ROIs were chosen to avoid contributions from spectral shadowing (absorption driven contrast). Scale bars = 250 µm. SC color scale = -1 to 1. DOP = 0.4 to 1.
Figure S3. OCTA enface view of before (A) and after (B) mouse leg GNBP injection. Colored arrows reference the same blood (vessels) visible before and after injection. Note the new OCTA signal on either side of the saphenous vein (green arrow) which appears after GNBP injection. (C-F) Cross-sections (blue-dotted line) of OCTA (C, D) and DOP (E, F) before and after GNBP injection. New OCTA and low DOP signal appears after injection on either side of the green arrow labeled blood vessel. (G) 5 mm OCTA enface view after injection. Animal leg movement during needle insertion into the foot resulted in a small shift in focal position before and after GNBP injection. Red box and arrows highlight related features in the full 5 mm post injection scan. Scale bars = 1 mm. Angio scale = 0 to 1. DOP = 0.4 to 1.
Figure S4. Comparison of (A) intensity, (B) DOP, and (C) spectral contrast between intralipid, 1 nM, and 6 nM GNBP<sub>1394</sub> concentrations. Capillary tubes were segmented and averaged across A-lines to produce a single depth profile (red dotted line). (D) SNR attenuation is higher in the 6 nM sample compared to 1 nM sample. (E) DOP remains constantly high in the intralipid control and equally low for both concentrations of GNBP<sub>1394</sub>. A small increase in DOP near the bottom of the capillary tube was observed due to low SNR. (F) Spectral Contrast starts strongly positive at both concentrations of GNBP<sub>1394</sub>, but decays more rapidly in the 6 nM sample. Both concentrations neutralize at greater depths due to low signal. Intralipid remains neutrally contrasting with some observed noise. Scale bar = 250 µm.

Figure S5. Evaluating cellular uptake of GNBPs after in vivo injection. (A) Microscopy image from an extracted popliteal lymph node with visible cellular uptake of GNBPs. Cells are H&E stained and illuminated with a 405 nm laser and imaged in transmission mode (absorption). (B and C) Respectively zoomed in areas showing cells and GNBPs. Absorption from GNBPs (black dots) can be observed both inside and outside of cells. Black arrows reference GNPB signal seen from within cells. The orange arrow highlights observed GNPB signal outside of cells. Scale bars = 5 µm.
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