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Phase contrast coherence microscopy based on transverse scanning

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Abstract

We present what we believe to be a novel approach to measuring optical path length differences with a precision of a few nanometers. The instrument is based on transverse scanning or en-face optical coherence tomography. Owing to the fast motion of the scanning beam over the sample, excellent phase stability in the transverse direction is achieved. Hence, phase changes caused by the varying optical path lengths within the sample arm occur with high frequency in the fast scanning direction. These changes are well separated from the rather slow phase changes introduced by jitter within the interferometer and can therefore be measured. The en-face imaging speed of the instrument is 40 fps (520×200 pixels). The measured precision of the method to detect small changes in optical path lengths was ~3 nm.

Phase contrast microscopy is a well-established tool to enhance image contrast of unstained samples. However, most techniques do not directly provide quantitative information on these phase changes. The use of low-coherence light sources enables the isolation of the region of interest within a depth range and avoids influences from nearby reflections [1-4]. Recently, high precision instruments utilizing Fourier domain optical coherence tomography (FDOCT) techniques have been introduced, which showed an excellent phase stability, because interference between two interfaces (top and bottom surfaces of a microscopic coverslip) is observed [5,6].

However, the acquisition speeds of en-face images of these systems are rather low because 3D volumes have to be recorded to extract this information. Moreover, these systems are limited to imaging with rather low NAs, because the reference reflection is located within the sample beam and is typically separated in depth by several hundreds of micrometers.

In this Letter we report a different approach that solves the above-mentioned limitations. The concept is based on the assumption that with the use of a high transverse imaging speed (of the order of several kilohertz), phase changes owing to jitter within the interferometer (or sample motion) can be separated from the phase changes introduced by the sample structure. The setup is similar to our previously introduced transversal scanning optical coherence tomography (OCT) instrument [7] and is shown in Fig. 1. In short a light source centered at 840 nm with a bandwidth of ~50 nm is employed. Hence light backscattered from regions that are outside the (round-trip) coherence length of ~6 μm in air is rejected by the system. The good phase stability along one transversal line that is needed for our method is achieved with the implementation of a resonant scanner operating at ~4 kHz. We used telescopes to

image the pivot point of one scanner onto the pivot point of the other scanner to reduce additional influences to the phase of the signal. One additional feature of the system is that OCT and confocal scanning laser microscopy (cSLM) images can be recorded simultaneously. In the current configuration the instrument uses a microscope objective with an NA of 0.5 (20×, OWIS) and is operated at a frame rate of 40 fps (520×200 pixels). All measurements are performed on an essentially flat sample (carrier glass plate) carrying microscopic structures [e.g., resolution test target (RTT), red blood cells]. Nevertheless, the technique can in principle be extended to 3D objects.

The signal measured with the dual balanced detector can be represented with $I(t) \propto 2(R_s R_r(t))^{1/2} \gamma_c(t) \cos(\phi(t))$, where γ_c denotes the coherence envelope and R_s, R_r represent reflectivity of the sample within the coherence volume and reference arm, respectively. The amplitude and phase of the signal are recovered using the Hilbert transformation.

To illustrate different influences on the phase of the signal we performed measurements on an RTT. We placed the coherence gate at the location of the surface of the RTT and recorded several en-face images. At this position the measured phase $\phi(t)$ [cf. Fig. 2(a)] of the interferometric signal consists of several terms,

$$\phi(t) = \phi^{\text{AOM}}(t) + \phi^x_{\text{scanning}}(t) + \phi^y_{\text{scanning}}(t) + \phi^{\text{jitter}}(t) + \phi^{\text{sample}}(t). \quad (1)$$

The first term is introduced by the net modulation frequency (or frequency difference) of the acousto-optic modulators (AOMs); the second and third terms are caused by the scanning of the beam over the sample (sample plane and coherence plane do not necessarily coincide). The fourth term is a random noise introduced by either jitter within the interferometer or sample motion. Only the fifth term (ϕ^{sample}) represents the phase change in interest. Therefore, all the other terms of Eq. (1) have to be eliminated to obtain this phase change. Note that a simple frequency analysis of the signal cannot separate the different phase terms, because the phase changes introduced by the AOMs and by the x scanning are occurring with a similar frequency as the phase changes introduced by the object structure.

