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2012 Meas. Sci. Technol. 23 035403

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Numerical correction of distorted images in full-field optical coherence tomography

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Received 21 July 2011, in final form 6 December 2011
Published 1 February 2012
Online at stacks.iop.org/MST/23/035403

Abstract
We propose a numerical method which can numerically correct the distorted en face images obtained with a full field optical coherence tomography (FF-OCT) system. It is shown that the FF-OCT image of the deep region of a biological sample is easily blurred or degraded because the sample has a refractive index (RI) much higher than its surrounding medium in general. It is analyzed that the focal plane of the imaging system is segregated from the imaging plane of the coherence-gated system due to the RI mismatch. This image-blurring phenomenon is experimentally confirmed by imaging the chrome pattern of a resolution test target through its glass substrate in water. Moreover, we demonstrate that the blurred image can be appreciably corrected by using the numerical correction process based on the Fresnel–Kirchhoff diffraction theory. The proposed correction method is applied to enhance the image of a human hair, which permits the distinct identification of the melanin granules inside the cortex layer of the hair shaft.

Keywords: full-field optical coherence tomography, interferometry, imaging systems, image analysis

(Some figures may appear in colour only in the online journal)

1. Introduction
Optical coherence tomography (OCT), as a noninvasive depth-resolved imaging technology, is capable of providing micron-scale imaging resolution [1, 2]. Recently, the range of applications has expanded in various fields such as biomedical diagnosis [3, 4], art inspection [5–9] and jewel evaluation [10]. Among the many OCT techniques, full-field optical coherence tomography (FF-OCT) is attractive owing to its high resolution not only in the axial but also in the transverse directions [11]. In addition, to get a three-dimensional (3D) image, FF-OCT requires only one-dimensional (1D) scanning (C-scan) owing to its use of a two-dimensional (2D) sensor array such as a charge-coupled device (CCD) [11, 12]. Therefore, we can expect high imaging speed and relatively low mechanical errors. However, when imaging the depth of a biological sample, the acquired en face image is degraded due to the refractive index (RI) of the sample, which is relatively high compared to that of its surrounding medium [13, 14]. Moreover, the high numerical aperture (NA) microscope objective lens (MOL) (0.3–1.0 NA), typically used to get a high transverse resolution, worsens this problem of image degradation.

In general, as the first process of system alignment, the focal plane of a FF-OCT system is set at the top surface of a sample, after which the imaging plane is located on the same surface by adjusting the reference arm of the system. In OCT, the imaging plane is the plane where the optical path length (OPL) difference between both arms of the system is within the coherence length of the light source. Further, in order to get depth images of the sample, the sample is usually shifted up or C-scanned by using a mechanical stage. However, the C-scan deviates the position of the imaging plane from the position of the focal plane due to the RI mismatch between the sample and the surrounding medium such as water or air [15]. To overcome this problem, Dubois et al [13] proposed the correction method...
Figure 1. Schematic of the FF-OCT system. BS: beam splitter, OL: microscope objective lens, CCD: charge coupled device, RM: reference mirror, PZT: piezo-electric transducer, NDF: neutral density filter, L: lens.

2. Experimental methods

Figure 1 shows the schematic of the FF-OCT system that is based on Linnik-type Michelson interferometry. A halogen illuminator is used as the broadband light source. The effective spectral bandwidth of the system is measured as 220 nm (FWHM), which gives the axial resolution of about 0.8 μm in water. The measured transverse resolution is 0.34 μm when using a 1.0 NA MOL. The input light is propagated via a fiber bundle and divided by a beam splitter (BS) into the reference and the sample arms, respectively. The back-reflected beam from the reference mirror and the one back-scattered from the sample are recombined at the same BS. When the difference in the OPLs of both arms is less than the coherence length of the source, interference occurs, which is then recorded with a CCD camera (VDS Vosskühler, 1024 × 1024 square pixels, 12 bits). The reference mirror is placed on a piezo-electric transducer (PZT), which gives rise to phase-shifted modulation of the interference. In both arms, water immersion MOLs are used. In the sample arm, a sample is moved up-and-down with a high-precision motorized stage for C-scanning. A neutral density filter (NDF) is mounted onto the reference arm in order to maximize the interference visibility. A piece of dummy glass is put on the sample arm to compensate the material dispersion caused by the NDF in the reference arm. The acquisition speed of the system was 5 Hz; five en face images could be taken per second.

