Ultrahigh-resolution full-field optical coherence tomography using spatial coherence gating and quasi-monochromatic illumination

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We developed an ultrahigh-resolution full-field optical coherence tomography (FF-OCT) microscope that is based on the spatial, rather than the temporal, coherence gating. The microscope is capable of observing three-dimensional microbiological structures as small as $0.4\mu m \times 0.4\mu m \times 1.0\mu m$ ($xyz$) using quasi-monochromatic light and a liquid crystal retarder. Unlike traditional FF-OCT systems, this microscope can be operated in high resolution for any preferable wavelength with minimized defocusing and dispersion effects. High-resolution images of an onion cell are presented. © 2012 Optical Society of America

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Optical coherence tomography (OCT) is a promising imaging modality first introduced at the end of 1991 [1]. OCT is a noninvasive imaging technology mainly associated with the production of high-resolution images of microbiological structures using harmless radiation. In the last couple of decades, OCT has caught a great deal of attention in many fields of research, particularly in medicine and biology. By now, OCT has been successfully applied to ophthalmology, biopsy, histology, cellular and subcellular imaging, dentistry, and more [2].

Among all of the OCT techniques, the ultrahigh-resolution "point-scanning" OCT systems [3] and the time-domain full-field OCT (TD-FF-OCT) [4] systems have been found to demonstrate the highest, subcellular resolution. Although ultrahigh-resolution "point-scanning" OCT systems demonstrate a higher sensitivity, they are much more expensive and, in general, lack the extremely high lateral resolution and the parallel detection obtainable by TD-FF-OCT systems.

TD-FF-OCT’s main advantages are its high three-dimensional (3D) resolution, speed, simplicity, and cost. TD-FF-OCT systems can use high-NA objective lenses and, therefore, achieve very high resolution also in the transverse direction. However, when a wide wavelength range is used together with very high-NA objectives, the interference signal contrast for deep layers is destroyed [5], a phenomenon usually termed the “defocusing effect.”

Our objective in this Letter is, first, to highlight the problem that arises when imaging with a high-NA objective and wide wavelength range. We then suggest that, for 3D ultrahigh-resolution FF-OCT imaging, a narrow wavelength range, rather than a wide range, should be used together with high-NA objectives. Under these circumstances, the short spatial coherence gating introduced by the high spatial frequency bandwidth is used for sectioning. This principle was used in the past to obtain ultrahigh-resolution 3D imaging of an integrated circuit [6]. However, the importance of the interplay between the spatial and temporal coherence gates was not discussed at all.

We demonstrate ultrahigh-resolution, narrowband TD-FF-OCT using high-NA objectives and a liquid crystal retarder (LCR). The fact that we use narrowband illumination minimizes the defocusing and dispersion effects and makes the chromatic requirements on the LCR and the other optical components much more tolerable. We achieved high contrast images with $0.4\mu m \times 1.0\mu m$ (lateral \times axial) resolution using near-IR narrow wavelength range illumination, $\Delta \lambda = 10\mathrm{~nm}$ and $\lambda = 710\mathrm{~nm}$. To the best of our knowledge, the lateral resolution reported here has never been demonstrated before in OCT while keeping the axial resolution in the ultrahigh range.

In Fig. 1, the Linnik-like optical microscopy system is depicted. A 150 W halogen lamp is used as the thermal light source (LS). The light propagates through a 50 $\mu m$ core optical fiber (OF) bundle and is projected onto a replaceable optical bandpass filter (BPF), followed by a diffuser (D). The diffused light is then condensed onto the aperture stop (AS) of the system. By means of the

![Fig. 1.](https://example.com/fig1.png)
projecting lens (L), the AS is imaged to the back aperture plane of the objective lenses (Oₜ and Oₙ; oil immersion ×100 objectives with 1.25 NA), whereas the field stop (FS) is projected to the back focal plane of the objectives. The light transmitted through lens L is polarized by the linear polarizer (P₁) before splitting at the beam splitter (BS). In each of the interferometer arms, a quarter-wave plate (Q) rotates the linear polarization of the transmitted light by 90°. The quarter-wave plate’s arrangement blocks any stray light reflected from the beam splitter facets. The LCR plate is positioned in the sample arm so that the extraordinary axis is aligned with the direction of the linear polarization of the transmitted light. After reflection from the reference mirrors [Rₛ and tilted mirror (TM)] and the object, the two beams are recombined in the BS and then transmitted toward the analyzer (P₂). The tube lens is used to project the interference image of the sample onto the CCD camera (Sony XC-EI50, 30 fps). To obtain the interference images, the sample (S) is positioned onto the sample mirror (Sₘ) and then scanned along the z axis with the linear motorized stage (M). The motorized stage is connected to a controller (C), and the LCR is connected to a function generator (FG).

The optical system can be operated both as an interference microscope and as an FF-OCT system. Note that, by changing the AS of the system, different effective NAs can be used for imaging. In addition, by changing the BPFs, different wavelengths and different bandwidths can be tested. The major differences of the current work from our recently published work [7] are in the setup configuration, the use of a LCR for demodulating the interference signal, the experimental demonstration of the interplay between temporal and spatial coherence gating, and the application of the method to biological samples.

