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High-resolution computed tomography of refractive index distribution by transillumination low coherence interferometry

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Abstract

We present a method to image refractive index distribution within a sample across 8mm dimension with high spatial resolution by a transmission low coherence interferometer. The relative strong forward scattering light is collected from which the parallel projections of refractive indices within the sample are obtained. A convolution back-projection algorithm is used to transform the projection-data set recorded at sufficient angular views into the spatial distribution of refractive indices within the sample. We experimentally demonstrate this method by imaging a phantom. We show that this method can achieve a precision of 0.01 in determining the refractive index and a spatial resolution of 40 μ m.

Detailed knowledge of the refractive index distribution within a heterogeneous turbid medium, such as biological tissue, is essential for better understanding of the light propagation in biological tissues. Such knowledge is also useful in further refining light-based diagnostic and therapeutic techniques as applied to healthcare. However, direct measurement of refractive index distribution within a tissue sample is currently technically challenging, partly because the light-transportation within tissue is severely limited by the efficient scattering and absorption of light. It is the refractive index that gives the imaging contrast mechanism for a number of high resolution optical imaging techniques, such as optical coherence tomography (OCT) and confocal microscopy. This is particularly true for transparent specimens, e.g., cells and early embryos of small animals, which typically exhibit low absorptive contrast. Recently, a number of papers describing refractive index tomography of biological tissues have been presented. Charrière *et al* proposed to apply digital holographic microscopy to image refractive index tomography of cells [1]. Zvyagin *et al* used a variant of OCT, bifocal optical coherence refractometry, to image the refractive index of the turbid media [2]. Boppart *et al* presented an approach to reconstruct the spatial distribution of refractive indices using OCT [3]. For all the methods that use OCT to map the refractive indices, the back scattering photons emerged from a sample were collected to reconstruct the refractive indices within the sample. However in the therapeutic window of the light-tissue interaction, the value of anisotropy parameter g is typically between 0.8 and 0.95 for the most tissues [4], implying that the light transportation in the tissue is weak in backscattering, but strong in forward scattering. The relatively weak backscattering places a constraint on the achievable imaging depth within biological tissues for the current available techniques. In this paper, we present an approach that utilizes the strong forward scattering characteristics of the light transportation within tissue in order to image refractive index distribution of relatively thick samples. The approach is realized by a transmission low

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coherence interferometer, a concept similar to OCT, which is termed here as transmission OCT (TOCT) for convenience. The concept of trans-illumination imaging has been explored previously to image deep tissue micro-structures [5,6] and functional information [7,8].

TOCT demonstrated in this paper is a variant of the spectral-domain optical coherence tomography [9], in which the light transmitted through, rather than backscattered from, the sample is detected by a high speed spectrometer. Assuming that the optical pathlength difference between the reference and sample beams in the spectral interferometer is initially matched in free space, and the refractive index distribution of the sample along the probe beam pass is $n(\mathbf{r})$, then the change of the optical pathlength for the light (ballistic photons) traveling through a sample can be written as:

$$\Delta z = \int (n(\mathbf{r}) - n_{air}) dl, \quad (1)$$

where n_{air} denotes the refractive index of air, and the integral is performed along the axial direction of the sample beam. Δz represents the optical pathlength change due to the projection of $n(\mathbf{r}) - n_{air}$ through the sample along the axial direction of the probe beam, i.e., the Radon transformation of refractive indices. If the projections within a cross section at sufficient views are collected through translating and rotating the sample, $n(\mathbf{r})$ can be reconstructed by use of the well-known convolution back projection algorithm [10],

$$n(\mathbf{r}) = n_{air} + \int_{-\infty}^{\infty} \int_0^{\pi} (p_{\theta}(l) * c(l)) \delta(x \cos \theta + y \sin \theta - l) dl d\theta, \quad (2)$$

where the asterisk denotes convolution, x and y are rectangular coordinates, θ is the angle from the y axis, l is the distance from the coordinates, $p_{\theta}(l) = \Delta z(\theta)$ is the projection of refractive indices at the view-angle of θ , and $c(l)$ is the spatial domain representation of an appropriate filter necessary for back projection algorithms. Compared with the backscattering configuration, such as conventional OCT system, the transmission configuration has an advantage of imaging a relatively thick biological sample because the scattering photons in the sample are strongly forward-directed. This arrangement can thus relax the limitation of achievable imaging depth by use of the conventional OCT to quantify the refractive indices by use of the backscattering photons. If a sample with an anisotropic factor of 0.9 is to be imaged and the system signal to noise ratio is fixed, then the possible thickness of the sample that can be imaged by TOCT is estimated about 9 times of that by the conventional OCT.