3. Principle and algorithm

3.1. Segregation of a focal plane from the imaging plane in FF-OCT

In a FF-OCT system, regardless of the location of the focal plane, an en face image is acquired at the imaging plane that is located where the OPL difference in both arms is within the coherence length of the source. We assume that the focal plane and the imaging plane are initially positioned at the top surface of the sample; thus, the system is in focus. In order to obtain the en face image of a depth of the sample, the sample is moved upward slightly so that the imaging plane is located below the top surface, thus, inside the sample. With
Figure 2. Schematic of the reference arm (a), the sample arm (b) and the sample arm shifted upward by a distance \( t \) (c). OL: microscope objective lens, \( n_0 \): RI of the surrounding medium, M: reference mirror, \( \Delta z_1 \): distance between OL and M in the reference arm, \( \Delta z_2 \): distance between OL and the top surface of a sample, IP: imaging plane, \( n_s \): RI of the sample, \( t \): moving distance of the sample, \( x_i \): distance from the sample surface to the new imaging plane, \( \Delta z_{ip} \): absolute moving distance of the imaging plane and \( t_s \): thickness of the sample. The red horizontal lines in (b) and (c) show that IP moves upward with the sample movement, but it differs from the physical shift of the sample.

Figure 3. The changes of the imaging plane and the focal plane (a), and the separated distance plotted in terms of the sample moving distance under the condition of \( n_s = 1.5 \) and \( n_0 = 1.33 \) (b). \( \Delta z_{fp} \): displacement of the focal plane, \( t \): moving distance of the sample, \( \Delta z_{ip} \): displacement of the imaging plane, \( \Delta l \): segregation distance between the imaging plane and the focal plane, OL: microscope objective lens, \( n_0 \): RI of surrounding medium, \( n_s \): RI of sample and \( t_s \): thickness of the sample.

The sample movement, the focal plane also becomes located inside the sample. However, both the shifted planes are not at the same position but segregated from each other when the RI of the sample is different from that of its surrounding medium.

Figure 2(a) shows that the OPL of the reference arm is \( n_0 \Delta z_1 \) where \( n_0 \) is the RI of the surrounding medium. Figure 2(b) shows that when the imaging plane is set on the top surface of the sample, the OPL of the sample arm is \( n_0 \Delta z_2 \). To capture OCT images, the condition \( n_0 \Delta z_1 = n_0 \Delta z_2 \) should be satisfied. However, when the sample having a RI of \( n_s \) is shifted upward by a distance \( t \), then the imaging plane moves below the surface of the sample by a distance \( x_i \) as shown in figure 2(c). Since the imaging plane is formed at the location where the OPLs of both arms are identical, we have

\[
x_i = (n_0/n_s)t. \tag{1}
\]

However, when \( n_s > n_0 \), the absolute location of the imaging plane is shifted upward (see the red horizontal lines in (b) and (c)), due to the RI mismatch by a distance of \( \Delta z_{ip} \) given by

\[
\Delta z_{ip} = t - x_i = \left( 1 - \frac{n_0}{n_s} \right) t. \tag{2}
\]

On the other hand, when the sample is moved upward with the condition of \( n_s > n_0 \), the focal plane is shifted downward from its original position due to the refraction at the boundary as shown in figure 3. When the NA of the MOL is not so high, the distance from the surface of the moved sample to the new focal plane \( x_f \) becomes approximately given as [15]

\[
x_f \approx \frac{n_s}{n_0} t. \tag{3}
\]

Therefore, we can calculate that the new focal plane is located below the original focal plane by a depth of \( \Delta z_{fp} \)

\[
\Delta z_{fp} = x_f - t = \left( \frac{n_s}{n_0} - 1 \right) t. \tag{4}
\]

Finally, we can see that the imaging plane and the focal plane are segregated by \( \Delta l \) with the sample movement of \( t \):

\[
\Delta l = \Delta z_{ip} + \Delta z_{fp} = \left( \frac{n_s}{n_0} - 1 \right) t = \frac{n_s^2 - n_0^2}{n_0 n_s}. \tag{5}
\]

