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Spectral estimation optical coherence tomography for axial super-resolution

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Abstract: The depth reflectivity profile of Fourier domain optical coherence tomography (FD-OCT) is estimated from the inverse Fourier transform of the spectral interference signals (interferograms). As a result, the axial resolution is fundamentally limited by the coherence length of the light source. We demonstrate that using the autoregressive spectral estimation technique to instead of the inverse Fourier transform to analyze the spectral interferograms can improve the axial resolution, which is named as spectral estimation OCT (SE-OCT). SE-OCT breaks the coherence length limitation and improves the axial resolution by a factor of up to 4.7 compared with FD-OCT. Furthermore, SE-OCT provides complete sidelobe suppression in the depth point-spread function, further improving the image quality. We demonstrate that these technical advances enables clear identification of corneal endothelium anatomical details ex vivo that cannot be identified using the corresponding FD-OCT. Given that SE-OCT can be implemented in the FD-OCT devices without any hardware changes, the new capabilities provided by SE-OCT are likely to offer immediate improvements to the diagnosis and management of diseases based on OCT imaging.

References and links

1. Introduction

Over 25 years optical coherence tomography (OCT) has become established as a high-resolution three-dimensional imaging modality for the diagnosis of various human diseases [1-3]. The introduction of Fourier-domain OCT (FD-OCT), including spectral domain OCT
and swept source OCT, has resulted in the rapid development of ophthalmic and intravascular imaging [4-6]. OCT can provide non-invasive and non-contact imaging of the elastic light scattering properties of a sample in three dimensions [1, 4, 7]. Without using exogenous contrast agents, OCT could potentially be used as the non-invasive ‘optical biopsy’ [7-9], complementing conventional, invasive biopsy and histopathology. However, this potential is still not fully achieved. One of the reasons is that the axial resolution of OCT is insufficient to identify micrometer-sized cellular-level structures. The typical axial resolutions of current ophthalmic OCT is 4–7 µm in air [9, 10].

The axial resolution of the current OCT technology is governed by the coherence length of the light source, which is further determined by the source center wavelength and the spectral bandwidth. Sustained efforts have been made to improve the axial resolution to 1–2 µm in air by using light sources with a shorter center wavelength and a broader bandwidth, enabled by the advances in laser technology, such as UHR-OCT and Micro-OCT [11-17]. This improved resolving power has enabled the visualization of cellular and extracellular structures in multiple organs, including the eye [11, 12], respiratory airways [15, 16], and arteries [14, 17].

Novel methods rather than the strategy of source bandwidth extension have been developed. Kulkarni et al. demonstrated that using a deconvolution technique to achieve 2 times axial resolution improvement [18]. Later Bousi et al. reported a 7 times axial resolution improvement using the modulated deconvolution [19]. Other methods, such as the maximum entropy method [20], the algebraic reconstruction [21] and the maximum a posteriori reconstruction [22] were also proposed to be applied to the OCT signal processing to enhance the axial resolution. However, regarding the widely application of OCT technique, the attempts to extract more information from the limited source bandwidth signal are still not widely recognized and worth more exploring.

In FD-OCT, the depth profile of the sample is encoded in the periodicities in the wavenumber space of a broadband illumination. These spectral interference signals (interferograms) are conventionally transformed into depth profiles of a sample using the inverse discrete Fourier transform (DFT), which provides a axial resolution limited by the coherence length of the light source [23, 24]. Although the DFT-based spectral analysis method provides reasonable results, it has two inherent limitations [25]: the low frequency resolution, which corresponds to the low axial resolution of FD-OCT; and the ‘frequency leakage’, which corresponds to the sidelobe artifacts in the OCT axial point-spread function (PSF). The sidelobe artifacts are the spurious ‘satellite’ peaks appearing in the axial PSF undermining the adjacent features in an OCT image [26]. Modern spectral estimation techniques have been developed as alternatives of the DFT-based method to alleviate its limitations [25]. Generally, these methods can be categorized into two classes: the parametric methods, such as autoregressive [25], autoregressive moving average (ARMA) [25], and the multiple signal classification (MUSIC) [27] etc., and non-parametric methods, such as Copan [28], the amplitude and phase estimation of a sinusoid (APES) [29], and the recently developed iterative adaptive approach (IAA) [30] etc. However, the potential of this class of powerful techniques has not been fully explored in OCT imaging [20].