The schematic of TOCT system used in this study is shown in Fig. 1. This TOCT system used a superluminescent diode with a central wavelength of 820nm and a spectral bandwidth of 50nm coupled into a single-mode fiber-optic Mach-Zehnder interferometer. Considering the transmission configuration, the system has a free-space axial resolution of $\sim 12 \mu\text{m}$. Together with the reference beam, the sample beam that transmitted through the sample was coupled via the coupler C_2 into a spectrometer (HR4000, Ocean Optics) for detection of spectral interferograms. The sample was immersed in water for index matching. An x - z axis translation stage (Physik Instruments, Germany) and a stepping motor were used to translate and rotate the sample in order to collect the data at different views.

A plastic tube was imaged to verify the proposed method. The tube was embedded in a nearly transparent gel cylinder made of 2% agar gel. The data collected by translating the

sample with a step of $20\mu\text{m}$ at one view within a cross section forms a parallel projection of the refractive indices across the probe beam path. A total of 400 such parallel projections were collected by rotating the sample through 360 degrees with a step size of 0.9 degree. The time required to collect the data representing one cross section was approximately 4.5h due to the slow scanning speed for the current system. This timing may be improved by use of a high speed scanning system. The spatial distribution of refractive indices was reconstructed by use of the convolution back-projection algorithm with Shepp-Logan filter and linear interpolation [10]. The computation time required to reconstruct one cross section of the sample was approximately 1.5min on a personal computer with a 1.8-GHz Pentium 4 processor.

One parallel projection of the refractive indices within a cross section is shown in Fig. 2, where the x axis denotes the translating positions and the parallel projection is displayed along the y axis. It can be seen that the signal due to the agarose gel is clearly distinguishable from that due to the background water. Therefore, we may conclude that the system is of at least 0.01 precision in determining the refractive index within the sample because the refractive index of the 2% agar gel is about 1.343 [11], while that of the water is 1.333. The strong refraction effect near the edge of the plastic tube results in abrupt changes in signals labeled as “a”. When the probe beam passes the edges of the tube, the refraction effect makes the refracted beam a relatively large divergence from the direction of the incident beam. For example, an incident angle of 80 degree results in a divergence of about 16 degree at the gel-plastic interface. Thus, the transmitted beams near the edges of the tube are difficult to be coupled into the detection spectrometer.

One typical reconstructed spatial distribution of the refractive indices within the scanned cross section is shown in Fig. 3. This image agrees well with the dimensions of the tube and the gel cylinder. The results indicate that the index variation less than 0.01 can be detected with this system.

The line profile of the reconstructed image at $x=8\text{mm}$ is shown in Fig. 4, where the two peaks labeled as “b” were resulted from the sharp variations of the projections near the edges of the tube as shown in Fig. 2. This sharp variation is also responsible for the straight-line artifacts seen in Fig. 3. For the case of imaging of tissues, this artifact may be less pronounced because it is less likely that a sharp interface exists within highly scattering biological tissues. To evaluate the spatial resolution for the system proposed, we cut across the profile with the half-amplitude and quarter-amplitude lines at points A_{1-4} and B_{1-4} , respectively. The sum of $(|B_1B_2|-|A_1A_2|)$ and $(|B_3B_3|-|A_3A_3|)$ is $\sim 40\mu\text{m}$. Thus, the spatial resolution of this system is better than $40\mu\text{m}$. The spatial resolution may be further improved by reducing the spot size of the probe beam, by increasing the number of translating and rotating steps, and/or by using a wider bandwidth of the illuminating light source.

In summary, we have demonstrated an approach of computed tomography of refractive index by use of Fourier domain transmission OCT. We have experimentally validated this approach by imaging of a plastic tube embedded within agarose gel. The reconstructed results demonstrate a precision of ~ 0.01 in determining the refractive index and a spatial imaging resolution of $40\mu\text{m}$. By collecting the relative strong forward scattering light rather than the relative weak backscattering light, the system is able to image the refractive index distribution of a biological sample with a relatively large size when compared with the conventional OCT approach. Such capability makes the proposed transmission OCT a potentially useful technique to image, for example, the embryos of small animals in 3D for better understanding of the biological functions of genes.

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Fig. 1. Schematic of the transmission OCT setup used in this study: SLD, superluminescent diode; C₁₋₂, coupler; PC, polarization controller; M, mirror; W, water; SM, x-z axis stage and stepping motor; S, sample; L₁₋₆, lens; T, water tank; SP, spectrometer.

Fig. 2.
Typical parallel projection of the refractive indices within one cross section of the sample.

Fig. 3. Reconstructed cross-sectional distribution of refractive indices within the tube sample surrounded with agarose gel.

Fig. 4. The line profile of the reconstructed image (Fig. 3) at $x=8\text{mm}$. The half-amplitude and quarter-amplitude lines cut across the profile at points A_{1-4} and B_{1-4} , respectively.