In general, the thickness of the image plane of FF-OCT is the same as the depth resolution of the system; a high axial resolution system has a thin image plane. Meanwhile,
the focal depth of an imaging system is inversely proportional to the square of the NA of the focusing lens; the system of high lateral resolution has a shallow focal depth. Therefore, the system having high axial and lateral resolutions is easily affected by the segregation of two planes. For an MOL of 0.3 NA, one-half of the focal depth is about 4.8 μm in water. However, when the sample is moved upward by 20 μm to image its internal structure, then the focal plane is segregated from the imaging plane by 4.8 μm (with the assumption that RIs of the sample and water are 1.5 and 1.33, respectively). It means that below 20 μm depth from the sample surface, the FF-OCT system gives a totally out-of-focus image. For the case with a 1.0 NA MOL, the sample movement of only 6 μm results in the focal plane segregation of 1.45 μm, which is much larger than one-half of its focal depth (0.44 μm). In figure 3(b), the segregation length Δh of equation (5) is plotted in terms of the sample movement distance.

The RIs of general biological samples have 1.35–1.7 distribution [18–20]. According to the research of G J Tearney’s group, the in vivo RI of stratum corneum is in the range of 1.5–1.55. The in vitro RIs of mesenteric adipose and dermis are 1.455 and 1.37–1.5, respectively [19]. Human hairs have 1.56–1.59 RIs although those are dependent on the hair color [20]. When the sample has RI near water, the focal plane segregation problem becomes less severe. However, even for this case, imaging the sample in air suffers the same problem.

3.2. FF-OCT imaging and numerical correction method

In a FF-OCT system, the light intensity at the CCD pixel located at a position (qx, qy) is generally given as

\[ I(q_x, q_y) = \tilde{I}(q_x, q_y) + A(q_x, q_y) \cos(\phi(q_x, q_y)). \]  

(6)

The first term \( \tilde{I}(q_x, q_y) \) is the average intensity of the light. \( A(q_x, q_y) \) is the amplitude and \( \phi(q_x, q_y) \) is the optical phase of the interference fringe. We adopt the four-integrating bucket method to get both the amplitude and the phase information from the CCD signal [21, 22]. To get them, sinusoidal phase modulation (SPM) is applied to the reference arm by a PZT and a series of interference images accumulated on the CCD during each quarter of the PZT oscillating period \( T \) are recorded. Then, the intensity of the CCD pixel signal is given as

\[ E_l(q_x, q_y) = \int \int \frac{\Gamma(x, y)}{(T/4)^4} \tilde{I}(q_x, q_y) + A(q_x, q_y) \cos[\phi(q_x, q_y) + \Omega \sin(\omega t \pm \theta)] \, dt, \quad l = 1, 2, 3, 4, \]  

(7)

where \( \Omega \sin(\omega t \pm \theta) \) is the phase modulation induced by the PZT. The optimal values of the modulation amplitude \( \Omega \) and the initial phase \( \theta \) were numerically calculated as 2.45 and 0.98, respectively [21, 22]. With the four CCD images taken in sequence, the amplitude and the phase information of the sample are simply calculated as

\[ A(q_x, q_y) = \frac{(E_1 - E_2 - E_3 + E_4)^2}{(E_1 - E_2 + E_3 - E_4)^2}, \]  

(8a)

and

\[ \phi(q_x, q_y) = \tan^{-1} \left[ \frac{E_1 - E_2 - E_3 + E_4}{E_1 - E_2 + E_3 - E_4} \right]. \]  

(8b)

When the imaging plane is the same as the focal plane of the FF-OCT system, we will have a well-focused image at the CCD plane. However, when the focal plane is segregated from the imaging plane, the captured image becomes out of focus as was explained with equation (5). Of course, an in-focus image can be obtained, at hand, by mechanically moving the CCD plane to the proper position along the optical axis. However, the already captured out-of-focus image, which is a blurred image, can be numerically refocused by using equation (8) without moving the CCD plane. From the en face amplitude and phase information taken at a depth plane, we can get the information at other depth planes by using the well-known Fresnel–Kirchhoff diffraction theory in the frequency domain [16, 17].

