Three-Dimensional Visualization of Lymph Node Morphology using OCT

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Abstract: We report the first demonstration of OCT for the three-dimensional visualization of lymph node morphology and microarchitecture from human and carcinogen-induced rat mammary tumor specimens.

OCIS codes: (170.1610 Clinical applications; 170.3880 Medical and biological imaging; 170.4500 Optical coherence tomography; 170.4580 Optical diagnostics for medicine)

1. Introduction

According to the American Cancer Society [1, 2], breast cancer is one of the most frequently diagnosed cancers in women with approximately 211,240 new cases (or 32% of all new cancer cases among women) of invasive breast cancer and 58,490 new cases of *in situ* breast cancer reported each year in the U.S. It is also the second leading source of cancer deaths in women with an estimated 40,870 deaths per year. Over the years, the decrease in the number of breast cancer deaths has largely been attributed to increased awareness, earlier detection, and improved treatment.

The sentinel lymph node biopsy (SLNB) is a surgical procedure that maps the lymphatic system and evaluates the status of the sentinel lymph node to determine whether a primary tumor in the breast has metastasized. This technique has revolutionized the management of melanoma and solid tumors that metastasize through the lymphatic system. The sentinel lymph node is the first node encountered in the lymphatic drainage pattern leading away from the tumor site in the breast. This procedure drastically reduces the number of lymph nodes removed in the axillary area in order to make a diagnosis. In addition to providing an equally valid diagnosis, the SLNB is an effective method for the staging of breast cancer, especially in assessing whether the cancer has metastasized.

2. Tracers for the SLN mapping

The SLNB is performed once the lymphatic system has been mapped using a different number of techniques ranging from the use of radioactive tracers to dyes (methylene blue, fluorescent dyes, quantum dots). One common method uses a radioactive tracer, technetium-99, and a dye, methylene blue, to locate the sentinel nodes. A small dose of technetium-99 is injected along with the blue dye into the breast at the primary tumor site and allowed to circulate through the lymphatic system for a period of 30 minutes to 8 hours. The dye aids in the physical visualization of node location while the radioactivity level is used as a diagnostic marker to guide the entire removal of the lymph node. Other methods include standard X-ray and computed tomography (CT). More novel contrast agents are being developed such as NIR quantum dots and indocyanine green (ICG) dye which allow a more accurate localization of the sentinel lymph node. Magnetic resonance imaging (MRI) contrast agents such as G6 have also been demonstrated in mouse models to give 3-D lymphatic drainage maps. Similar to the methylene blue, these contrast agents take an extended period of time to circulate through the lymphatic system. Lymphazurin dye (1%) is currently our proposed dye of choice to complement OCT imaging as its absorption spectrum is in the visible range (500-700 nm) outside of the laser bandwidths typically used for OCT (700-900 nm, 1280-1370 nm). This dye is currently used clinically to map the lymphatic system in breast cancer, melanoma, and gastrointestinal tumors with a circulation time of 30-60 minutes.

3. Optical Coherence Tomography

OCT may provide beneficial alternatives to current methods for lymph node assessment, and subsequently affect the staging procedure as a low-cost and minimally invasive technique that is capable of imaging morphological structures at cellular resolutions as well as visualizing the micro-structures of lymph nodes in tissue without the use of radioactive tracers. In comparison to other optical imaging techniques, OCT is capable of imaging 1-2 mm in depth in highly-scattering samples, allowing for the real-time *in vivo* evaluation of the tissue prior to its resection.

The OCT system in this study used a Nd:YVO_4 pumped titanium:sapphire laser with a center wavelength of 800 nm and a 100 nm bandwidth yielding an axial resolution of ~ 2 μm in tissue. In the sample arm, a 20 mm achromatic lens was used to focus 10 mW of light down to a 15 μm spot size (transverse resolution). The interferometer employed a single-mode 50/50 fiber optic splitter (Gould Fiber Optics, Inc.) coupling the signals from the sample arm and a galvanometer-based reference delay (30 scan lines per second). Spatial scanning in the X-Y plane was accomplished via a pair of galvanometer-mounted mirrors (Cambridge Technology, Inc.). Time-domain detection was achieved via a dual-balanced detection scheme using a 125 kHz auto-balanced photoreceiver (New Focus Inc., Model #2007) and data acquisition was performed by a dedicated computer cards (National Instruments, Model #PCI-6110, PCI-6711) with a 10 MHz sampling rate, a 12-bit quantizer, and a ±5V input range. Data acquisition in the spectral domain was achieved using a diffraction grating with 830 grooves / mm and blazed for 828 nm (Richardson Grating Laboratory, Rochester, NY) to disperse the light, and a lens to focus onto a line scan camera (Model #L104K, Basler Vision Technologies). The time domain and spectral domain systems have measured SNRs of 100dB and 90dB and acquisition rates of 10 lines/sec and 29,000 lines/sec, respectively. The rat lymph nodes were imaged under time-domain while the human samples were imaged under spectral-domain.

