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Jannick P. Rolland
University of Central Florida

Panomsak Meemon
University of Central Florida

Supraka Murali
University of Central Florida

Kevin P. Thompson

Kye-sung Lee

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Gabor-based fusion technique for Optical Coherence Microscopy

Jannick P. Rolland1,2*, Panomsak Meemon2, Supraja Murali2, Kevin P. Thompson3, and Kye-sung Lee1

1 The Institute of Optics, University of Rochester, Rochester, NY 14627, USA
2 CREOL, The College of Optics and Photonics, University of Central Florida, Orlando, FL 32816, USA
3 Optical Research Associates, Pasadena, CA 91107, USA

* rolland@optics.rochester.edu

Abstract: We recently reported on an Optical Coherence Microscopy technique, whose innovation intrinsically builds on a recently reported - 2 µm invariant lateral resolution by design throughout a 2 mm cubic full-field of view - liquid-lens-based dynamic focusing optical probe [Murali et al., Optics Letters 34, 145-147, 2009]. We shall report in this paper on the image acquisition enabled by this optical probe when combined with an automatic data fusion method developed and described here to produce an in-focus high resolution image throughout the imaging depth of the sample. An African frog tadpole (Xenopus laevis) was imaged with the novel probe and the Gabor-based fusion technique, demonstrating subcellular resolution in a 0.5 mm (lateral) x 0.5 mm (axial) without the need, for the first time, for x-y translation stages, depth scanning, high-cost adaptive optics, or manual intervention. In vivo images of human skin are also presented.

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OCIS codes: (110.4500) Optical coherence tomography; (110.1085) Adaptive imaging; (120.3890) Medical optics instrumentation.

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1. Introduction

Optical Coherence Tomography (OCT) is a technology capable of depth sectioning of biological tissue at micrometer scale resolution. As a result of the large effort put forth by the scientific and industrial community to develop broader and broader source spectra, OCT has achieved (since the mid 1990s) remarkable axial resolution, starting in 1995 with mode-locked solid state lasers [1] and recently with supercontinuum sources [2,3]. The first demonstration of high lateral resolution associated with the terminology of Optical Coherence Microscopy emerged in 1994 [4]. Ultrahigh-resolution OCT was first demonstrated in vivo in 1999 with simultaneously up to ~1 µm axial resolution in tissue and 3 µm lateral resolution [5]. The high lateral resolution throughout the depth of the sample was achieved by not only increasing the numerical aperture (NA) of the imaging optics but also refocusing into the sample with depth to overcome the decrease in depth of focus (DOF) that varies as the inverse square of the NA [6]. In [5] and [6], the imaging was performed with Time-Domain OCT (TD-OCT) using scanning stages. In that approach, it is worth noting that the sample point being imaged is always first positioned on-axis with respect to the imaging optics, thereby requiring only spherical aberration and color correction, and stage zone-focusing is performed. Since then, an open challenge has been to demonstrate high lateral resolution without scanning stages, because it is only when we can move the technology off the scanning stages that we open a path for in vivo clinical applications seeking histology grade image quality.
Fourier Domain OCT (FD-OCT) methods have been developed [7] to dramatically improve the imaging speed over TD-OCT. Furthermore, utilizing full-field illumination, the technique of parallel acquisition of 1D time-encoded spectral interference signals without lateral scanning was demonstrated to achieve high speed and mechanical stability volumetric imaging. Using a fixed high NA objective, the depth of acquisition was limited to a couple of hundred micrometers [8]. Intrinsic to the method of FD-OCT is that no refocusing may be applied given that an entire A-scan is acquired per measurement. Throughout the literature to-date, whether FD-OCT or TD-OCT is implemented, low NA imaging optics that allow longer DOF at the expense of lateral resolution is utilized and clinical imaging is carried out at lateral resolution in the 10-20 µm range [9]. A new paradigm is needed to achieve micron level lateral resolution throughout the depth of imaging of the sample, at high speed, without any scanning stages.

