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Simultaneous multiwavelength laminar optical tomography

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Abstract

Spatially resolved reflectance measurements can be used to characterize the depth-resolved optical properties of superficial tissues. However, until now, rapid acquisition of multiwavelength data has been hindered by multiplexing problems. We report on a novel multiwavelength laminar optical tomography system capable of acquiring data from multiple source-detector separations at three wavelengths simultaneously. Such data can allow *in vivo* depth-resolved spectroscopic imaging of absorbers, such as oxy- and deoxyhemoglobin, or of multiple fluorophores, that is unaffected by motion artifacts at frame rates exceeding 100 Hz. The system design and phantom validation studies are presented.

Laminar optical tomography (LOT) is an optical imaging technique capable of high-resolution depth-resolved measurements of living tissue. LOT uses a noncontact reflectance-measurement geometry and can acquire high-density measurements over fields of view exceeding 3 cm at over 100 frames per second. LOT offers measurement sensitivity to depths of 2 mm as well as the ability to quantify the depth-dependent concentrations of chromophores and fluorophores [1]. Previously reported uses of LOT include imaging of a rodent brain and heart [2,3], where its ability to resolve oxy- and deoxyhemoglobin, or the fluorescence of voltage sensitive dyes, allowed measurement of the function of intact tissues. Additional applications of LOT that we are exploring include imaging skin lesions to investigate lesion boundaries and depth of invasion.

Until now, multiwavelength LOT data acquisition has been hindered by the need to time-multiplex each laser wavelength using shutters. The resulting slower frame rates and motion artifacts can be significant drawbacks for imaging fast hemodynamic responses, since data at each wavelength will be acquired at a slightly different time. Simultaneous acquisition is also beneficial for clinical imaging, where patient motion artifacts are likely and short scan times are desirable.

This Letter describes a new LOT system that can simultaneously acquire spectroscopic measurements of chromophores, such as melanin and oxy- and deoxyhemoglobin [3], and fluorophores, such as collagen, metabolites, and exogenous contrast agents [2]. This approach allows point-by-point spectroscopic analysis and image frame rates of up to 100 frames per second. The image quality of raw LOT data rivals that of a CCD camera but simultaneously provides quantitative measures at three wavelengths, fluorescence data, and depth-resolved information.

The fundamentals of LOT have been described previously [1]. Briefly, LOT relies on detecting light that has been scattered within tissue and then emerges at multiple distances (between 0 and 3 mm) away from a scanning spot. The emitted light contains information about the tissue's

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optical properties and depth-dependent structure. In a similar way to confocal microscopy, LOT acquires measurements by scanning a laser beam over the tissue using galvanometer mirrors. Within the tissue, the light is scattered such that a fraction is re-emitted from the tissue's surface. This emitted returning light is descanned by the galvanometer mirrors and directed toward a photodetector array. A confocal microscope would use a pinhole aperture to block off-axis light in this plane. However, LOT captures this returning off-axis light because the signal detected at a position adjacent to the returning beam's focus corresponds directly to light emerging from different distances away from the scanning beam on the tissue. The wider the offset between the beam's focus and the detected light, the deeper, on average, the scattered light has traveled.

The implementation of the new system is shown in Fig. 1. Three lasers, 488, 532, and 638 nm (85-BCD-030-115, 85-GCA-020, 56RCS004/HS, Melles Griot), are collinearly aligned into a polarization-maintaining single-mode fiber (PM460-HP, Thorlabs). The fiber delivers the beam to a polarizing beam splitter, passing only *P*-polarized light, through a three-line dichroic filter (Di01-T488/532/638, Semrock) and onto 6 mm *x-y* galvanometer mirrors. The galvanometer mirrors, capable of scanning 4500 lines per second, raster scan the beam through scan and objective lenses and onto the tissue. Light emerging from the tissue travels back through the lenses and is descanned by the galvanometer mirrors. Two different detection paths are used to measure the fluorescence and backscattered multiwavelength light. Fluorescent light is reflected by the dichroic filter and focused onto a linear array photomultiplier tube (PMT) (R5900U-01-L16, Hamamatsu). Backscattered multiwavelength light passes through the dichroic filter and is reflected by the polarizing beam splitter before being focused onto a slit. The polarizing beam splitter suppresses *P*-polarized specular reflections and reflects only *S*-polarized light.

In our previous system [1] returning light was coupled into a linear fiber array, with each fiber delivering light from a different offset to an avalanche photodiode. Multiwavelength measurements required the lasers to be switched on and off via shutters. The system presented here instead incorporates a dispersive element to separate the returning light by wavelength and a two-dimensional (2D) array PMT to measure the different wavelengths of light and all offsets simultaneously. For this approach to work, returning light must first be focused at a slit and then allowed to continue toward a diffraction grating that separates the light into red, green, and blue lines. These color-separated lines of light are focused onto an 8×8 channel 2D array PMT (H7546B, Hamamatsu).

The PMT array elements are connected to a custom-built 32 channel transimpedance amplifier (TIA) whose outputs are read by four eight-channel data acquisition boards (PCI-6133, National Instruments) capable of 3 MS/s per channel. A graphical user interface written in MATLAB allows the user to view data and set scan parameters, such as field of view, number of pixels, sample rate, and the number of frames. In the current configuration the illuminated spot scans a field of view of up to 20×20 mm with a maximum source-detector (S-D) offset of 2 mm (these limits can be varied by changing the system's magnification). The system can acquire a 200×200 pixel (source-position) image at 23 frames per second and a 45×45 pixel image at 100 frames per second.

