



IMAGING LYMPHATIC DRAINAGE

Application Note

Abstract

The tracking of a contrast agent's lymphatic drainage enables further case studies of metastasis progression in lymph nodes, which is crucial for evaluating cancer staging and prognosis of patients. Using a healthy nu/nu nude mouse, a 50 μ l 1:1 mixture of glycol-chitosan coated gold nanoparticles (GC-AuNPs) and indocyanine green (ICG), both of concentration 50 μ g/ml, was injected subcutaneously into the mammary right fat pad. Approximately 17 hours after injection, the mouse was photoacoustically (PA) scanned using a TriTom system. The results show that the injected GC-AuNPs/ICG contrast has drained from the mammary fat pad into the right subiliac lymph node.

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Introduction

Malignant primary tumors spread cancerous cells by metastasizing through the lymphatic system or through vasculature. The first lymph nodes that metastatic cells drain from the primary tumor are designated the sentinel lymph nodes [1]. In order to effectively track the progression of metastasis *in vivo* through non-invasive imaging techniques, an experiment that visualizes injected contrast drainage to the right subiliac lymph node (Figure 1) is performed. This first study will drive future studies with cancer bearing mice and investigate how effectively metastasis can be tracked using contrast agent lymphatic drainage.

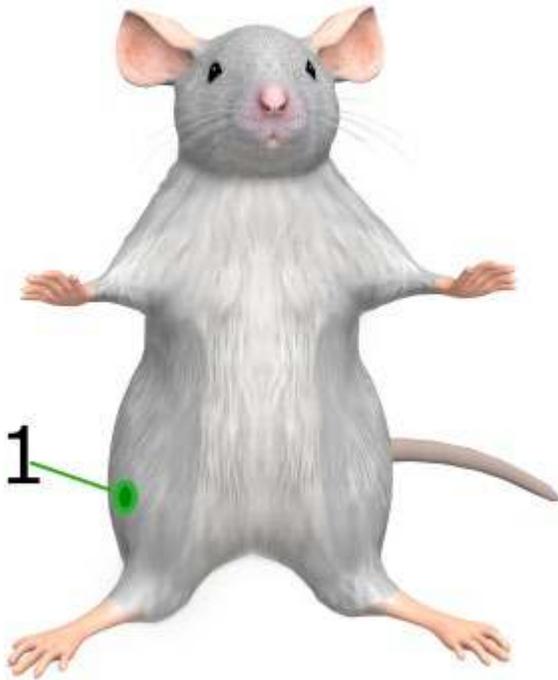


Figure 1: (1) The location of the right subiliac lymph node in a mouse.

Materials and Methods

Contrast Agent Synthesis

A 1:1 mixture of glycol-chitosan coated gold nanoparticles (GC-AuNPs) and indocyanine green (ICG) was used as a non-targeted dual-contrast agent. A sample of 50 μ l ICG (Sigma Aldrich, St. Louis, MO) of concentration 50 μ g/ml was mixed with a sample of 50 μ l of GC-AuNPs of concentration 50 μ g/ml, the recipe for the GC-AuNPs has been detailed by our collaborators [2]. The contrast agent, of volume 50 μ l,

was injected into the mouse's right mammary fat pad 17 hours before imaging.

Mouse Model

A healthy female nu/nu nude mouse (Charles River Laboratories, Wilmington, MA) was housed at the Georgia Institute of Technology's animal facility. The imaging procedure was approved by GIT's Institutional Animal Care and Use Committee (IACUC) and was performed in accordance with federal guidelines.

Imaging *in vivo*

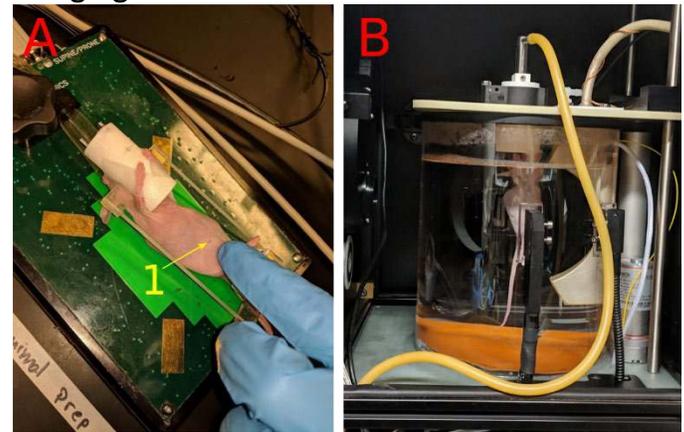


Figure 2: Mouse subject preparing to be scanned (A), the RSLN stained by the GC-AuNPs/ICG is identified (1). The mouse mounted into the TriTom's imaging chamber (B).

The contrast agent mixture is injected subcutaneously into the right 4th mammary fat pad of the mouse; 17 hours after injection, drainage can be visually observed to the right subiliac lymph node (RSLN)(Figure 2:A1). The TriTom imaging platform was prepared with the water in the imaging chamber at temperature $T = 37.0 \pm 0.5$ °C. The mouse subject was anesthetized using isoflurane gas and placed into a mouse restrainer, resting on a pad heated to 37°C (Figure 2:A). The mouse holder is then mounted onto the rotational stage of the TriTom (Figure 2:B) with the anesthesia fed into the breathing cylinder through tubing. Several 3D PA tomography (PAT) scans were initiated, each rotating the mouse 360° while acquiring 360±5 frames of PA data, for multiple wavelengths between 690-900 nm. Fluorescence (FL) image data was simultaneously acquired during 770 nm excitation scan.