Being fully aware of this limitation, OCT system developers have initiated the development and in some instances the assessment of several dynamic focusing schemes to overcome the inadequate lateral resolution across the imaging depth [10–12]. Quasi-Bessel-beam imaging in biological tissue was reported with demonstrated 4 mm DOF imaging at invariant resolution at a reduced sensitivity of ~80dB [13]. In FD-OCM, even though the entire reflectivity profile along the depth can be collected at once from the inverse Fourier transform of the spectral interference signal, the use of a high NA objective lens leads to clear distinctions between in-focus and out-of-focus regions across the depth. Imaging techniques similar to C-Mode scanning in ultrasound imaging have been investigated to recover an out-of-focus portion [14,15]. The purpose of these studies was to demonstrate feasibility of high lateral resolution and as such the re-focusing was applied by manual translation of the objective lens. In [15], volumetric merging of OCT data were presented that adopted the methods reported in [14] of manual segmentation of the C-Mode images followed by the summation of the segmented images to form a high-resolution image.

Finally, an approach consisting in using multiple beams to image in parallel at a few depth planes has also been developed, and a four beam implementation and image fusion have been demonstrated [16]. Each beam is focused at a different depth and the NA of the beam is chosen to achieve a DOF equal to one quarter of the total desired focal depth. The NA also determines the lateral resolution. The advantage of this multiple beam approach is faster speed of acquisition since four images in depth are acquired simultaneously. The fused image from the four beams has been shown however to present discontinuities between the depth regions caused by differing signal-to-noise ratios present in the OCT images from neighboring beams, an inherent property of the technique. To eliminate the discontinuities, a blending approach was recently developed as reported in [16] that consisted in splitting the images into their low and high spatial frequency components using a single pole filter applied in each direction. The high frequency components were obtained by subtracting the low frequency filtered images from the originals. A fused image was then obtained by adding back a weighted average of the low-frequency images. The depth region over which this was done was chosen to visually eliminate intensity discontinuities. Some challenges associated with the technique include the requirement of sufficient source power to accommodate four beams operating at high signal to noise ratio and sensitivity. Also, the presence of crosstalk between the channels has been observed and traced back to scattered light from the reference mirror entering adjacent channels.

We recently presented a solution for high invariant resolution 3D imaging using embedded voltage-addressable variable focusing elements, such as a liquid crystal lens [17] and a liquid lens within a fixed optics [18,19]. One of the designs led to a custom microscope for skin imaging that not only involves non-moving parts for refocusing along the depth, which promotes a robust prototype for clinical environments, but also was designed to compensate for aberrations and dispersion in the system. The dynamic focus microscope enables as-built measured resolutions at focus of 2 µm throughout a 2 mm cubic sample, with
lateral resolution independently set by the NA of the microscope [19]. The optics was designed and optimized for a Titanium: Sapphire (Ti:Sa) femtosecond laser with a broadband spectrum (120nm bandwidth) centered at 820 nm. An axial resolution of 2.5 µm in air was achieved as determined by the source parameters. In addition, at the chosen NA of 0.2, we measured a 100 µm DOF at each focus point using a 10% Modulation Transfer Function (MTF) criterion at 177 lp/mm corresponding to a two-point separation of 2.8 µm [20]. The measured DOF enables us to quantitatively and independently set the number of focused zones for a given imaging depth.

In this paper we report on the image acquisition enabled by the above summarized optical probe and the automatic data fusion method developed to render an in-focus high-resolution image throughout the imaging depth of the sample.

2. Mathematical methods

The use of high NA imaging optics with a high-speed variable focus capability within an OCM experimental setup [19], combined with a Gabor-based image fusion algorithm is referred to as Gabor Domain OCM (GD-OCM) [21], to distinguish the acquisition and processing from FD-OCM. The fusion algorithm is now described.

