Quantitative Phase Imaging Using Swept Source

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Abstract: We demonstrate a technique to obtain quantitative field information in the broad visible wavelength range. We verified the capability of the system by measuring the refractive indices dispersion of polystyrene microspheres and BSA solution.

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1. Introduction

A quantitative phase microscopy (QPM) has become a promising technique in biological studying [1]. Due to the non-invasiveness, no needs for exogenous labels for imaging, and the quantitative imaging features, QPM has been extensively used in several researches in studying pathophysiology of cells and tissues [2-8].

Recently spectroscopic QPM techniques have been introduced [9-12]. The spectroscopic QPM does not only provide structural information about a sample but also enables to address chemical information via dispersion, wavelength-dependency of refractive index. Spectroscopic QPM has been enabled by using several lasers, selecting the specific wavelengths from a white-light source with band-pass filters, or utilizing a spatial light modulator [3-5]. However, conventional spectroscopic QPM techniques had limitations: the restricted number of wavelengths or a poor spectral resolution.

Here we demonstrate a swept-source quantitative phase microscopy, which can obtain full-field quantitative phase images for a wide range of visible spectrum (~ 300 nm) with a high spectral resolution (< 10 nm). With the present system, we measured the amplitude and phase images of individual microscopic polystyrene beads immersed in phosphate buffered saline (PBS) solution and 30% bovine serum albumin (BSA) solution. From retrieved field information, we obtained refractive index (RI) dispersions of polystyrene and BSA solution. We also applied this technique to red blood cells and obtained refractive index increments and molar extinction coefficients of hemoglobin (Hb) of intact human red blood cells (RBCs.).

2. Experimental setup

The experimental setup was comprised of a wavelength swept source and a diffraction phase microscopy (DPM) as shown in Fig. 1.

Figure 1. This setup comprised of custom-built swept source (dot line) and DPM (dash line).

GM: galvo mirror, M: mirror, L: lens, G: grating

The DPM is a common-path QPM that provides stable phase image measurements [13, 14] [ref]. The optical imaging condition for DPM does not depend on the wavelength of the illumination used, which enables a direct combination with a swept-source illumination. We have used a custom-built swept source based on a rotating 1-D galvano mirror equipped with a xenon lamp as a white light source. The white light from the lamp was divided into a visible spectrum by a transmission holographic grating. Then each spectrum was focused by a lens, of which the narrow spectral band is then selectively filtered out by passing a pinhole. By varying the angle of the galvano mirror, the spectrum of the band-passed beam is systematically controlled. The angle of the galvano mirror was controlled by a function generator that is synchronized with a sCMOS camera which measures holograms.

The voltage dependency of selected wavelength λ could be expressed as

$$\lambda = d \sin(2\beta V + \theta_0) , \quad (1)$$

where d is a grating separation, β is a rotation angle per a unit voltage, V is the voltage applied to the galvano mirror, and θ_0 is a calibration constant. In our case, the following values are used: $d = 1.67 \mu\text{m}$, $\beta = 2^\circ/V$, and $\theta_0 = 20.6^\circ$. Since the function generator was synchronized with the camera, a series of images can be obtained at high speed in a wide range of visible wavelengths (450 – 750 nm)

3. Results

To verify and calibrate the instrumentation, we first imaged spectroscopic phase images of individual microspheres. Polystyrene beads with a mean diameter of $3 \mu\text{m}$ are immersed in phosphate-buffered saline (PBS) solution, and the quantitative phase images are measured over the visible wavelength range (250 nm spectral bandwidth with 3.3 nm spectral resolution.) Fig. 2(a) shows four representative phase images of a bead. The phase delays $\Delta\phi$ are related with the optical path length differences as following,

$$\Delta\phi(x, y; \lambda) = \frac{2\pi}{\lambda} \Delta n(\lambda) h(x, y) , \quad (2)$$

where Δn is a RI difference, and h is a sample thickness. Once we measured phase images over a wide range of spectra, the RI difference of the bead was calculated. Since the RI of PBS solution was known [ref], the RI of polystyrene could be retrieved. The averaged RIs of 10 bead samples over the wavelengths of 450 – 750 nm are shown in Fig. 2b, which are consistence with reported values [6].

Then we measured the RI dispersion of 30% Bovine Serum Albumin (BSA) solution. To quantitatively measure the RI dispersion values, we have places the polystyrene beads with known RI values, which is then utilized for the separation of RI values and a sample thickness from measured phase images (please refer to Eq. 2.). The measured RIs of 30% BSA solution over the wavelengths of 450 – 750 nm is also shown in Fig. 2b, which is consistence with the reported values [7].

Figure 2. (a) The phase delay maps of a polystyrene bead with a diameter of $3 \mu\text{m}$ immersed in PBS solution. Four phase images measured at representative wavelengths are shown. (b) Comparison of the measured RIs over a wide range of wavelengths and literature values. Shaded area represents standard deviations of 10 samples.

Finally, the RI dispersion of human RBCs was measured. The RI of a RBC, n_{RBC} , can be expressed as

$$n_{RBC}(\lambda) = n_w(\lambda) + \alpha(\lambda) \cdot C + n_x , \quad (3)$$

where α is a refractive index increment, C is the Hb concentration of RBCs, n_w is the RI of water, and n_x is the RI of non-Hb molecules in RBCs. Because the thickness distribution of a RBC is unknown, the dispersion RIs of this type of non-spherical samples cannot be directly obtained. Nonetheless, the refractive index increments and molar extinction coefficients can be quantitatively obtained. If the phase map of a RBC at a specific wavelength is divided by another phase map of the RBC at a different wavelength, C and h can be removed, since the values for C and h are wavelength-independent. From Eqs. (2) and (3), we can derive a following equation:

$$\frac{\lambda \cdot \Delta\phi(x, y; \lambda)}{\lambda' \cdot \Delta\phi(x, y; \lambda')} = \frac{\Delta n(\lambda) \cdot h(x, y)}{\Delta n(\lambda') \cdot h(x, y)} = \frac{\alpha(\lambda)}{\alpha(\lambda')} . \quad (4)$$

Therefore, by assuming the value for α is known at a wavelength, the values for α at all other wavelengths can be measured. The result is shown in Fig. 3(a). In addition, the amplitudes of light fields can also be measured as well as phase images, from which the extinction coefficients over a wide range of wavelengths can be retrieved. The measured molar extinction coefficients of Hb inside intact individual RBCs (results are not shown.)

4. Discussion and conclusion

We demonstrate the quantitative phase microscopy with a swept source for measuring the dispersion of RIs. This system covers almost all visible range from 450 to 700 nm. The center wavelength of the swept source is systematically controlled by rotating the 1-D galvo mirror. The real and imaginary components RIs of microscopic samples were quantitatively measured from the spectral full-field phase image measurements. The present method could be utilized as a refractometer for a micro-size sample over a wide range of wavelengths.

We also measured the dispersions of the RI increments and the extinction coefficients of non-spherical samples over a wide range of spectra. Since these dispersive characteristics of biomolecules are different from each other, this technique could also be used to identify proteins or molecules without using exogenous labeling agents. In addition, the amount of phase delays are proportional to the concentration of non-aqueous samples, furthermore, this could be used to quantitatively measure the dry mass of the sample [15].

Finally we also note that a galvo mirror can rotate at a very high speed up to a few kHz, we expect that dynamic measurements of full-field spectroscopic light field information will be possible if the power of light source is high enough for detection. In our demonstrations, the acquisition time for a single image at a specific wavelength was about 0.05 second but it will be dramatically reduced by using high power light source such as a supercontinuum laser.

5. References

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