In this paper, we present to use the autoregressive spectral estimation method to replace the conventional inverse DFT for the depth profile retrieval, to achieve a super-resolution factor of up to 4.7 as well as completely sidelobe artifacts suppression. We name this method spectral estimation OCT (SE-OCT). The term ‘spectral estimation’ often refers to the estimation of the frequency components of a temporal or spatial signal. But in our application, the spectral estimation is used to estimate the frequency components of the interferograms in the optical spectral domain, hereby producing the depth profiles of the sample. We further find that the super-resolution performance of SE-OCT is depended on the signal-to-noise ratio (SNR) of the feature of interest. This superresolution technique is readily incorporated into FD-OCT devices and may make it possible for a cellular-level inspection of the tissue microstructures.
2. Methods

A typical spectrometer based OCT imaging system is depicted in Fig. 1(a). In this scheme, the light source provides broadband illumination to both the sample arm and the reference arm. Spectral interference occurs between photons that are reflected from the reference mirror and those that are backscattered from the refractive-index inhomogeneities of the structures within the sample. The spectral interference signals (interferograms) are detected using a spectrometer, followed by uniformly resampling in the wavenumber space (k-space) (Fig. 1(b)).

The only difference between SE-OCT and FD-OCT is in the spectral interference signal analysis. In brief, the FD-OCT approach uses inverse DFT to retrieve depth information from the k-space interferograms. In SE-OCT, the k-space interferograms are reshaped to a uniform distribution, and then modeled to an autoregressive process, followed by calculation of the depth profiles using the autoregressive model parameters (Fig. 1(c)).

Specifically, in SE-OCT, two signal pre-conditioning steps were necessary before applying the autoregressive spectral estimation techniques to the interferograms. First, the interferograms were linearly sampled in the k-space as which was normally done in FD-OCT. Second, the k-space interferograms were further reshaped to a stationary (uniform distributed) series. The reshaping could be done by a pixel-to-pixel division of the source spectrum, since the interferogram was modulated by the spectrum shape of the light source. An uniform reshaping of the interferograms before modeling can satisfy the stationary requirements of the autoregressive process, thus improving the estimation fidelity. At the edges of the source spectrum where the light intensity was small, dividing the small intensity value in the reshaping process would significantly amplify the noise. To avoid this, we digitally truncated the source spectrum, keeping the spectrum where the magnitude was larger than 10% of the largest magnitude. Such a truncation would slightly reduce the spectrum bandwidth. But the influence was small since most SLD sources have a hat-shape spectrum and the small spectrum edges contribute little to the entire bandwidth.

After pre-conditioning, the uniform reshaped k-space interferogram was fitted to an autoregressive model, which can be expressed recursively as [31]

$$x_n = - \sum_{m=0}^{\alpha} a_m x_{n-m} + n_n$$  \hspace{1cm} (1)
where \( x_n \) is the data sequence of the interferogram, \( n_a \) is the white noise sequence with a variance of \( \sigma^2 \), driving the \( p \) order autoregressive process. \( a_m \) and \( \sigma^2 \) are the parameters to be estimated.

Estimating the parameters of an autoregressive model is the key step in the autoregressive modelling. Several approaches have been developed to fit a data sequence to an autoregressive model, such as the Burg’s approach, the Yule-Walker approach, the covariance approach, and the modified covariance approach [32]. A previous study conducted by Takahashi et al. [20] used the maximum entropy method, which can be proved as a mathematical equivalent to the autoregressive spectral estimation, with the model parameters estimated by Burg’s method. However, Burg’s method suffers from the peak-splitting problem [33], which may cause misinterpreting of the underlying layer structures [20]. In this study, we used the modified covariance method [31] to estimate the autoregressive parameters. This method minimizes both the average forward and backward prediction error and assumes no windowing on the sequence, which does not have the peak-splitting issue [33].

The order selected for autoregressive modeling significantly affects the result of SE-OCT. This problem was investigated in [20]. Briefly, a too low order results in fewer details in the image, while a too high order leads to increased spurious peaks. The appropriate order is also related to the method of autoregressive parameter estimating. In our application, we found that a good balance could be met setting the order to be the \( 1/3 \) of the length of the interferogram samples.