Let us consider a conventional FD-OCT configuration. The spectral electric field reflected from the reference and the sample arms can be expressed, respectively, as

$$\hat{E}_R(k) = K_R \hat{E}_o(k) r_r \exp(ikz_r),$$

$$\hat{E}_S(k) = K_S \hat{E}_o(k) \int_{-\infty}^{\infty} r_s(z_s) \exp(ikz_s) dz_s, \quad (2)$$

where the caret denotes a function in the spectral domain, $\hat{E}_o(k)$ represents the electric field emitted from the light source, $K_R$ and $K_S$ are real numbers representing total losses in the two paths, $r_r$ is the reflectivity of the reference mirror, $r_s(z_s)$ represents the sample reflectivity profile along the depth, and $z_r$ and $z_s$ are round-trip optical path lengths along the reference and sample arms, respectively [22,23]. Consequently, together with Euler’s formula for cosines and complex exponentials, a spectral intensity as detected by the spectrometer can be expressed as

$$\hat{I}_D(k) = |\hat{E}_R(k) + \hat{E}_S(k)|^2, \quad (3)$$

$$\hat{I}_D(k) = \tilde{S}(k) \cdot \left[ K_R r_r \int_{-\infty}^{\infty} r_s(z_s) \cos(k(z_s-z_r)) dz_s \right. + \left. K_S \int_{-\infty}^{\infty} r_s(z_s) \exp(ikz_s) dz_s \right] \cdot (4)$$

where $\hat{I}_D(k)$ is the detected spectral intensity, and $\tilde{S}(k) = |\hat{E}_o(k)|^2$ denotes the power spectral density of the light source. For a simplification, let’s assume that we can remove the DC and auto-correlation terms (1st and 3rd terms), and there is no loss in both paths ($K_R = K_S = 1$). In addition, since only the optical path length difference is of interest, we set the optical path length difference $z_D = z_s - z_r$ and define $r_s(z_D)$ as the sample reflectivity profile associated with the optical path difference. Therefore, the spectral interference signal can be expressed as
\[ \hat{I}_{\text{int}}(k) = 2r_R \hat{S}(K) \int_{-\infty}^{\infty} r_S(z_D) \cos(kz_D) dz_D, \quad (5) \]

Furthermore, the function inside the integral can be represented in terms of a complex representation or an analytic signal [24] as

\[ \hat{I}_{\text{int},c}(k) = 2r_R \hat{S}(K) \int_{-\infty}^{\infty} r_S(z_D) \exp(ikz_D) dz_D, \]

\[ = 2r_R \hat{S}(k) \mathfrak{F}\{r_S(z_D)\}, \quad (6) \]

where \( \mathfrak{F}\{f(x)\} \) denotes the Fourier transform operator applied to \( f(x) \). Consequently, the inverse Fourier transform of Eq. (6) denoted \( I_{\text{OCT}}(z_D) \) yields

\[ I_{\text{OCT}}(z_D) = 2r_R \cdot \mathfrak{F}^{-1}\{\hat{S}(k)\} * r_S(z_D), \quad (7) \]

where the symbol * denotes the convolution operator. Equation (7) expresses the FD-OCT signal as a reflectivity profile \( r_S(z_D) \) convolved with a coherence function, defined as \( \mathfrak{F}^{-1}\{\hat{S}(k)\} \), the axial point spread function.

The low (< 0.1) NA objective lens that is typically used in FD-OCT to ensure long DOF is replaced in GD-OCM by a higher NA variable focus objective (e.g. NA ≥ 0.2). The illumination power is peaked at the focus point of the objective and rapidly drops as a function of the distance away from that point [25–27]; but most importantly, the lateral resolution is maintained in an even smaller region around the focus point, known as the DOF, which may be based on a variety of image quality criteria including the one we selected, which, as expressed earlier in this paper, is the region over which the MTF exceed 10% at 177 lp/mm. This region may be shown to be equivalent to about five times the conventional quarter wave criteria established by Rayleigh, which means that we tolerate about 1.25 waves in defocus aberration [25]. Using a window localized at a given focus and extended in depth, we impose with the Gabor domain technique that only the back-scattering events that happen around the focus, approximately inside the DOF region defined above, will contribute to the final image. Using the expression for the spectral reflected field given by Eq. (2) together with the GD-OCM imaging property, the electric field from the sample arm can be written as

\[ \hat{E}_S(k; z_{so}) = K_s \hat{E}_o(k) \int_{-\infty}^{\infty} g(z_s - z_{so}) r_s(z_s) \exp(ikz_s) dz_s, \quad (8) \]