We performed experiments to verify the new system's multispectral and depth-sensitive measurement capabilities. An agarose gel phantom was made using 2% agarose and sufficient bovine hemoglobin (100714, MP Biomedicals), and intralipid (I141, Sigma) to provide background absorption and reduced scatter coefficients at 532 nm of 0.2 and 1 mm⁻¹, respectively. In our first set of experiments, 4×4 mm pieces of 100 μm thick red, green, and blue transparency films (C-Line Products, Inc.) were embedded in the phantom at depths of 0.2, 0.4, and 0.6 mm (see Fig. 2). These depths were controlled using 200 μm sheets of agarose

created by sandwiching the liquid agarose mixture between two glass slides and cooling until solidified [4]; 200×200 pixel images of a 20×20 mm region were acquired at 23 frames per second. The data from 30 frames were averaged.

Figure 2 (top right) shows “raw” LOT images acquired at 638, 532, and 488 nm. The 638 nm data show highest contrast for the green and blue absorbers while the 488 and 532 nm data show higher contrast for the green and red absorbers, respectively. A red-green-blue (RGB) (638-532-488) merge of this raw data is also shown. While the RGB merge provides a photographlike image for visualization of the phantom, it should be noted that for raw LOT data, each laser wavelength has slightly different depth sensitivities.

Figure 2 (middle row) shows RGB-merged LOT raw data for three different S-D offsets. A small piece of transparent ruler (with millimeter gradations) was placed onto the phantom's surface for scale. Superficial absorbers in the 0.25 mm S-D separation image show strongest contrast; however, the deeper absorbers are less apparent. As the S-D offset increases, the photons probe deeper into the tissue and the deeper absorbers become more apparent. The wider offset images also have a shadow to the right of each absorber and on the tic marks of the ruler. This shadow occurs because less light is detected both when the laser spot is scanned over the absorber and when the imaged position of the detector scans over the absorber. For wider separations and superficial objects, this effect creates a shadow whose offset is equal to the S-D separation. For deeper objects, the shape and amplitude of this shadow is characteristic of the shape and depth of the buried object and constitutes part of the quantitative depth-specific information provided by LOT. Figure 2 (bottom row) shows simulated measurement sensitivity functions for detectors positioned at 0.25, 0.5, and 1 mm from the source. These sensitivity functions represent the probable light path within the background agarose gel and were generated using Monte Carlo modeling [1,5].

We expect that “raw” LOT images such as these will provide valuable subsurface contrast for a wide range of *in vivo* imaging applications, including dermal and intrasurgical imaging. These images are analogous to those shown for modulated imaging (MI) in [6], although MI faces significant multiplexing limitations for high-speed multispectral imaging. LOT has the advantage of high frame-rate “single-shot” acquisition of depth-resolved and multispectral data.

LOT's measurements constitute a “tomographic” data set containing quantitative information about the depth and optical properties of the tissue being imaged [7]. Therefore, a second set of experiments was performed to demonstrate LOT's ability to quantify the properties of an absorber at different depths. Absorbers, composed of 0.2 mm thick squares of 2% agarose, intralipid, and instant coffee, were embedded in the phantom at depths of 0.2, 0.4, and 0.6 mm (see Fig. 3). The absorption and reduced scattering coefficients of these inclusions at 532 nm were 5 and 1 mm⁻¹, respectively (mimicking the optical properties of a melanotic skin lesion). The phantom was imaged using LOT with the raw data from 30 frames averaged. Monte Carlo modeling of the phantom geometry was performed for comparison with the LOT measurements [5].

Figure 3 shows a drawing of the phantom and plots of the LOT measurements and simulated values. The plots show the fractional change in measurements $(M_a - M_o)/M_o$, where M_a and M_o are the averages of 10×10 pixel regions overlaying each absorber and a background region, respectively. The LOT measured data are in agreement with the simulation data. We confirmed this using a look-up table approach, where forward data were simulated for all three depths and a range of absorption contrast from 1 to 10 mm⁻¹. By fitting our data to these simulated values, we were able to unequivocally predict the depth of each object correctly and accurately estimate its absorption properties to within 20%.

In summary, we have presented a novel LOT system that allows simultaneous multiwavelength imaging of tissues at high frame rates. The system design and experimental validations were shown. We are currently using this system for high-speed imaging of functional activity in the rodent brain and for clinical studies of malignant skin lesions.

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Fig. 1.

(Color online) Schematic of LOT system. Collinearly aligned laser light is raster scanned over the tissue surface. Galvanometer mirrors descanned the returning light. Multiwavelength light is separated using a diffraction grating and focused onto a 2D PMT array.

Fig. 2.

Color Phantom. Top left, schematic of agarose gel-based phantom with embedded colored transparency film absorbers. Top right, simultaneously acquired raw LOT multiwavelength data individually and merged to form an RGB image. Middle row, RGB-merged LOT data for three S-D offsets; deeper absorbers become more apparent at wider S-D offsets. Bottom row, Monte Carlo sensitivity functions for the same three S-D separations.

Fig. 3.

Agarose gel based phantom with three absorbers embedded at different depths. Plot shows the fractional change in measurement $\Delta M/M_0$ for LOT data (solid curves) and Monte Carlo simulation data (dotted curves) for each absorber depth.