The frequency contents of the interferogram, or the depth profiles of backscattering light intensity \( I_{sl}(z) \) can be calculated with the model parameters by [31]

\[
I_{sl}(z) = \frac{\sigma^2 \Delta k}{1 + \sum_{m=0}^{\infty} a_m \exp(-jzm\Delta k)}
\]

where \( z \) is the depth, \( \Delta k \) is the sampling interval of the interferogram in the k-space. In the practical calculation of SE-OCT images, \( I_{sl}(z) \) was continuous along the \( z \) axis and had a range of \(-\pi / \Delta k \leq z \leq \pi / \Delta k \) according to the Nyquist–Shannon sampling theorem. To produce the digital image, we sampled the function \( I_{sl}(z) \) to a discrete series within its range at a length 8-times longer than that of the input interferogram. Finally, an averaging down sampling was performed to resize the image to a suitable aspect ratio.

3. Results

To characterize the resolution superiority and sidelobe suppression of SE-OCT, we conducted two phantom studies, the air wedge and calibration particles. To demonstrate the clinical potential of SE-OCT, we imaged the rat corneal endothelium ex vivo. The OCT imaging system (Fig. 2) used in this study had a resolution of 2.5 \( \mu \text{m} \times \) 2.5 \( \mu \text{m} \times 1.3 \mu \text{m} \) (\( xy \times z \)) and assumed the scheme shown in Fig. 1 (a) with the following additional features. (1) Two diode array sources and two combined spectrometers were employed to achieve a coherence-length limited axial resolution of 1.3 \( \mu \text{m} \) in air [17]. The method to combine two spectrometer bands are presented in [17]. (2) By physically or numerically reducing the detected spectral bandwidth, we could also reproduce the axial resolution of the clinical FD-OCT devices, specifically, \( \sim 4 \mu \text{m} \) for rat corneal tissues. In our study, the images of FD-OCT with coherence-length limited axial resolution of 1.3 \( \mu \text{m} \) (for short, noted as 1.3-\( \mu \text{m} \) FD-OCT) served as the ground truth, together with the histology image, validating and confirming the structures in bandwidth-reduced SE-OCT and FD-OCT images.
3.1 Characterization of axial resolution using an air wedge

An air wedge was formed by stacking two optical flats, with a small angle between them. This simple phantom is ideal for characterizing the axial resolution, because the spacing between the two air-glass interfaces of the wedge decreases linearly to 0 along the transverse beam scanning direction (scan length). We numerically reduced the detected spectral bandwidth by applying a ‘tight’ Gaussian window to provide a coherence-length limited axial resolution of 4.8 μm in air. With the same bandwidth-reduced interferograms, SE-OCT provided significantly greater axial-resolving power than FD-OCT (Figs. 3 (a), 3 (b) & 3 (c)). Note that the fringe patterns (blue arrows in Figs. 3(a) & 3(b)) in the transverse direction were formed by the coherence superposition of reflected wavefronts of the upper and lower surfaces of the air wedge, which are known as speckles in the axial direction.

In autoregressive spectral estimation, the magnitude of the estimated spectrum is not linearly related with the original spectrum [33]. Therefore, the full-width at half-maximum of the axial point-spread function was not a valid indicator of the axial resolution of SE-OCT. So we defined the resolution of OCT as the minimum distance where two interfaces were resolvable. Using this definition, the axial resolution of both FD-OCT and SE-OCT could be characterized and compared by plotting the measured wedge thickness as a function of the true thickness (Fig. 3 (d)). The first total constructive interference between the two reflected wavefronts, which demarcates resolvable thickness and unresolvable thickness, occurred at a true thickness of 4.77 μm in the FD-OCT image (Fig. 3 (d)). By contrast, SE-OCT clearly resolved the two interfaces up to a true thickness of 1.03 μm – a resolution 4.7-times better than FD-OCT. Note that this result was under the condition of a signal-to-noise ratio of 45 dB of each surface.
It was reported that the signal-to-noise ratio affects the results of autoregressive spectral analysis [34]. Therefore, it is important to investigate the influences of the noise on the super-resolving power of SE-OCT. We firstly conducted a numerical analysis in which normally distributed random noise was added to a simulated interferogram. We found the SE-OCT axial resolution was higher than the coherence-length limited axial resolution over the entire tested range of signal-to-noise ratio, and the superresolution factor increased as this ratio increased (Fig. 3 (e)). We then conducted imaging experiments using signal-to-noise ratios of 35 dB to 45 dB, and the results matched our numerical prediction very well (Fig. 3 (e), squares and circles).
3.2 Verifying superresolution using calibration particles