where \( g(z_s - z_{so}) \) denotes the window whose shape will be described in section 3.1. In all cases, the window shape will be approximated by a function that concentrates around the position \( z_s \) equals to \( z_{so} \) and has a finite width, which is much narrower than the function \( r_s(z_s) \). The window serves as a weighting function along the depth centered at the focal plane of the imaging optics located at \( z_{so} \). We shall again assume that there is no loss in both paths \( (K_R = K_S = 1) \). In a similar manner to the case of FD-OCT, the detected spectral interference signal is now given as

\[ \hat{I}_{\text{int},c}(k; z_{do}) = 2r_R \hat{S}(k) \int_{-\infty}^{\infty} g(z_d - z_{do}) r_s(z_d) \exp(ikz_d) dz_d, \quad (9) \]

The spectral interference signal is now a function of two variables, and the integration term is in the form of a local Fourier transform or Gabor transform where the DOF serves as a weighting window whose center may be shifted by varying the focal length of the dynamic
focus probe. In the process of reconstruction of the full depth profile, we define a new function

$$\hat{F}(k; z_{D0}) = \int_{-\infty}^{\infty} g(z_D - z_{D0}) r_s(z_D) \exp(ikz_D) dz_D,$$

(10)

Hence the depth profile can be reconstructed by means of an inversion of the local Fourier transform [24] defined by

$$r_s(z_D) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \hat{F}(k; z_{D0}) g(z_D - z_{D0}) \exp(-ikz_D) dz_D dk,$$

(11)

where we define $$\|g\| = \left[ \int_{-\infty}^{\infty} |g(z_D)|^2 dz_D \right]^{1/2}$$ for the purpose of normalization of the window.

Using the concept of the Gabor’s signal expansion [24], the sampled version of Eq. (11) can be expressed as

$$r_s(z_D) = \sum_{m=-\infty}^{\infty} \sum_{n=-\infty}^{\infty} \hat{F}(k_n; m\delta z_D) g(z_D - m\delta z_D) \exp(-ik_n z_D),$$

(12)

where the signal $$\hat{F}(k_n; m\delta z_D)$$ corresponds to an interference fringe of the detected spectral interference signal acquired for every shift of the focal plane of the dynamic focusing probe by an amount of $$\delta z_D$$. Equation (12) can be rearranged into a desired form as

$$r_s(z_D) = \sum_{m=-\infty}^{\infty} g(z_D - m\delta z_D) \sum_{n=-\infty}^{\infty} \hat{F}(k_n; m\delta z_D) \exp(-ik_n z_D).$$

(13)

The inner sum is now in the form of an inverse discrete Fourier transform. By defining the second sum in Eq. (13) as $$r_{s,m}(z_D, m\delta z_D)$$, which represents a backscattering event that occurs within the optical path length difference $$z_D$$ and within the DOF of the dynamic focusing objective when the focal plane is shifted by an amount of $$m\delta z_D$$, Eq. (13) can then be rewritten as

$$r_s(z_D) = \sum_{m=-\infty}^{\infty} g(z_D - m\delta z_D) r_{s,m}(z_D, m\delta z_D),$$

(14)

By inserting Eq. (14) into Eq. (7), we get

$$I_{OCT}(z_D) = 2r_k \cdot \mathcal{F}^{-1}\{\hat{S}(k)\} \ast \sum_{m=-\infty}^{\infty} g(z_D - m\delta z_D) r_{s,m}(z_D, m\delta z_D),$$

(15)

$$I_{OCT}(z_D) = \sum_{m=-\infty}^{\infty} g(z_D - m\delta z_D) \mathcal{F}^{-1}\{\hat{S}(k)\} \ast r_{s,m}(z_D, m\delta z_D),$$

(16)

$$I_{OCT}(z_D) = \sum_{m=-\infty}^{\infty} g(z_D - m\delta z_D) I_{OCT,m}(z_D, m\delta z_D),$$

(17)
where \( I_{OCT,m} (z_D, m\delta z_D) = 2r_R \cdot 3^{-1} \left( \hat{S}(k) \right) \ast r_{s,m} (z_D, m\delta z_D) \), is the signal acquired at focus position shifted by an amount \( m\delta z_D \). Equation (17) expresses that the full depth profile of the sample reflectivity can be reconstructed from multiple images acquired by shifting the focal plane along the depth direction, which from this point on will be referred to as GD samples.