Because of the stability of the carrier frequency generated by the AOM, we can eliminate the first term simply by calculating the phase difference of the measured signal with a signal of the same frequency. The result [cf. Fig. 2(b)] mainly shows the dominating phase change introduced by x scanning over the flat surface of the RTT. It should be noted here that the flat surface of the RTT is regarded as “zero” height plane, and all the phase changes are measured with respect to this reference plane. To minimize the influence of the other terms we used the following approach. We first unwrapped the phase using the standard procedure to eliminate 2π jumps along the x direction. As can be seen in Fig. 2(c) the slopes of the unwrapped phase vary from line to line because of the jitter and phase changes introduced by y scanning. In the next step we want to eliminate the jitter of the interferometer (caused by path length changes in the sample and reference arm). To eliminate this jitter we have to measure it at first. Ideally this would be carried out on a perfectly flat sample surface. However, our sample contains, in addition to the flat surface, the object structure (chromium steps of the RTT, blood cells in a later experiment), which generates high-frequency phase fluctuations and therefore causes erroneous jitter measurements. In the first step, we measure the jitter (that will be eliminated in a later step) using a procedure similar to the Doppler OCT [8] by calculating the phase difference between adjacent transverse lines. The phase difference between the m th and $(m+1)$ th lines can then be expressed by ($m=1,2, \dots, n$)

$$\phi_m - \phi_{m+1} = \Delta\phi_{m+1} = \phi_m^{\text{jitter}} - \phi_{m+1}^{\text{jitter}} + \phi_m^y \text{ scanning} - \phi_{m+1}^y \text{ scanning}. \quad (2)$$

As mentioned above a residual influence of the object structure would distort the calculation of the jitter. This influence is minimized in the next step; we removed the high-frequency fluctuations (caused by the object structure) by fitting the calculated phase difference with a polynomial of third order, which was empirically determined to be best suited for describing the function in Eq. (2). (Here we rely on the fact that the phase changes caused by scanning of the beam across the object structure occur on a shorter time scale than the phase changes caused by the jitter.)

If we add the first fitted phase difference to the second fitted phase difference, we obtain a phase difference consisting only of the phase of the first line and the phase of the third line (phase of the second line is eliminated),

$$\Delta\phi_1 + \Delta\phi_2 = \phi_1^{\text{jitter}} - \phi_3^{\text{jitter}} + \phi_1^y \text{ scanning} - \phi_3^y \text{ scanning}. \quad (3)$$

The sum of the first three phase differences contains only the phase of the first line and the phase of the fourth line and so forth,

$$\sum_{k=1}^m \Delta\phi_k = \phi_1^{\text{jitter}} - \phi_m^{\text{jitter}} + \phi_1^y \text{ scanning} - \phi_m^y \text{ scanning}. \quad (4)$$

Therefore, we can add the sum of the phase differences to the corresponding unwrapped phases (after removing AOM influence, see above) of each line to obtain a phase map that contains the object structure of the m th line, the phase jitter from the first line, the phase change owing to x scanning (identical for all lines), and a constant phase offset from the first line caused by the y scanning,

$$\phi_m + \sum_{k=1}^m \Delta\phi_k = \phi^x \text{ scanning}(t) + \phi_1^y \text{ scanning}(t) + \phi_1^{\text{jitter}}(t) + \phi_m^{\text{sample}}. \quad (5)$$

Figure 2(d) shows the result of this procedure that clearly eliminates the differing slopes visible in Fig. 2(c). However, the remaining phase is dominated by the phase change introduced by x scanning, which is now the same for all transverse lines.

To remove the first three terms on the right-hand side of Eq. (5), we averaged all the transverse lines along the y direction and fitted this average with a polynomial of fifth order before subtracting from each transverse line [cf. Fig. 2(e)]. In the final step we corrected all images (cSLM, OCT intensity, and OCT phase) for image distortion caused by the sinusoidal motion of the x scanner [cf. Fig. 3(c)].

To test the performance of our system we imaged a U.S. Air Force RTT. The focus and the coherence gate are set to the surface of the RTT. Figure 3(a) shows the cSLM image, and Fig. 3(b) shows the OCT intensity image. Note the inhomogeneous intensity distribution on the glass surface within the OCT image that is caused by the intersection between the coherence plane and the plane of the RTT (only the diagonal from the upper left corner to the bottom right corner lies within the peak of the coherence function). Despite this mismatch of planes the phase image [Fig. 3(c)] clearly shows the phase change introduced by the different height (and a possible phase shift introduced by reflection) of the chromium layer that demonstrates the elimination of all other influences.

