**Priority Report**

**Imaging of Human Lymph Nodes Using Optical Coherence Tomography: Potential for Staging Cancer**

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**Abstract**

Histologic assessment is the gold standard technique for the identification of metastatic involvement of lymph nodes in malignant disease, but can only be performed ex vivo and often results in the unnecessary excision of healthy lymph nodes, leading to complications such as lymphedema. Optical coherence tomography (OCT) is a high-resolution, near-IR imaging modality capable of visualizing microscopic features within tissue. OCT has the potential to provide in vivo assessment of tissue involvement by cancer. In this morphologic study, we show the capability of OCT to image nodal microarchitecture through an assessment of fresh, unstained ex vivo lymph node samples. Examples include both benign human axillary lymph nodes and nodes containing metastatic breast carcinoma. Through accurate correlation with the histologic gold standard, OCT is shown to enable differentiation of lymph node tissue from surrounding adipose tissue, reveal nodal structures such as germinal centers and intranodal vessels, and show both diffuse and well circumscribed patterns of metastatic node involvement. Cancer Res; 70(7); 2579–84. ©2010 AACR.

**Introduction**

The lymphatic system is a key constituent of the human immune system and provides a critical pathway for the metastatic dissemination of malignancies. It consists of multiple lymphoid tissue structures interconnected by a network of vessels through which lymph circulates. Lymph nodes, one class of lymphoid structures, contain functionally and morphologically distinct compartments: the cortex, paracortex, and medulla. Lymph nodes are surrounded by a stromal capsule, perforated by afferent channels, allowing lymph to enter sinuses which pass through the various lymphoid compartments, before exiting through an efferent lymphatic vessel. The cortex, the outer portion of the node, contains B-cell lymphoid follicles. After appropriate antigen stimulation, primary follicles enlarge and form germinal centers, in which the B cells develop the ability to generate antibodies against specific antigens. The paracortex is that part of the node located between the follicles. Paracortical tissue is populated chiefly by T-lymphocytes and contains vascular spaces which are portals for lymphocyte trafficking. The medulla, the deep or central portion of the node, contains cell-rich cords interspersed among medullary sinuses. The sinuses converge to form efferent lymphatic channels at the hilum, the point at which arteries and veins enter and exit the node.

The presence or absence of metastatic deposits in axillary lymph nodes is critical in clinical staging and is one of the most important prognostic indicators in individuals with invasive breast carcinoma (1). At present, this requires microscopic examination of excised nodes. Although dissection of all or most regional axillary nodes (axillary clearance) may be of therapeutic benefit in some individuals with breast cancer, the procedure may result in lymphedema (2). Techniques such as sentinel lymph node biopsy aim to limit the extent of initial regional node sampling to minimize such complications. However, such techniques still require excision of the node.

Recent research has highlighted the use of new optical imaging modalities to identify malignant cells. Examples include the use of confocal microscopy using gold nanoparticles conjugated with a monoclonal antibody as a contrast agent (3); angle-resolved low-coherence interferometry to characterize cancerous cells (4) and their change in response to chemotherapy (5); and fluorescence imaging to identify metastatic growth of tumor cells (6) and differentiation of normal and malignant lymphocyte populations (7).

Optical coherence tomography (OCT; ref. 8) is a high-resolution optical imaging modality with many applications, including the assessment of breast carcinomas (9). It is conceptually similar to ultrasound, but uses reflections of low-power, near-IR light instead of sound waves. OCT has the potential to provide in vivo assessment of lymph node metastatic involvement.

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involvement and preliminary work has shown images of a human cervical lymph node specimen (10). The use of OCT may avoid unnecessary excision of benign, uninvolved nodes, and reduce the incidence of lymphedema.

This article presents the first morphologic study demonstrating the capabilities of OCT imaging of human lymph nodes. Through the use of custom software enabling correlation of the OCT data with histology, we identify the appearance of structural features in benign nodes and in nodes containing metastatic deposits of invasive breast carcinoma. To our knowledge, this report includes the first published OCT images of cancer metastases within human axillary lymph nodes.

**Materials and Methods**

**Imaging.** OCT, described in detail in refs. (8, 11), acquires images of tissue by illuminating a biological sample with a focused beam of nonionizing, near-IR light, and detecting the component of this light that is backscattered (reflected). Backscattering from different depths within the tissue is separated through a process referred to as interferometry, giving a narrow, one-dimensional depth scan into the tissue at a specific location. A three-dimensional data volume is constructed by acquiring a sequence of adjacent scans, at different locations over the area to be scanned. OCT does not require the introduction of an exogenous contrast agent. Different tissues have different scattering and absorption properties, allowing OCT to provide high-resolution morphologic imaging.