3. Automatic fusion technique

3.1 Operational methods

Given the formalism provided in section 2, we have designed an automatic fusion algorithm that is capable of extracting and fusing the acquired GD samples. Each GD sample is multiplied by a weighting window, whose width is based on the measured DOF of the dynamic focusing probe, as we will describe, and the center is shifted to the position that corresponds to the focus position of that particular GD sample. When combined with the variable focus capability of the custom-designed dynamic focusing probe, the image resolution, which is quasi-invariant throughout the imaging depth, can be reconstructed.

To evaluate the imaging capability using the dynamic focus OCM probe and the Gabor-based fusion technique, we acquired multiple images from the same 0.5 mm x 0.5 mm (lateral x axial) portion of an African frog tadpole (Xenopus Laevis) at five different focus positions. Five images were taken at a shifted focal plane with 100 \( \mu \)m separation that corresponded to the measured DOF. The data in each B-scan or 2D slice contained 500 A-scans with a sampling interval of 1 \( \mu \)m. The measured lateral resolution was 2.8 \( \mu \)m using a 10% MTF criteria at the two edges of the DOF. For each image, the high lateral resolution and sensitivity were observed (as expected) only within a certain region around the focal plane (i.e. within the DOF).

A trapezoidal shape that is a flat top with linear transition at both edges as shown in Fig. 1 was chosen as the windowing function to define and weight the DOF region. The center and width of the windows were determined automatically and applied dynamically for each focus position as now detailed. The window’s center and width were first estimated from the relation between the applied voltage and the focal shift for each acquired image. The algorithm was then applied to determine the optimal focus position defined as the center of mass (CM) of the averaged reflectivity profile for each image over a certain depth range around the estimated focus position. Specifically, the averaged reflectivity profile along the depth was calculated by taking the ensemble average along the B-scan of each image as \( r_j (z) = \left( 1/N_j \right) \sum_z r_j(x,z) \), where \( r_j(x,z) \) represents the \( j^{th} \) cross-sectional image, \( x \) and \( z \) are lateral and axial pixels, respectively, and \( N_j \) is a number of A lines/frame. Then, the \( CM_j \) of each profile \( j \) was calculated around the estimated focus position using

\[
CM_j = \sum_z z \cdot r_j(z)/\sum_z r_j(z).
\]  

The left half-maximum point of the \( j^{th} \) window \( HM_{j,L} \) and the right half-maximum point of the \( j^{th} \) window \( HM_{j,R} \) are given by

\[
HM_{j,L} = 0.5 \left( CM_j + CM_{j-1} \right), \quad HM_{j,R} = 0.5 \left( CM_j + CM_{j+1} \right).
\]  

The window, which we set to be trapezoidal in shape, can be constructed as
where \( T \) is a transition width of the window, which is a free parameter. We established that a transition zone of about 20 pixels provides both a sharp cutoff for out of focus imagery together with smooth transition between the fused zones. The width of the transition zone is however quite insensitive to variations up to +/-10 pixels. These linear transitions provide smooth fusion at boundaries. Summing all windows together yields an overall flat response, thus preserving the original intensities in the images around each focus point.

3.2 Imaging system parameters

The system built for the purpose of validating the feasibility of the technique consists of a Titanium:Sapphire femtosecond laser centered at 800 nm with 120 nm FWHM (Integral, Femtolaser Inc.), a broadband custom-made 80/20 (NSF-DARPA/PTAP) fiber coupler, a Fourier-domain optical delay line in the reference arm to compensate the overall dispersion of the system, and a commercial spectrometer with a 3648 line CCD array (HR4000, Ocean Optics Inc). Spectra are acquired with an exposure time of 50 µs and 4ms readout time corresponding to a readout speed of 250 spectra/s; thus 500 spectra that form one frame are acquired in precisely 2.025 seconds. Using Labview software, performing the FFT on 500 spectra currently takes less than 5 seconds, and other operation times are negligible. Five frames are thus acquired and processed in about 35.5 seconds, accounting for the 100 ms refocusing of the liquid lens between frames. The fusion algorithm is currently implemented in Matlab and embedded in the Labview code and takes about 400 ms, thus yielding a current processing time of less than 36 seconds.