To quantify the phase stability and the associated error of the path length difference of the system, we used the following formula [6]: $\Delta x \approx \lambda_0 / 4n\pi(1/\text{SNR})^{1/2}$ (Δx is the error of the

path length difference, SNR is the signal-to-noise ratio, λ_0 is the central wavelength, n is the refractive index of the surrounding medium). With the SNR that was obtained from the measurement with the RTT (~48 dB), we calculated the error of the path length difference with 0.3 nm. Without scanning we measured an error introduced by a phase noise of 0.5 nm, which is in good agreement with the calculated value. However, the standard deviation of Δx obtained from 40 successive frames of the RTT was measured with 3 nm. This increase in the error of the path length difference might be caused by a fast jitter that is introduced by the scanning mirrors.

To demonstrate the applicability of the method for biological samples we imaged human erythrocytes obtained from a dried blood smear. The confocal arrangement of the system already enables the visualization of the typical shape of red blood cells in the cSLM and OCT intensity images [cf. Figs. 4(a) and 4(b)]. However, quantitative assessment of the varying path length differences can be provided only by the phase image [Fig. 4(c)]. One drawback of the method that still has to be solved is the generation of artifacts [cf. small horizontal lines in Fig. 4(c)] that are caused by the low signal intensity from some locations. At these locations a (from frame to frame) reproducible phase jump can be observed, that is, extended by the algorithm to a horizontal line.

In conclusion, we have introduced what we believe to be a new method based on transverse scanning or en-face OCT for the quantitative coherence phase microscopy. The en-face imaging speed of the instrument is more than ten times faster than the comparable coherence microscopy systems based on FDOCT. Since with the proposed method no reference plane close to the sample is needed, the system might be applicable for *in vivo* imaging (e.g., human cornea).

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References

1. Koyama K, Iwasaki A, Tanimoto M, Kudo I. Rev. Sci. Instrum. 1996; 67:2584.
2. Barty A, Nugent KA, Paganin D, Roberts A. Opt. Lett. 1998; 23:817. [PubMed: 18087351]
3. Hitzenberger CK, Fercher AF. Opt. Lett. 1999; 24:622. [PubMed: 18073803]
4. Yang C, Wax A, Georgakoudi I, Hanlon EB, Badizadegan K, Dasari RR, Feld MS. Opt. Lett. 2000; 25:1526. [PubMed: 18066267]
5. Joo C, Akkin T, Cense B, Park BH, de Boer JE. Opt. Lett. 2005; 30:2131. [PubMed: 16127933]
6. Choma MA, Ellerbee AK, Yang CH, Creazzo TL, Izatt JA. Opt. Lett. 2005; 30:1162. [PubMed: 15945141]
7. Pircher M, Baumann B, Goetzinger E, Sattmann H, Hitzenberger CK. Opt. Express. 2007; 15:16922. [PubMed: 19550983]
8. Zhao YH, Chen ZP, Saxer C, Xiang SH, de Boer JF, Nelson JS. Opt. Lett. 2000; 25:114. [PubMed: 18059800]

Fig. 1. Scheme of the experiment. LS, light source ($\lambda=840$ nm, $\Delta\lambda=50$ nm); AOM, acousto-optic modulator; DC, dispersion compensation; BS, beam splitter; L1-L6, lenses.

Fig. 2.
(Color online) Different steps of the evaluation procedure. (a) Measured en-face phase map, (b) en-face phase map after subtracting the phase introduced by the AOMs, (c) en-face phase map after unwrapping, (d) en-face map after correction of the jitter between the lines, (e) en-face map after removing all phase influences, and (f) enlargement of the rectangle in (e).

Fig. 3.
(Color online) Images of a U.S. Air Force resolution target. (a) cSLM image, (b) OCT intensity image, (c) phase image (color bar, measured phase in radians; white bar represents $20\ \mu\text{m}$).

Fig. 4.
 (Color online) Images of human erythrocytes. (a) cSLM image, (b) OCT intensity image, (c) phase image (color bar, measured optical path length difference in nm; white bar represents $20\ \mu\text{m}$), (d) enlargement ($\sim 2\times$) and perspective surface view of the region marked with a rectangle in (c).