High-dynamic-range quantitative phase imaging with spectral domain phase microscopy

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Phase microscopy for high-dynamic-range quantitative phase-contrast imaging of a transparent phase object was demonstrated. Using a common path Fourier domain optical coherence tomography system, this technique is capable of displacement measurement with a sensitivity of 34 pm. The limitation of 2π ambiguity restriction was overcome by the use of a phase retrieval approach performed in spectral domain. Two-dimensional quantitative phase imaging of human neonatal dermal keratinocyte cells was demonstrated to evaluate the performance of the system for cell imaging. © 2009 Optical Society of America

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High-sensitivity and high-speed quantitative phase measurement to retrieve nanometer or subnanometer scale variation is important for applications, such as subcellular dynamics studies and high resolution material inspection. Quantitative phase microscopy based on interferometric techniques are widely developed, including phase shifting interferometry [1], digital holographic microscopy [2], Fourier phase microscopy [3], Hilbert phase microscopy [4], and optical coherence tomography (OCT) [5], etc. The recent application of spectral domain phase microscopy with the Fourier domain OCT (FDOCT) technique for phase measurement has resulted in excellent phase stability, high sensitivity, and imaging speed, because FDOCT does not require mechanical reference scanning and is able to reject common mode noise with common path configuration [6,7].

In phase measurement with FDOCT, the oscillation of the fringes in spectral domain due to optical path difference (OPD) between the reference and sample arms is detected. The phase information is extracted from the complex depth-resolved profile, which is obtained by Fourier transformation of the spectral fringes. Since the phase oscillates 2π rad at every shift of half a wavelength of the OPD, high-sensitivity phase measurement of the fringes can provide an ultrahigh accuracy measurement of the OPD. However, the measurement of OPD with FDOCT systems is restricted to less than half a wavelength owing to the 2π ambiguity. To measure OPD longer than half a wavelength, phase unwrapping algorithms are needed. Conventional phase unwrapping algorithms require that the phase shift varies slowly and gradually [8], which limits the dynamic range of the phase measurement in the case of large phase discontinuity. Phase unwrapping by synthesizing a beat wavelength between two wavelengths was demonstrated for correction of the 2π ambiguity [9,10]. However, additional phase noise or spikes were generated.

In this Letter, we demonstrate a spectral domain phase microscopy technique that is capable of high-dynamic-range quantitative phase-contrast imaging by overcoming the limitation of 2π ambiguity. Unlike conventional spectral domain phase microscopy, the presented technique retrieves phase information in spectral domain instead of depth domain by Hilbert transformation of the detected fringe signal. The discontinuous phase change in depth domain can be converted to a gradually varying phase shift in spectral domain with this approach.

Figure 1 shows the setup of the spectral domain phase microscopy. A superluminescent diode (SLD) with a center wavelength of 890 nm and an FWHM bandwidth of 150 nm was used as the light source. The light was focused by a 20× objective lens on the sample, which rested on the top surface of a coverslip. The backreflected light from the bottom surface of the coverslip acted as the reference for common path configuration to eliminate phase turbulence due to aberration.
to environment variation. The interference fringe was detected by a high-performance 2048 pixel spectrometer which was set at 20,000 A lines/s.

In a spectral domain phase microscopy system, the detected intensity can be expressed as

\[ I(k) = S(k)R_R + S(k)R_S + 2S(k)\sqrt{R_R R_S} \cos(2k \Delta d + \theta), \]

(1)

where \( k \) is the wavenumber; \( S(k) \) is the spectral density of the light source; \( R_R \) and \( R_S \) are the reflectivities from the reference surface and the sample, respectively; \( \Delta d \) denotes the OPD between sample and reference arms; and \( \theta \) is the arbitrary phase. The first and second terms in Eq. (1) represent the reflected intensities from the reference surface and the sample, respectively, and the last term represents the interference between the reference and the sample beams. In a conventional spectral domain phase microscopy, the phase information \( \psi \) is extracted by Fourier transformation of the detected spectral intensity and by taking the argument of the transformed depth-resolved complex function. The OPD was calculated to be \( \Delta d = \lambda_0 \psi / (4 \pi) \), where \( \lambda_0 \) is the center wavelength of the source. This method directly retrieves phase shift and, therefore, experiences the limitation of \( 2\pi \) ambiguity. In this Letter, the spectral fringe \( I(k) \) was first transformed from wavenumber to depth space by fast Fourier transformation. The process of narrow-bandpass filtering was adopted to select the positive term of the complex depth function and reject the noise from the dc term and multiple reflections. The subsequent inverse fast Fourier transform step acquired the complex spectral signal as

\[ \tilde{I}(k) = 2S(k)\sqrt{R_R R_S} \exp[j(2k \Delta d + \theta)]. \]

(2)

The phase term of \( \tilde{I}(k) \) can be retrieved as

\[ \varphi(k) = 2k \Delta d + \theta. \]

(3)

In our setup with a 2048 pixel spectrometer, the phase term is a discrete function:

\[ \varphi(k_i) = 2k_i \Delta d + \theta \quad (i = 0 \sim 2047). \]