3.3 Results

Figure 2(a) shows images acquired at five different focus positions along the depth. It can be seen that a clear structure can be observed only within an area close to each focal plane. The extracted in-focus portions from each acquired image are shown in Fig. 2(b). The filtered images shown in Fig. 2(c) were then summed to form a high-resolution image with extended DOF image as shown in Fig. 2(d). An image acquired using the same probe by GD-OCM and that acquired with conventional FD-OCM image are shown in Fig. 3(a) and (b), respectively.
Fig. 2. (a) OCM Images of an African Frog Tadpole (Xenopus laevis) each acquired with the optics focused every 100 µm apart in depth from 0 to 0.5 mm; the red arrow points to a dominant feature (b) A plot of each window profile superimposed on top of an averaged reflectivity profile (c) Filtered images (d) The filtered images were then summed using a Gabor-based fusion algorithm.
To validate high-resolution capability across the full-field of view of the probe, the 2 mm lateral dimension sampled at 1 \( \mu \)m interval of fat cells of an ex vivo human tissue obtained from breast reduction surgery was acquired using the same setup. The fused image was reconstructed from six refocus steps with a focal shifted distance of about 60 \( \mu \)m per step covering approximately 500 \( \mu \)m imaging depth range in high resolution as shown in Fig. 4. Finally, in vivo imaging of human skin is shown in Fig. 5.

### 3.4 Discussion

The image acquired by GD-OCM shown in Fig. 3(a) provides better details compared to that of a conventional FD-OCM image shown in Fig. 3(b) acquired using the same probe. If we
consider Fig. 2 and the image in position 4 that contributed to the GD-OCM image, a red arrow points to a dominant feature that could be thought to be problematic in accurately computing the placement of the window. It is important to note that this feature is out of focus in the 3rd position image. While a dominant feature could create a critical issue if the window placement was computed based on an entire depth scan, it is important to note that the computation of the centroid location is carried out only within the DOF region around the estimated location of the focus based on an initial calibration of the probe. Thus strong reflections outside the estimated focus range are excluded. While a slight shift in the placement of the centroid for a specific image may still occur, the approach has been found to be robust to the precise placement of the window when computed within the estimated focus position of the focusing probe.

Depth of imaging in skin *ex vivo* and *in vivo* was also included to benchmark performance of 2D imaging with the probe in its current implementation. To fully appreciate the imaging capability in skin however, we are in the process of upgrading all components of the system to enable imaging at even higher frame rates, in 3D, where en face images may be reconstructed as well to further evaluate the achieved lateral resolution with depth in skin.

4. Summary and future work

GD-OCM is a developing imaging technology that builds on the high speed imaging provided by FD-OCT, the high lateral resolution of OCM, the ability of real-time (i.e. 100 ms for each focus position) refocusing of a custom-designed dynamic focusing microscope objective, and a Gabor-based fusion algorithm to produce a high lateral resolution image throughout the depth of imaging. Driven by the capability of the technique to image at high speed, the fusion algorithm we developed is capable of automatic reconstruction of the full depth image (the fused image) based on the concept of the inverse local Fourier transform and the Gabor’s signal expansion. The Gabor-fused image provides a final image that is high resolution and invariant over the cross-section area. The technique requires no human intervention and adaptively determines the width and position of the window for each fused image. The fusion process involves only summation and multiplication, and is therefore fast.

Real-time fusion and display of an invariant high-resolution image is possible when combined with a high acquisition speed camera. In order to realize a real time imaging capability, we are building a custom designed spectrometer utilizing a high speed CMOS camera (spL8192-70km, Basler Vision Technologies) with readout speed of 70k spectra/s [28]. This new spectrometer has the potential capability of achieving imaging speed of ~140 frames/s for 500 A-lines/frame. For 500 A-lines per frame and 5 imaging zones, an acquisition speed of 28 frames/s (i.e. 140/5) may be achieved. The proposed technology is capable of high-resolution volumetric imaging and has the potential to impact many other areas of surgery and diagnostic applications.

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