Figure 4 shows the coordinate system for the numerical correction analysis in a FF-OCT system. The complex information \( \Psi(X, Y, Z) \) of equation (8) taken at the CCD plane is out of focus. The correction plane, in which the image will be numerically refocused, is separated by \( Z \) from the CCD plane. The complex information \( \Psi(X, Y, Z) \) at the correction plane, from the Fresnel–Kirchhoff diffraction theory, is numerically obtained as

\[ \Psi(X, Y, Z) = \frac{1}{i \lambda Z} \int \int \Gamma(x, y) \times \exp \left[ ikZ + ik \left( \frac{(X - x)^2 + (Y - y)^2}{2Z} \right) \right] \, dx \, dy. \]  

(9a)
By defining the impulse response function \( h(x, y; Z) \) as
\[
\begin{align*}
\Psi(x, y, Z) &= \Gamma(x, y) \otimes h(x, y; Z), \\
F[\Psi(X, Y, Z)] &= F[\Gamma(X, Y)] \times F[h(x, y; Z)],
\end{align*}
\]
The Fourier transform of a convoluted function is given as
\[
\Psi(X, Y, Z) = \Gamma(X, Y) \otimes h(x, y; Z).
\] (9c)
The Fourier transform of the impulse response function is simply Fourier transformed as [23]
\[
F[h(x, y)] = \exp(ikZ) \times \exp(-i\pi \lambda Z(\xi^2 + \eta^2)),
\] (9e)
with the mathematical relationship of
\[
F[\exp(i \pi (p^2 x^2 + q^2 y^2))] = \frac{i}{|pq|} \exp \left( -i \pi \left( \frac{\xi^2}{p^2} + \frac{\eta^2}{q^2} \right) \right),
\] (9f)
where \( p^2 = q^2 = \frac{\lambda}{2} \). Therefore, the Fourier transform of the field in equation (9a) is represented by
\[
\begin{align*}
F[\Psi(X, Y, Z)] &= \exp(ikZ) \exp(-i\pi \lambda Z(\xi^2 + \eta^2)) F[\Gamma(X, Y)],
\end{align*}
\]
where \( \xi \) and \( \eta \) indicate the spatial frequencies and the phase factor \( \exp(ikZ) \) is the complex constant. Finally, by taking the inverse Fourier transform of it, we have the complex field at the correction plane as
\[
\begin{align*}
\Psi(X, Y, Z) &= \frac{1}{2\pi} \int F[\exp(-i\pi \lambda Z(\xi^2 + \eta^2))] F[\Gamma(X, Y)] d\xi d\eta,
\end{align*}
\] (9h)

To confirm the defocusing phenomenon in FF-OCT, experiments were performed with a USAF 1951 resolution target, after which the proposed correction algorithm was applied to obtain numerical refocusing. The resolution target was imaged face down, so that the chromium-coated test bars were imaged through a 1.585 mm thick glass substrate. The soda lime glass substrate of the target had a RI of 1.513 at 645 nm, and a pair of water-immersion MOLs of 0.3 NA was used. Finally, as a biological sample, a human hair was imaged with water-immersion MOLs of 1.0 NA and the refocusing process was carried out.

First, as shown in figure 6(a), the stains on the top surface of the reversed target were imaged, which indicates that the imaging plane and the focal plane were located at the top surface of the substrate. Subsequently, the bottom surface of the substrate, where the test bars were coated, was imaged
by shifting up the resolution target. When the shift was 1.8 mm, we could see the bar patterns of the target as in figure 6(b). The target-shifting distance gives good agreement with that (1.803 mm) calculated with equation (1) (finding \( t \) with \( x_i = t_i \)). Figure 6(c) is the enlarged image of the red rectangular box region in figure 6(b), which shows that the image is heavily out of focus as expected. The edge of the image is blurred and the diffraction pattern is clearly observed.

To acquire the focused image of the bar pattern optically, the reference beam of the FF-OCT system was blocked and the sample was shifted up and down. When the resolution target was shifted up by 1.390 mm from the initial position, we obtained the clear image shown in figure 6(d), which is well matched with the one (1.393 mm) calculated with equation (3) (finding \( t \) with \( x_f = t_f \)). In short, the coherence-gated image shown in figure 6(b) was obtained at the target position of 1.8 mm but the focused imaging was captured at the target position of 1.390 mm. The discrepancy distance between the imaging plane and the focal plane was as much as 0.410 mm.

Figure 6(e) is the numerically corrected test bar from the blurry original FF-OCT image of figure 6(b). The enlarged image (figure 6(f)) shows that the bar pattern became much clearer and more similar to the optically focused image of figure 6(d).

Figure 7. Criterion metrics for the AMP. When the correction distance \( Z \) is 34.2 mm, the criterion value of the AMP metrics is minimum.