(4)

The spectral range \( \Delta \lambda \) of the spectrometer is 200 nm. The corresponding spectral space \( \delta k = k_{i+1} - k_i \) of the spectrometer is about 8 cm\(^{-1}\). With the sample resides on the top surface of a coverslip, the typical OPD \( \Delta d \) is around 210 \( \mu \)m. Therefore, the phase step \( \delta \varphi = \varphi(k_{i+1}) - \varphi(k_i) \) is 0.34 rad. Figure 2 shows the phase term as the function of a wavenumber with the top surface of a coverslip as the sample. With a phase step of 0.34 rad, the phase function can be easily unwrapped as shown in Fig. 2(b). A least-square algorithm was used to calculate the slope of the phase function, which is proportional to the OPD. By calibration with a standard thickness sample, an OPD can be determined precisely. This approach is capable of absolute OPD determination; however, it suffers from a larger displacement error compared with the direct phase retrieval method. Suppose phase stability of the system is \( \Delta \phi \), the displacement error by calculating the slope of the phase function will be \( \lambda_0^2 \Delta \phi / 4 \pi \Delta \lambda \), while the displacement error with the direct phase retrieval method will be \( \lambda_0 \Delta \phi / 4 \pi \). Hence the displacement error is amplified by a factor of \( \lambda_0 / \Delta \lambda \), which is around 5 in our setup. In our experiment, the slope of the phase function was used as the reference for removal of \( 2\pi \) ambiguity, while the OPD was determined with one of the phase components \( \varphi(k_i) \), as

\[ \Delta d = \frac{\varphi(k_i)}{2k_i} + \frac{\pi}{2} \left\lfloor \frac{\varphi'}{2\pi} \right\rfloor, \]

(5)

where \( k_i \) is one of the discrete wavenumbers and \( \varphi' \) is the phase determined with the slope of the phase function, which is used for the determination of the integer multiple of \( 2\pi \).

The minimum detectable displacement is dependent on the phase error in the system. To evaluate the phase stability of the system, a stationary microscope coverslip with a thickness of 210 \( \mu \)m was used as the sample. 1024 A-scans were averaged to determine the phase difference between adjacent A-lines of the interference between the top and bottom surfaces. The phase variations are demonstrated in Fig. 3 showing a phase stability of 0.48 milliradians. The corresponding displacement sensitivity of the system was calculated to be 34 pm in free space. The theo-

![Fig. 2. (Color online) Measured phase in wavenumber space with the top surface of a coverslip as the sample. (a) Wrapped phase, (b) unwrapped phase.](image)

![Fig. 3. (Color online) Probability distribution of measured phase variations with a microscope coverslip as the sample.](image)
retical phase sensitivity is determined by the signal-to-noise ratio (SNR) of the phase measurement system as \[6,7\]
\[
\langle \Delta \phi^2 \rangle = \frac{1}{\text{SNR}}.
\] (6)

The signal-to-noise ratio of our system was measured to be 70 dB. The corresponding theoretical phase stability was calculated to be 0.32 milliradians, which is consistent with our measured value.

To evaluate the capability of the system to remove \(2\pi\) ambiguity, we imaged patterns with thickness steps of more than half a wavelength. Standard SU-8 photolithography technique was used to fabricate the patterns on a glass slide. A coverslip was placed in front of the target as the reference and adjusted to be parallel to the surface of the sample. The OPD between the surfaces of the sample and the coverslip was measured as shown in Fig. 4. Figure 4(a) illustrates the profile of the OPD of the target in one direction measured with the phase unwrapped in wavenumber space in comparison with the OPD calculated with the conventional direct phase retrieval approach. The discontinuously changed phase shift shown in Fig. 4(b) reveals that the \(2\pi\) ambiguity cannot be corrected with the conventional phase unwrapping algorithm. Figure 4(c) shows 3D phase images of the pattern. The residual spikes in the image were due to scratches during fabrication with photolithography. With the phase retrieval approach performed in wavenumber space, the spectral domain phase microscopy system could measure a large range of displacements, ranging from the minimum measurable displacement of 34 pm to the maximum measurable displacement of 2 mm, which is the imaging range of the system and determined by the spectral resolution of the spectrometer.

To demonstrate the performance of the system in cell imaging, living human neonatal dermal keratinocyte cells were used as samples. Figure 5 shows two-dimensional quantitative phase imaging of the cells. The image covering a sample area of 150 \(\mu\)m \(\times\) 150 \(\mu\)m was acquired in 0.3 s.

In summary, a spectral domain phase microscopy capable of high-dynamic-range quantitative phase-contrast imaging was developed. The phase stability of the system was measured to be 0.48 mrad corresponding to a minimum measurable displacement of 34 pm in free space. \(2\pi\) ambiguity was corrected by retrieving the phase in spectral domain. A phase object pattern with discrete displacements was imaged, demonstrating the high-dynamic-range capability of the system. Two-dimensional quantitative phase imaging of human neonatal dermal keratinocyte cells was also presented to evaluate the performance of the system for cell imaging.

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