4. Tissue Specimen

The *N*-methyl-*N*-nitrosourea (NMU) carcinogen was injected intraperitoneally in virgin female rats to induce mammary tumors [3]. This is one of the most widely used animal models the study of the development of breast cancer. Mammary carcinogenesis in this animal model closely resembles that of the human breast carcinogenesis including hormone dependency and histopathological features. Invasion of the regional lymph node chain has been observed with this rat model, and distant metastases to the lung have also been reported. Similar patterns of invasion and metastasis are also observed in the human case. The induced tumors emulate the carcinogenesis of human ductal carcinoma, first as ductal carcinoma *in situ*, then as locally invasive disease, and finally as metastatic disease to the liver, lung, and spleen. To provide clinical relevance, late-stage human lymph nodes were imaged under OCT. The cervical lymph node specimen was resected from an 80-year-old female patient with Stage 4, T4N2b squamous cell carcinoma of the oral cavity.

5. Results

Mesenteric rat lymph nodes were imaged *in vitro* using OCT. A total of 256 2-D images of each specimen were acquired in 10 μm spatial increments to construct the 3-D images. Detailed structure can be seen in the left set of OCT images in Figure 1, including the lymphoid follicles located at the outer part of the node, the adipose tissue in which the lymph node is embedded, and the nuclei of adipocytes, which are displaced to the edge of the cells by the central lipid collection. In the right set of images, detailed internal structures are apparent including the capsule, a strong fibrous tissue enclosing the lymph node, the cortex, the outer part of the node containing dense masses of lymphocytes, and the medullary sinuses, the central section of the node composed of lymphoid elements with large sinusoids.

The late-stage metastatic human lymph nodes are shown in Figure 2. The images on the left side contain a region infiltrated with tumor. The inhomogeneous scattering regions in the OCT images are a result of the destruction of the lymph node architecture, and contrast the optical scattering properties observed in the OCT images of normal nodes. The images on the right show regions of necrotic tumor tissue bordered by adipose tissue. Clear microstructural and cellular scattering differences are noted when compared with the normal lymph nodes.

The observed internal lymph node architecture is clearly identifiable when imaged from the external surface, and strongly correlated with the histology, indicating that lymph nodes are a promising target as a clinical OCT application. Despite the depth penetration limits of OCT imaging, this study shows that relevant features are accessible from the surface and that in many cases the entire node can be visualized in 3-D [4]. Reactive nodes increase significantly in size but also have larger reactive follicles near the surface, which are amenable to OCT imaging. Appropriate minimally invasive OCT forward-imaging devices, such as those previously reported, suggest *in vivo* imaging is a feasible means by which to assess nodal structure allowing for the selective removal of metastatic lymph nodes. Recent development in high speed image acquisition such as spectral-domain OCT and optical frequency domain imaging (OFDI) makes 3-D imaging more practical for the clinical setting.