Within this study, two different OCT scanners were used to obtain three-dimensional data sets. Eighteen samples were scanned with a time-domain OCT scanner developed in-house, with a central wavelength of 1,320 nm, source bandwidth of 101 nm, and resolution in air of \( \sim 11 \text{ \mu m} \) in both axial and lateral dimensions. Images were acquired with an \( X-Y \) field of view of \( 6 \times 3.7 \text{ mm} \) and an imaging depth of approximately 1.6 mm. An additional 12 samples were imaged with a swept-source OCT system (ThorLabs) with a central wavelength of 1,325 nm, spectral bandwidth of 100 nm, and resolution of \( \sim 5 \text{ \mu m} \) in air.
lateral resolution of 15 μm and axial resolution of 12 μm in air. The X–Y field of view was 10 \times 10 \text{mm} and the imaging depth approximately 3 mm. Comparable results were observed with both imaging systems.

**Preparation and processing of lymph nodes.** Informed consent was obtained from the patients and the study approved by the Human Research Ethics Committee of Sir Charles Gairdner Hospital. Surgical procedures were performed by two of the authors (S. Hamza and C. Saunders). Thirty axillary lymph nodes were obtained from 19 patients undergoing axillary clearance or sentinel node biopsy. Within 15 min of excision, the fresh tissue was cut in 2-mm slices and kept hydrated in PBS until it could be imaged. During imaging, nodes were mounted on a glass plate. Glycerol was used to reduce the refractive index mismatch and improve coupling of the light by displacing any air gaps between the node and the glass plate.

After scanning, each node was fixed, embedded in paraffin, and sectioned in accordance with standard laboratory procedures, and H&E sections prepared. The H&E sections were digitally micrographed using a ScanScope XT system (Aperio Technologies), and matched against the three-dimensional OCT imaging volume using in-house viewing software developed in C++. The software allowed for the extraction of an arbitrary imaging plane from the OCT volume, allowing an optimal correspondence with histology. Representative images of seven lymph nodes were then selected to illustrate anatomic and pathologic features visible with OCT. High-resolution versions of these H&E histology and OCT images have been included in the online Supplementary Data for this article.

**Results**

A strong visual correlation between OCT and histology was identified in 91% of lymph node samples. Characteristic features of the microarchitecture visible under both OCT and histology were used to establish correspondence.

Lymph node 1 (Fig. 1, top row) shows corresponding H&E histology (left) and OCT images (right) of a benign node. There is a clear distinction between the node and surrounding adipose tissue, and the honeycomb structure of adipose cells is visible in the OCT scan. A small lymphovascular space within the node has been highlighted (B). The empty lumen appears as an area of low backscattering, appearing as light gray in OCT, with a thin delineation of more highly scattering stroma in the vessel wall. Variations in the OCT intensity could be attributed to the various lymphoid tissue compartments within the node.

Lymph node 2 (Fig. 1, bottom row) shows a benign, reactive node. The cortex contains prominent germinal centers (G), giving rise to a characteristic circular texture in the OCT image. Because this image was slightly axially displaced from the optimal focal plane of the OCT system, cytoplasmic membranes of individual adipose cells are not well defined. However, they are texturally distinguishable from cortical tissue. Two regions of highly scattering stroma from the node capsule have been circled, staining eosinophilic (light pink) in the H&E image, and appearing highly backscattering (dark) in the OCT image.

Lymph node 3 (Fig. 2) highlights the value of three-dimensional OCT imaging. Each OCT scan consisted of a three-dimensional volume acquired from the fresh tissue.
This is in contrast to histology, which provides a two-dimensional image of a thin (~5 μm) slice of fixed, stained tissue. Figure 2 shows two perpendicular planes taken through the OCT volume. In the OCT literature, these imaging planes are referred to as en-face and B-scan images, and are conceptually similar to axial and sagittal planes in other medical imaging modalities. The dashed line in each image shows the intersection of the two perpendicular planes. Note that the en-face image has been rotated to match the H&E image, rendering the dashed line slightly off the vertical axis. Intersections with a blood vessel are visible (B1, B2, and B3). The B-scan OCT image (Fig. 2, bottom right) shows a view longitudinal to the blood vessel, in which the vessel lumen appears as a tubular area of low backscattering (white), delineated by a thin vessel wall (dark gray). The three-dimensional nature of the vessel visible in this image reveals that B1 and B2 are distinct intersections with a single vessel. Note that if such images were acquired in vivo, then the blood vessel lumen would appear as a dark tubular structure due to the blood’s high scattering coefficient for near-IR light (12).