The proposed correction method was used for the FF-OCT image of a human hair fiber as a biological sample. Figure 8 shows the en face image of the human hair taken 6 \( \mu \)m below its top surface. It is well known that a human hair is constructed with the cuticle layer, the cortex and the medulla. The cuticle layer is the outermost layer of the hair and the cortex is the main body within the cuticle layer. The medulla is the central pillar of the hair. In the cortex, melanin granules having round shapes are distributed [25–27].

Figure 8(a) shows the cuticle layer and the cortex part of the human hair. The enlarged image (figure 8(b)) shows that a bunch of melanin granules are located inside
Figure 8. *En face* image of a human hair taken $6 \, \mu m$ below the top surface; the obtained FF-OCT image (a) and its enlarged image (b), the numerically refocused image (c) and its enlarged image (d), (e) is the line image taken along the arrow in the red circle of (b), and similarly (f) is the line image of the chosen single melanin granule in (d) taken along the arrow.

the cortex. However, the image, especially of the melanin granules, is not clear enough; it appears a little bit blurred. Moreover, the image-blurring phenomenon makes it impossible to differentiate adjacent melanin granules. Therefore, the proposed numerical algorithm was applied and the image of figure 8(c) was obtained. Figure 8(d) shows the enlarged image of the refocused region in figure 8(c). In these figures, we can see that the melanin granules are plainly distinguished from each other. To confirm the focusing enhancement more clearly, a line profile (figure 8(e)) was taken across one melanin granule in the red circled region of figure 8(b) along the arrow, and also figure 8(f) from figure 8(d). The line image for the single melanin granule clearly shows that the numerical correction method offers details of the image and a much more focused image.

However, a hair fiber has a cylindrical surface, not a flat one. Moreover, the index distribution over the internal area of the hair is different. It means that we cannot correct the whole part of an *en face* image only with a single distance $Z$. Therefore, we optimized the distance $Z$ to have good focusing only at the partial region in figure 8(a), the red box. Figure 9 shows the normalized criterion values taken in terms
of Z with a step of 0.2 mm. The minimum value of the AMP was obtained at $Z = 10.2$ mm. Of course, the well-focused images of figures 8(c) and (d) were obtained at $Z = 10.2$ mm.

5. Conclusion and discussion

We have proposed a numerical correction method that can refocus the already taken but distorted or defocused en face images in FF-OCT. It has been experimentally confirmed that the en face FF-OCT image of the depth of a biological sample could be degraded by the RI of the sample itself. Due to the RI mismatch between the sample and its surrounding medium, the focal plane of the imaging system was segregated from the imaging plane of the coherence-gated system. For a sample of 1.5 RI immersed in water, with a sample movement of only 20 $\mu$m, the segregation length reaches 4.8 $\mu$m, which is the same as one-half of the focal depth of a 0.3 NA MOL. Considering that the FF-OCT system uses high NA optics to obtain high resolution, in general, the focal plane segregation from the imaging plane should be corrected or compensated by some means. As a numerical correction method, a refocusing algorithm based on the well-known frequency domain Fresnel–Kirchhoff diffraction theory and the integrating-bucket method has been proposed. With a USAF 1951 resolution target, it was experimentally shown that the FF-OCT system could not get images of both sides of the target only by scanning the sample along the depth. The image of the test bar taken through the soda lime glass substrate was blurred due to the RI of the substrate. With the substrate of 1.585 mm thickness and 1.513 RI, the segregation length was measured as long as 0.410 mm, which well matched the calculated value of 0.410 mm. The problem of focal plane segregation from the imaging plane could be solved by refocusing the already obtained FF-OCT image with the proposed algorithm. The proposed algorithm has been confirmed by imaging a human hair as a biological sample. The image showed that a bunch of small melanin granules were inside the cortex layer, but a little bit blurred. The image was numerically corrected or re-focused by the proposed refocusing algorithm, which allowed us to observe the melanin granules in a little more detail. The proposed method provides a focused image from an already taken out of focus, thus blurred, FF-OCT image only by computer processing without using any additional or mechanical operations. Therefore, we can expect improvement in the ease of operation, measurement robustness and system speed eventually.

Acknowledgments

The research was supported by a grant from the institute of Medical System Engineering (iMSE) in the GIST, Korea and the Korea Science and Engineering Foundation (KOSEF) NCRC grant funded by the Korea government (MEST) (no R15-2008-006-02002-0).

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