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Backscattering spectroscopic contrast with angle-resolved optical coherence tomography

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Abstract

Backscattering spectroscopic contrast using angle-resolved optical coherence tomography is demonstrated as a powerful method for determining scatterer diameter with subwavelength resolution. By applying spectroscopic digital processing algorithms to interferograms acquired in the frequency domain with a wavelength-swept laser centered at 1295 nm, it was shown that differences in wavelength-dependent backscattering from 0.3 and 1 μm diameter microspheres can be clearly resolved. The observed backscattering spectra were found to be consistent with Mie theory. High levels of speckle noise reduction achieved by angular compounding increased the spatial resolution at which backscattering spectra could be accurately differentiated.

Optical coherence tomography (OCT) and its recent derivative, optical frequency domain imaging, are powerful tools for measuring depth-resolved reflectance noninvasively. With standard image reconstruction methods, OCT is color-blind, however, in the sense that it is not directly sensitive to the spectral dependencies of backscattered light. In comparison, spectroscopic OCT (sOCT) allows this information to be extracted from interference spectra by using digital signal processing methods [1]. As such, sOCT has the potential for generating contrast for chromophores and scatterers with characteristic spectral features. It has recently been observed with spectroscopic spectral-domain optical coherence microscopy that biological tissue can be a rich source for spectroscopic contrast [2], although the underlying contrast mechanisms are still not well understood. One exciting possibility, supported by theoretical arguments [3], is that scatterer size can be a potent contrast mechanism in sOCT. Contrast for scatterers at a 0.1-10 μm level is particularly desirable for optical diagnosis, as the size of intracellular organelles is correlated with important pathologies, such as cancer. Recent developments in spectroscopic OCT have largely been focused on imaging with light sources operating in the range of 800 nm, where hemoglobin [4] and exogenous agents such as nanocages and photodynamic therapy-related dyes [5,6] have absorption peaks. The penetration of 1300 nm light within tissue is significantly higher than of 800 nm light, which makes it attractive for future applications of spectroscopic OCT in highly scattering tissues such as those found in the gastrointestinal tract.

One of the main challenges in conducting sOCT is the presence of speckle noise, which obscures subtle differences in backscattering spectra from tissue. Resulting from the coherent addition of waves with random phases, speckle patterns vary as a function of wavelength [7], thereby degrading spectroscopic information. In conventional OCT, speckle manifests itself as a grainy texture and limits the signal-to-noise ratio (SNR) to near-unity values. Averaging across uncorrelated realizations of speckle noise is essential for achieving high spectral

resolution [8]. This is typically performed across multiple depth and transverse locations, with a corresponding loss in spatial resolution.

The recent advent of angle-resolved OCT, which allows the acquisition of images at hundreds of different backscattering angles, enables high levels of averaging to be performed in the angular domain instead of, or in addition to, the spatial domain [9]. This feature is advantageous, as angular averaging does not necessarily involve a substantial loss in spatial resolution. In this Letter, backscattering spectroscopic imaging is demonstrated using angle-resolved OCT. A lines acquired from different angles were averaged for speckle reduction, resulting in significantly improved backscattering spectral and spatial resolution.

An optical phantom consisting of agar gel (0.5% by weight) and polymer microspheres (Duke Scientific) was created. The phantom was designed to be homogeneous in the axial direction, but it consisted of two sections that were immediately adjacent to each other in the transverse direction. The microspheres in the first section were 0.3 μm in diameter; those in the second section were 1 μm in diameter. The density of the polymer microspheres in the 1 μm section was chosen to be 6.6 times higher than that of the 0.3 μm section, so that the backscattering coefficients of the two sections were approximately the same. During imaging, the phantom was oriented so that its surface normal was at a 15° angle with respect to the incident beam to minimize specular reflection from the surface.

The angle-resolved OCT system, as previously described [9], consisted of an interferometer in which backscattered sample and reference light was detected with a line scan camera. The line scan camera allowed the simultaneous acquisition of interference spectra from 400 backscattering angles across the range of $\pm 10^\circ$. With the source wavelength swept linearly in time from 1230 to 1360 nm, the depth dependence of the reflectance was encoded in the Fourier domain. The focused spot size was 14 μm (FWHM) in air; the axial resolution of the system was 11 μm in air.

Analysis of the interference spectra was performed with and without spectroscopic methods. With the spectroscopic-sensitive method, five different A lines were generated by separately multiplying the entire interference spectrum by five nonoverlapping Hanning windows. The chosen number of spectral windows represented a compromise between the increased spectral information obtained from having more windows, and the increased axial resolution obtained from having fewer ones. The windows were of equal size in wavenumber space (k space). A Fourier transform was applied to each windowed spectrum, and the magnitudes of the resulting complex reflectance values were obtained [1]. The application of this procedure to all A lines yielded five optical frequency-domain images corresponding to spectral regions centered at wavenumbers $\{k_1, \dots, k_5\}$. With the spectroscopic-insensitive method, which we denote the “standard” method, only one A line was generated from each interference spectrum; a Hanning window spanning the entire spectrum was applied. With both of the methods, background subtraction and interpolation to equispaced k space values were performed as preprocessing steps prior to spectral windowing. All processing steps were performed separately for interference spectra acquired at different angles. Angular compounding was accomplished by averaging the magnitudes of the Fourier-transformed data; subsequently, the square of the average magnitude was computed. As a final processing step, a Gaussian spatial filter with standard deviation of 5 image pixels was applied in the transverse image dimension, parallel to the surface of the tissue phantom.