Fig. 4. Comparison between SE-OCT and FD-OCT analyses of polystyrene calibration particles, from the same interferogram data. (a, b & c) Cross-sectional images of calibration particles in water using FD-OCT (a), Gaussian reshaped FD-OCT (b) and SE-OCT (c). Scale bar: 20 µm. Insets at the left corner in (a, b & c): magnified views of two particles. Inset at the right corner in (a): the spectrum of the source used. Inset at the right corner in (c): a presentative image obtained by 1.3 µm axial resolution OCT. (d) Original (left), Gaussian reshaped (middle) and uniform reshaped (right) interferograms from one location after background subtraction. (e) Depth profiles in the images of FD-OCT (red), Gaussian reshaped FD-OCT (black) and SE-OCT (blue) at the location indicated by the red (a), gray (b), and blue (c) vertical lines in (a, b & c). The coherence-length limited resolution (CLLR) of FD-OCT was 4.1 µm before spectral reshaping, which was measured the air water interface. The nominal optical height (3.2 µm) of the polystyrene particles fell below the CLLR and cannot be resolved by FD-OCT. By contrast, SE-OCT could resolved the two surfaces of the beads and correctly measured the size. Yellow arrows: two particles were overshadowed by the sidelobe artifacts of the water surface in FD-OCT and can be seen in Gaussian reshaped FD-OCT and SE-OCT. Orange circles: spurious peaks.

To verify that the superresolution of SE-OCT is also valid for spherical phantoms that resemble biological cells, we imaged polystyrene calibration particles (80177, Fluka, diameter 2 µm) in water (Figs. 4 (a), 4 (b) & 4 (c)). To physically reduce the light source spectral bandwidth, we used a superluminescent diode array with the spectral band from 755 nm to 860 nm, corresponding to a measured coherence-length limited axial resolution of 4.1 µm. The nominal optical height of the polystyrene particles was 3.2 µm with a refractive index of
1.58 at 810 nm. FD-OCT was unable to resolve the top and bottom surfaces of the particles (Fig. 4 (a)). By contrast, with the same spectral bandwidth (Fig. 4 (d)), SE-OCT could clearly resolve the top and bottom surfaces of each particle (Fig 4 (c) and inset) and correctly measure the height of the particles (Fig. 4 (e)), demonstrating the superior axial resolution. Note that due to the limited NA of the objective lens, the particles seem like two horizontal lines in OCT images. This is also the case using the high axial resolution OCT (Fig. 4(c) right up corner inset) and not an artifact of SE-OCT.

Since the spectral shape of the light source was non-Gaussian (Fig. 4 (d)), sidelobe artifacts appeared in FD-OCT images (Figs. 4 (a), 4 (e)), which seriously degraded the image quality. In FD-OCT practices, a Gaussian window is usually applied to the interferograms to suppress the sidelobe artifacts at the cost of axial resolution degradation (Fig. 4 (b)) [26, 35]. In the SE-OCT images, we found the sidelonges were also completely suppressed, allowing clearer visualization of relatively weakly scattering particles (Fig. 4 (c), yellow arrows) which would otherwise be overshadowed by the sidelonges of neighboring strongly scattering objects (Fig. 4 (a), yellow arrows), without affecting the resolution. We also found that in SE-OCT, superresolution and sidelobe suppression were achieved without affecting the noise floor or signal strength (Fig. 4 (e)), although spurious peaks might appear as additional noise (Fig. 4 (c), orange circles and Fig. 4 (e)).

3.3 Biological tissue imaging: the rat cornea ex vivo

The corneal endothelium plays a vital role in maintaining corneal transparency. Therefore, it is important to visualize and evaluate the morphology of endothelial cells when diagnosing corneal decomposition and during pre-operative and post-operative assessment of corneal and intraocular surgeries. Unfortunately, current ophthalmic OCT, with a typical coherence-length limited resolution of 4-7 µm [9, 10] is unable to clearly visualize this monolayer.

To acquire corneal images from fresh rat eyeballs, immediately after cessation of vital signs, both eyes were enucleated from a normal adult female Sprague Dawley rat. A 3-dimensional image was acquired at the center of the cornea. The eyes were then fixed using 4% neutral buffered formaldehyde for routine paraffin-embedded histology.