PA and FL Reconstruction

The acquired PA data was reconstructed into 40x40x30 mm volumes with a voxel size of 0.1 mm using a filtered back projection method. The FL data was reconstructed

into a 40x40x30 mm volume with a voxel size of 0.1 mm using a radon transform algorithm.

Results

3D PAT

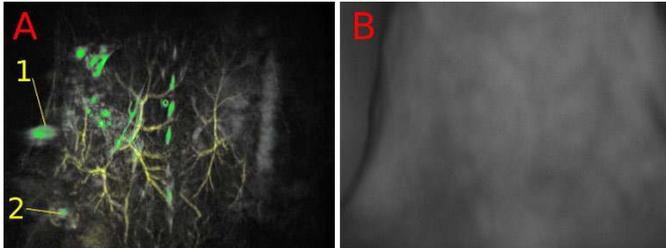


Figure 3: PAT volume (A) of the mouse's skin (532 nm, yellow) and high PA amplitude objects (770 nm, green). The RSLN (1) and injection site (2) are marked. Optical image for anatomical reference (B).

The 3D PAT reconstruction volume shown in Figure 3:A has the detected contrast in the RSLN marked (Figure 3:A1). Two volumes are superimposed: a 532 nm excitation scan's reconstruction in yellow and a 770 nm excitation scan's reconstruction in green. 532 nm is used for reconstructing superficial vessels on the mouse's skin as well as visualizing the skin's topography. The 770 nm excitation is used to induce both a PA and FL response from the ICG component of the GC-AuNPs/ICG contrast mixture.

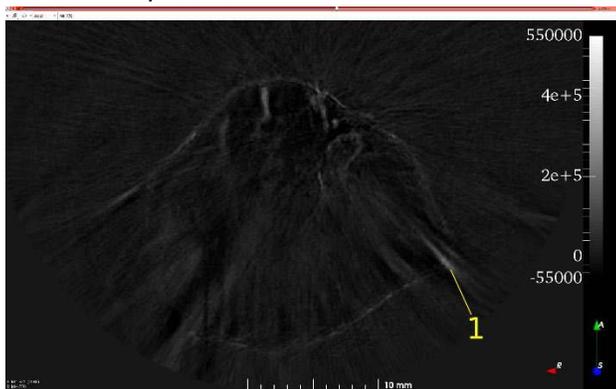


Figure 4: Single axial slice of the 770 nm reconstruction with the 532 nm reconstruction boundary for reference. The RSLN (1) is marked.

The slice view of the 770 nm reconstruction shown in Figure 4 features the RSLN (Figure 4:1) as the higher PA amplitude object in the slice.

Fluorescence Validation

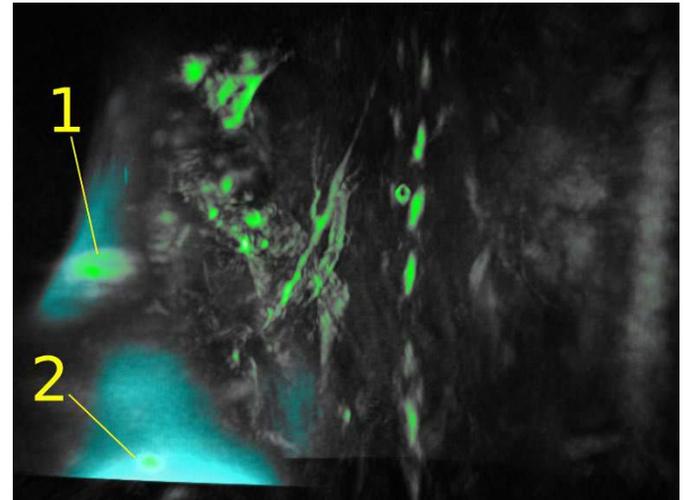


Figure 5: FL volume (770 nm, blue) and high PA amplitude objects (770 nm, green). The RSLN (1) and injection site (2) are marked.

The PA and FL volumes are overlapped in Figure 5, with the FL volume shown in blue. The FL data validates that the ICG component of the contrast agent has drained into the RSLN (Figure 5:1). The injection site, which is barely detected at the deeper anatomy 770 nm PAT volume, is visible in the FL reconstruction (Figure 5:2).

References

- [1] Jones, Dennis, et al. "Growth and Immune Evasion of Lymph Node Metastasis." *Frontiers in oncology* vol. 8 36. 21 Feb. 2018, doi:10.3389/fonc.2018.00036
- [2] Dumani, Diego S., et al. "Ultrasound-Guided Immunofunctional Photoacoustic Imaging for Diagnosis of Lymph Node Metastases." *Nanoscale*, vol. 11 24, 22 May 2019, pp. 11649–11659., doi:10.1039/c9nr02920f.