Speckle notwithstanding, variations in the magnitudes of the complex reflectances $S(k_i, z_j)$ obtained from different spectral windows derive primarily from three wavelength-dependent quantities: the laser source intensity $G(k_i)$, backscattering, and attenuation due to forward scattering and absorption. In the case where the reflectance derives from depths near the surface

of the sample, so that the third quantity can be neglected, $S(k_i, z_j)$ can be approximated as being proportional to the backscattering coefficient, μ_b :

$$S(z_j, k_i) \propto G(k_i) \mu_b(z_j, k_i).$$

To remove the confounding effects of the term $G(k_i)$, reflectance magnitudes $S(k_i, z_j)$ were divided by a reference spectrum, yielding normalized backscattering spectra. The reference spectrum was obtained by averaging reflectance magnitudes corresponding to the top surface of a microscope coverslip that was mounted on top of the phantom.

Spectroscopic contrast at individual image pixels was obtained by measuring the change of μ_b with respect to wavelength. Specifically, linear fits of the normalized backscattering spectra were performed by using the method of least squares. Least-squares analysis of spectroscopic data has previously been shown to be a powerful method of speckle noise reduction [10]. The slopes from the linear fits were depicted as red, green, and blue colors, overlaid onto the log-transformed, standard OCT images. With this color scheme, an increase in backscattering at longer wavelengths corresponds to red pixels, and a decrease, to blue pixels; intermediate slopes were observed at green pixels.

Standard OCT images obtained with a single backscattering angle yielded very little contrast between the two phantom sections due to the similarities of the respective backscattering coefficients at the center wavelength of the laser source spectrum, as shown in Fig. 1(a). With angular compounding across 64 angles, shown in Fig. 1(b), subtle intensity differences within the two sections were apparent. These differences can be attributed to inhomogeneities in the distributions of microspheres. The backscattering spectra from the two sections were markedly different, however. Indeed, on average, the backscattering was seen to increase with wavelength in the section with 1 μm microspheres, whereas a decreasing relationship was observed in the section with 0.3 μm microspheres. The results are in good agreement with Mie theory, presented in Fig. 2.

Spectroscopic contrast images generated without angular compounding show color variations across the two sections that are in accordance with the averaged backscattering spectra from the two sections: red colored pixels predominate over blue ones in the section with 1 μm scatterers, with the opposite pattern observed in the section with 0.3 μm scatterers [Fig. 3(a)]. However, colors within the two sections were not homogeneous, with erroneous values directly attributable to speckle noise. Angular compounding across 1, 4, 16, and 64 angles resulted in SNR increases of 1.0, 1.9, 2.5, and 2.5, respectively. Given that the SNR increases proportionally to the square root of the number of uncorrelated samples, it can be concluded that for this phantom, SNR increases were limited by angular correlations in the backscattered field.

The SNR improvements achieved by angular compounding greatly improved the spectroscopic contrast between the two regions. The greatest improvement in contrast was observed between the case without angular compounding and that with angular compounding across four angles [Fig. 3(b)]. Improvements based on increasing the number of angles from 4 to 16 were small [Fig. 3(c)]. No appreciable difference was observed with an increase from 16 to 64 angles [Fig. 3(d)], which is consistent with the SNR's having been approximately equal in these cases.

Our results demonstrate that scatterer diameters can be differentiated with subwavelength accuracy, given narrow particle size distributions within each phantom section. As such, the results are analogous to those obtained with optical scatter imaging, in which scatterer diameter is inferred from the ratio of images collected at high and low numerical aperture in central

dark-field microscopy [11]. Biological tissue is known to contain a broad continuum of particle sizes, however. Differences between backscattering spectra resulting from different particle size distributions typically decrease as the widths of the distributions increase, so that the inverse problem of inferring distributions from backscattering spectra may not always be well posed. As the diameter of scatterers becomes significantly smaller than the wavelength, backscattering spectra approach the inverse fourth-power limit of Rayleigh scattering; we may expect only slight differences in color for scatterers within this regime. In general, the accuracy at which particle size distributions can be recovered with sOCT is a complicated problem that depends on both the sizes of the scatterers and the parameters of the spectroscopic algorithms. The use of angle-resolved OCT to improve spectroscopic contrast in biological samples will therefore require further analysis of these issues.

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Fig. 1. OCT images of the phantom obtained (a) from a single angle and (b) from compounding across 64 angles. The arrow indicates the boundary between the sections of the phantom containing $1\ \mu\text{m}$ spheres (left) and that with $0.3\ \mu\text{m}$ microspheres (right). The transverse extension of the images is 5 mm. The scale bar indicates $100\ \mu\text{m}$ in the axial direction within the phantom.

Fig. 2.

The backscattering cross section of 1 μm spheres increases by a factor of 2 across the 130 nm tuning range of the source laser, whereas that of the 0.3 μm spheres decreases by a factor of 0.8. The measured values, obtained by averaging backscattering spectra from corresponding sections of the agar tissue phantom (circles), are in good agreement with theoretical values obtained from Mie Theory (solid and dashed). The data were scaled uniformly so that the theoretical values at the shortest wavelength were unity.

Fig. 3.
 (Color online) Spectroscopic OCT images of the phantom obtained (a) from a single angular sample and by averaging across (b) 4, (c) 16, and (d) 64 angular samples. The pixels are color coded according to the observed change in wavelength-dependent backscattering. The arrow indicates the boundary between the sections of the phantom containing 1 μm spheres (left) and 0.3 μm microspheres (right).