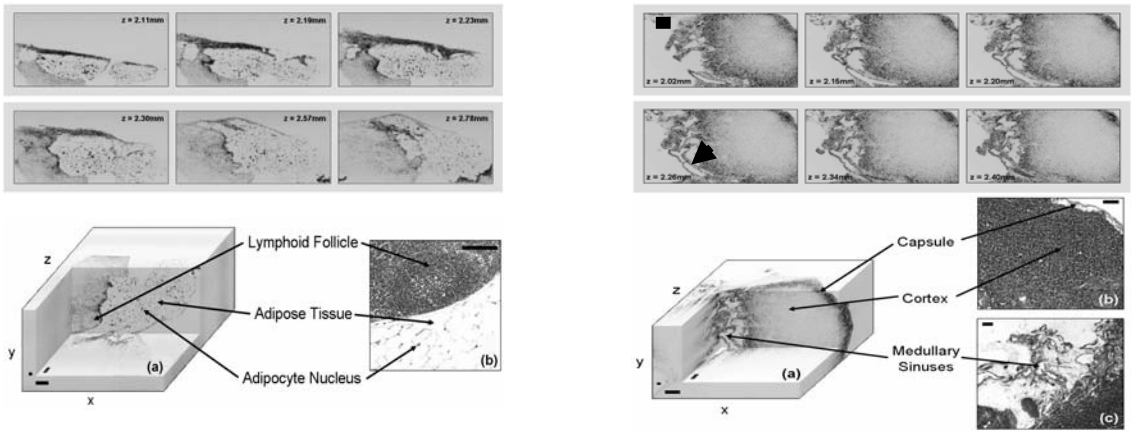

Fig. 1: The lymph node was translated in 10μm increments in the X-Z plane to acquire each 2-D image comprising the 3-D data set. Pixel dimensions in the 3-D set are 1,10,3 μm in the x, y, and z directions. Scale bars = 100μm. Left: Images of an *in vitro* rat lymph node showing the boundary between mammary adipose tissue and lymph node with visible structures, including adipose tissue and a lymphoid follicle. Right: Images of *in vitro* rat lymph node showing internal structures with visible structures, including the capsule, cortex, and medullary sinuses. Top: Sequence of 2-D OCT slices. Bottom: (a) 3-D OCT Reconstruction. (b, c) Corresponding H&E.

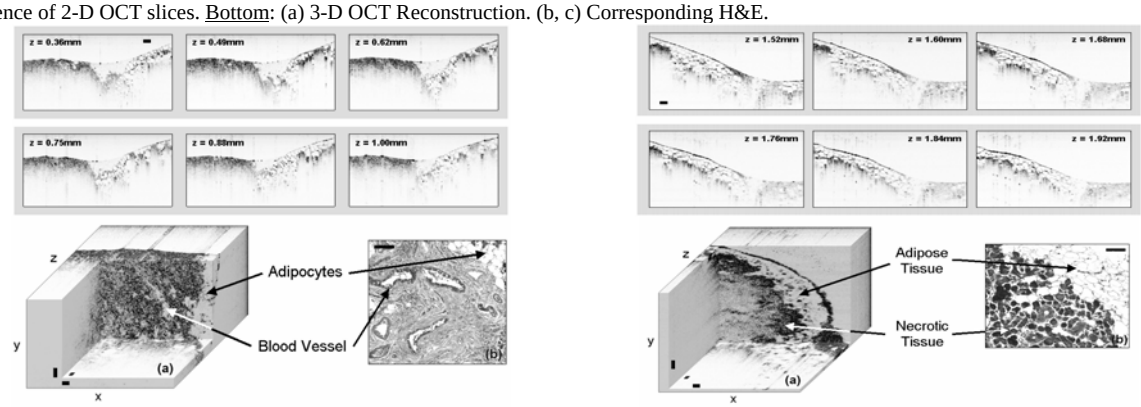

Fig. 2: Left: Images of metastatic human lymph node bearing infiltrating squamous cell carcinoma showing the structure of blood vessels and squamous cell growth. Top: Sequence of 2-D OCT images. The lymph node was translated in 7μm increments in the X-Z plane to acquire each 2-D image comprising the 3-D data set. Pixel dimensions in the 3-D set are 2, 7, 3 μm in the x, y, and z directions. Right: Images of human metastatic lymph node bearing infiltrating squamous cell carcinoma with evident regions of advanced necrosis. The lymph node was translated in 8μm increments in the X-Z plane to acquire each 2-D image comprising the 3-D data set. Pixel dimensions in the 3-D set are 2, 8, 3 μm in the x, y, and z directions. Top: Sequence of 2-D OCT slices. Bottom: (a) 3-D OCT Reconstruction. (b) Corresponding H&E. Scale bars = 100μm

6. Conclusions

We have demonstrated the potential of OCT for 3-D imaging of lymph node morphology with high resolution. This method may be well-suited for identifying suspect or sentinel lymph nodes intraoperatively, such as during breast tumor resection and staging. Using computationally-constructed 3-D OCT data sets, many detailed internal structures were easily identified including the lymphoid follicles, the cortex, the capsule, and the medullary sinuses. When a lymph node becomes a reactive node or contains metastatic tumor cells, the physical size, tissue composition, and optical scattering properties change, which should all be evident under OCT. Given further study, OCT in combination with current lymph node mapping procedures may be able to provide physicians with real-time intraoperative evaluation and staging of metastatic breast cancer. Current ongoing studies include using OCT to detect the presence of micro-metastasis, the optical characterization of the various stages of infiltration, and more importantly, determining the optical differences between normal non-reactive nodes, benign reactive nodes, and malignant reactive nodes.

7. References

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