To stress the important difference between wideband and narrowband illumination TD-FF-OCT, we present a typical experimental result obtained when scanning a thin film with the interference microscope in air (n₀ = 1); see Fig. 2. Different BPFs and different effective NAs were used to demonstrate the destructive result forced by the defocusing effect. For this purpose, a 4.8 µm thin film of SiO₂ on Si was investigated under four different coherence conditions. In Fig. 2(a), the scan was done using a BPF with a Gaussian-like shape having a central wavelength at 580 nm and FWHM of 10 nm with NAeff ~ 0.8 in air. Under these conditions, the spatial coherence gate lₛ ~ λ/NA² [7] is much shorter than the temporal coherence gate lₜ ~ 2 ln(2) · λ²/Δλπ [2]. As it is seen, a strong peak denoting the location of the SiO₂–Si interface is located at ~3.2 µm away from the air–SiO₂ interface peak. This result shows that, when the spatial coherence gating is dominant, the position of the second peak is in accordance with Δz_SCAN = ℓ/nSiO₂ = 4.8/1.5 ~ 3.2 µm [7]. In Figs. 2(b) and (c), the bandwidths of the BPFs were gradually enlarged to 30 nm and then to 55 nm; as a result, the second peak contrast is severely destroyed. The destruction of the interference signal for the deep interface [Figs. 2(b) and (c)] is due to the fact that the narrow widths of the spatial and temporal coherence envelopes are comparable but their centers are axially separated due to the defocusing effect. As the interference envelope is given approximately by their multiplication, the contrast is severely destroyed when the offset between their centers is larger than the sum: (lₛ + lₜ)/2. To further clarify this behavior, in Fig. 2(d), the AS was closed to achieve NAeff ~ 0.3 in air, while leaving the 55 nm BPF in place (note that, now, lₜ ≪ lₛ). Note that the second peak is now located 7.6 µm away from the first peak. As it is well known from white-light interferometry, the layer thickness is determined by dividing the scanning space with the refractive index, that is, Δz_SCAN = ℓ · nSiO₂ ~ 7.6 µm.

This experiment demonstrates the problem that arises when using both a high-NA objective lens and wideband illumination for imaging multilayered objects (both lₛ, lₜ are narrow and comparable). It also demonstrates that, for high-resolution FF-OCT imaging, better fringe contrast is obtained by relying only on the short longitudinal spatial coherence length while keeping the temporal one long enough; in view of Fig. 2(b), TD-FF-OCT systems that use LEDs (FWHMLED ~ 20–30 nm) are not considered narrowband. It is important to mention that some researchers have shown how this effect can be compensated [8] by using suitable a index matching material along with changing the reference arm length; however, such procedures require further complication from the system point of view.

The narrowband (narrow wavelength range), full-field interference output signal of a single microstructure located within a weakly attenuating sample is given by Eq. (1) [7]. For simplicity, we assumed that an index matching material is used. In Eq. (1), Iₛ is the DC level, γ is the contrast [Eq. (2)], A is the axial response (envelope) function [Eq. (3)], Δz is the scanning distance, ℓ is the distance of the microstructure from the sample top.
surface, $f_z$ is the axial spatial carrier frequency [Eq. (4)], and $\beta$ is the phase delay produced by the LCR:

$$I(x', y', \Delta z, \beta) = I_0(x, y) \times \{1 + \gamma(x, y)A[\Delta z - t(x, y)] \cos[2\pi f_z(\Delta z - t(x, y)) + \beta]\}\}$$

(1)

$$\gamma(x, y) = 2\sqrt{R_R R_S(x, y, \lambda)}/[R_{scat}(x, y) + R_R + R_{Stotal}(x, y)]$$

(2)

$$A[\Delta z - t(x, y)] = \sin e\left\{n_0 NA^2 x / \lambda \left[\Delta z - t(x, y)\right]\right\}$$

(3)

$$f_z = n_0 \lambda / (1 - NA^2/4).$$

(4)

In Eq. (1), $(x, y)$ and $(x', y')$ are the lateral coordinates in the object and image space, respectively. In Eq. (2), $R_R$ is the reference reflectivity, $R_S$ is the sample microstructure reflectivity, and $R_{Stotal}$ and $R_{scat}$ are the total reflectivity coefficients of all resolvable and irresolvable microstructures, respectively. In Eqs. (3) and (4), $n_0$ is the index matching material and $NA$ is the numerical aperture in air. By changing the LCR phase from $\beta = 2\pi$ to $\beta = 1.5\pi$ and then to $\beta = \pi$, we obtain the en face OCT images for each point of the object $E_{WF}(\Delta z) = I_0/A(\Delta z - l)$. By accumulating $N$ images of each phase, the signal-to-noise ratio (SNR) and sensitivity of the system are enlarged. In TD-FF-OCT, the SNR is proportional to the square of the contrast [9]; therefore, high contrast signals drastically reduce the necessary number of images $N$ and improve the imaging speed significantly. We estimated the dynamic range and the sensitivity in our system ($N = 10$) to be 54 and 70 dB, respectively [4]. To obtain the cross-section tomograms, the en face images are squared and normalized. In Fig. 3 (a), we present the edge spread function (ESF) obtained from a Ronchi rulings target with 200 line pairs per millimeter (lp/mm). According to the diffraction limited resolution of an edge, our system is operating with an effective NA of 1.05. The reduction in the NA is attributed to an underfilling illumination of the back aperture plane of the objectives. In Fig. 3(b), the axial response of the system is presented. In Figs. 4(a)–(c), we present OCT images of an onion cell boundary (walls) with high resolution and, in Figs. 4(d) and (e) we show a comparison between bright-field and OCT images by imaging a nucleus of an onion cell. It should be mentioned, however, that vertical surfaces with low scattering are not very effectively detected by this method, as with many other optical methods.

To conclude, in this work we have demonstrated ultra-high-resolution FF-OCT with spatial coherence gating using quasi-monochromatic illumination and an LCR. The method can be applied in any preferable wavelength with ultrahigh 3D resolution. Another advantageous application of the method is in cases where strongly dispersive samples are investigated.

References