In the images of 1.3-µm FD-OCT, the corneal epithelium, stroma, Descemet’s membrane, and endothelium can be clearly seen in the representative cross-sectional images (Figs 5 (a) & (a’)). The corneal endothelium and Descemet’s membrane can be observed as two weakly scattering layers defined by three strongly scattering interfaces: the interface between the stroma and Descemet’s membrane, the basolateral surface of the endothelium, and the apical surface of the endothelium. These structures were confirmed by clear images of the hexagonally shaped endothelial cells in the corresponding reconstructed en face images (Fig. 5 (a’’)), and a representative histology image (Fig. 5 (d)).

To reproduce the axial resolution of a typical ophthalmic OCT, we digitally truncated the spectral bandwidth by applying a relatively ‘tight’ Gaussian window to the interferograms detected by the spectrometer, thereby producing images with a coherence-length limited resolution of 4.1 µm (Figs 5 (b) and 5 (b’)). In these 4.1-µm FD-OCT images, the characteristic anatomical features including Descemet’s membrane and the surfaces of endothelium could not be clearly seen. In addition, the contrast of the corresponding en face image (Fig. 5 (b’)) was degraded with respect to the 1.3-µm axial resolution image even though the transverse resolution was not altered.

By contrast, with the same spectral bandwidth, SE-OCT was able to break the coherence-length resolution limitation, eliminate the sidelobe artifacts, and improve the image quality to be comparable to 1.3-µm FD-OCT images (Figs. 5 (c) and 5 (c’)). The contrast of the en face image (Fig. 5 (c’’)) was also enhanced due to higher axial resolution. In SE-OCT images (Fig. 5 (c’’)), the thickness of Descemet’s membrane and the endothelial layer was measured to be 3.1 µm and 2.3 µm, respectively (refractive index = 1.37).
4. Discussion

In this study, we improved the axial resolution of OCT through modeling the reshaped spectral interferograms to autoregressive processes. The results indicated SE-OCT could provide more detailed structure information than conventional FD-OCT. In cornea imaging, SE-OCT allows the evaluation of corneal endothelial damage by visualizing the endothelial layer and measuring its thickness. This technical advance can help surgeons plan the targeted replacement of corneal tissue and confirm the benefit of selective transplantation of the endothelial layer, i.e., endothelial keratoplasty [36].

In FD-OCT, using the inverse DFT for depth profile retrieval assumes the spectral data out of light source spectral band to be zeros. From this point, spectral estimation is a more reasonable way to obtain the depth profile. In SE-OCT, the autoregressive modeling procedures estimate the interferogram data out of the light source spectral band, thereby...
virtually broadening the spectral bandwidth in favor of the axial resolution. However, the spectral interferogram is actually formed by composition of periodicities in wavenumber space, thus is not a true autoregressive process. Therefore, we have to use a high order autoregressive model to approximate the OCT interferogram. This approximation may lead to two drawbacks. One is that the estimated signal magnitude, or light intensity in the SE-OCT images, may not be proportional to the true intensity backscattered from the samples. Take bead 1 in Fig. 4 (e) as the example, the signal intensity of its two surfaces has a big difference which should be small. This issue may not affect the sample morphology presented in SE-OCT images, but quantitative analysis based on the image intensity should be carefully taken. The other drawback is that spurious peaks appearing in SE-OCT images (Figs. 4 (c), orange circles & 4 (e)) as additional noise, especially when a high order was used. However, we found this one-pixel width noise may not significantly affect the image quality and can be easily suppressed by frame averaging, which is widely used in OCT images to suppress the speckle noise.

The performance of SE-OCT depends on the signal-to-noise ratio of the microstructure of interest (Fig. 2 (e)). Specifically, SE-OCT shows better performance for the highly scattering microstructures, such as those demonstrated in Fig. 5, than the weekly scattering microstructures. This SNR dependence may limit the application of SE-OCT to relatively highly scattering microstructures. Nevertheless, over the SNR range > 15 dB, SE-OCT demonstrates superior axial resolution than the corresponding FD-OCT. In summary, near-term future developments for SE-OCT will be testing the efficacy of cellular-level imaging in clinical OCT systems in vivo. Moreover, improvement of processing speed using parallel computing and achieving nanometer axial resolution using supercontinuum generation [13] could help this method become even more attractive for wide usage.