Lymph node 4 (Fig. 3, top) contains metastasis from a ductal breast carcinoma. The histology shows aggregates of malignant cells effacing the node architecture in an irregular fashion. Areas of uninvolved cortex (C) are visible in the OCT image as lighter areas of lower backscattering tissue, interspersed between darker regions of highly backscattering, tumor-permeated tissue (T).

Lymph node 5 (Fig. 3, bottom) also exhibits metastasis from a ductal breast cancer. The diffuse pattern of metastatic involvement gives rise to variations in the OCT image. Corresponding regions of tumor (T) and residual cortical tissue (C) have been identified in the histology and OCT images. Residual cortex is again found to be lower backscattering than malignant regions.

Lymph node 6 (Fig. 4, top) shows metastatic involvement by a ductal breast carcinoma. A relatively well-circumscribed metastasis is visible in both histology and OCT (T). In addition, a small centrally necrotic metastasis is illustrated (N). The capsule of the node is delineated by a layer of highly backscattering stroma (S), appearing as a darker region in the OCT image.

Lymph node 7 (Fig. 4, bottom) displays a well-delineated metastasis (T) from a ductal breast carcinoma. The remainder of the node consists of predominantly uninvolved tissue. A thin area of low backscattering cortex (light gray in OCT) is visible near the top of the node (C), adjacent to a region of the paracortex (PC). The paracortex appears as high backscattering regions (dark). These sinuses aggregate in the medulla (M), corresponding to a darker region in the center of

Figure 3. Top left, node 4 H&E; top right, node 4 OCT; bottom left, node 5 H&E; bottom right, node 5 OCT. T, areas effaced by diffuse sheets of metastatic tumor cells; C, uninvolved cortex. Insets in H&E images show the morphology of the metastatic carcinoma at higher magnification.
the node. Note that the black outline at the edge of the node, immediately above the labeled cortex, is an imaging artifact caused by the \textit{ex vivo} sample not sitting flat on the imaging plate during scanning.

\section*{Discussion and Conclusion}

The OCT images presented here show the potential of OCT for tissue differentiation. The honeycomb structure of adipose cells is clearly visualized. Characteristic patterns are also observed for both blood vessels and germinal centers. In addition, the three-dimensional structure of features such as blood vessels can be visualized. Stroma is found to be highly backscattering, in agreement with previously reported results (13). Areas of malignancy generally appear as more highly backscattering than healthy cortical tissue. Quantitative studies by Wax and colleagues (4, 5) suggest that this is due to changes in size and texture of cell nuclei as a result of neoplastic transformation.

Direct morphologic comparison between histologic and OCT images is made challenging by imaging artifacts in both types of images. The absolute intensity values in an OCT data set are a function of both tissue type and imaging parameters such as tissue depth, power and incident angle of the light source, imaging optics, and the effects of overlying tissue. Parametric image processing techniques for OCT (14) have been proposed to remove these imaging variations and to provide absolute quantification of the distinction between healthy and malignant tissue. In addition, OCT imaging is performed on fresh tissue, whereas subsequent histologic analysis requires fixation and further processing. Histologic samples undergo both shrinkage and deformation (15), primarily during sectioning and mounting, with tumor and normal tissues affected to different degrees (16).

To increase contrast between healthy and malignant tissue, ongoing research is exploring the use of optical contrast agents. Such contrast agents typically consist of gold nanoparticles with distinctive optical properties, conjugated to biological molecules (17). Some early work exists specifically describing such OCT contrast agents for breast cancer cells (18).

One difficulty with OCT when imaging structures deep within the body is its poor penetration depth in tissue, which is typically only 2 to 3 mm. However, several groups have shown that an OCT probe may be miniaturized and encased in a medical needle (19, 20). Such an imaging probe could be used interstitially to image lymph nodes \textit{in vivo}. As OCT needle probes directly couple to the tissue without an air gap, they have no need for a refractive index–matching medium such as glycerol.

An attractive feature of imaging techniques such as OCT is the potential to monitor changes in lymph node structure in response to therapy. This has potential therapeutic value in the neoadjuvant treatment setting.

In conclusion, the potential to image metastatic deposits using OCT opens the possibility of new \textit{in vivo} techniques for the assessment of lymph node involvement in breast cancer. In this preliminary morphologic study, we have presented the first published OCT images of cancer metastasis within human axillary lymph nodes, and have identified characteristic backscattering patterns. Interpretation of the
results was facilitated through one-to-one comparison with histologic slices. The results of the ex vivo work presented here establish a basis for pathologic features to be assessed in larger OCT clinical studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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