Characterization of tissue cells by full information from spectral interferogram

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A new method for characterizing living tissue cells is demonstrated using both phase and amplitude information derived from the spectrally resolved interferogram in a single measurement. The effect of the light source spectral distribution can be cancelled out with the help of the zero order spectrum of the Fourier transform of the interferogram. The ratio of amplitudes between the two interference beams is acquired without this effect. The group delay, the first and second order dispersions, and the absorption, etc., for the full wavelength range can be measured. The results of the culture medium and the HeLa living cells are given. In addition, the measured values of $d^2n/d\lambda^2$ and absorption of the distilled water are also provided for comparison.

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Spectral interferometry has been used to measure the dispersion of optical components, media, photonic devices, and fibers[1−5]. Dispersion should be avoided in optical coherence tomography (OCT) because it causes the degradation of axial resolution. To avoid this problem, many methods have been suggested to suppress the effect of dispersion on image quality[6,7]. Nevertheless, dispersion could also be used for some diagnostic and measurement purposes, for example, the group velocity dispersion of water and lipid may be used to characterize plaque morphology[8]. Recently, second-order dispersion has been used to measure the concentration in aqueous solutions[9]. The optical dispersion of DNA in films has also been measured from interference fringes in absorption and reflection spectra[10]. Spectrally resolved white-light interferometry (SRWLI), a kind of spectral interferometry, has been used to measure ocular dispersion[11]. This technique is simple, highly efficient, and needs just a single measurement for the full wavelength range; it also does not need to measure a single wavelength at a time.

Compared with temporal interferometry, spectral interferometry is faster, has high signal-to-noise ratio due to parallel data acquisition, and is insensitive to external environments because it has no moving parts; its disadvantage is that the spectrometer cannot resolve the denser interference fringe when the wavelength moves further away from the zero-dispersion wavelength of a sample[4]. However, this can be overcome by changing the zero-dispersion wavelength through the change in the optical path difference (OPD) between the two arms of the interferometer, excluding the sample. Dispersion measurement not only provides the information on the refraction index of biological tissues at different wavelengths $n(\lambda)$, it also provides information on the derivatives $dn/d\lambda$, $d^2n/d\lambda^2$, and $d^3n/d\lambda^3$, and even higher order derivatives in a single measurement. The additional information may provide us with more opportunities to diagnose biological tissues. Dispersion can be acquired from the phase of the spectrally resolved interferogram; the amplitude is also directly derived out of the interferogram, so the full information can be applied to characterize the sample. In Ref. [11], the maximum and minimum intensities near certain wavelengths have been used to derive the relative phase; this study also found that the relative phase is unwrapped by the rule that the phase at a fringe peak is equal to an integer multiple of π. Furthermore, the relative phase can be adjusted to the actual phase. In Ref. [4], the relative phase has been directly obtained from the interferogram by the rule stating that phase difference between adjacent positive fringe peaks is 2π.

In this letter, the Fourier transform method is used to simultaneously derive the phase and amplitude information from the spectrally resolved interferogram[12]. A new method, which cancels out the effect of the light spectral distribution on the ratio of amplitudes between the two interference beams, is advanced with the help of the zero-order spectrum of the Fourier transform of the interferogram. Furthermore, the absorption of the sample could be derived from the ratio of the amplitudes. In the proposed method, the dispersion and absorption of distilled water are measured and compared with the results presented in Refs. [13,14]. Then, we report the results of the dispersion measurements of living HeLa cells in the culture medium.

The detailed description of the experimental setup has already been presented in Ref. [5]. The experimental setup consisted of a Michelson interferometer based on a superluminescent light-emitting diode (SLED) broadband light source with a nominal central wavelength of $\lambda_c = 1316$ nm and a bandwidth of $\Delta\lambda = 65$ nm. The detector was a spectrometer with an actual resolution of 0.94 nm.

The recorded interferogram can be written as[5]

$$I(\nu) = I_0(\nu)\{1 + r(\nu)^2 + 2r(\nu)\cos[\phi(\nu)]\}, \quad (1)$$

where ν is defined as $1/\lambda$, λ is the wavelength, $I_0(\nu)$ is the light source spectral distribution, and $r(\nu)$ is the ratio of the amplitudes of the two superposed interference beams. The phase $\phi(\nu)$ can be expressed as[5]

$$\phi(\nu) = 2\pi\nu\{2d[n(\nu) - 1] + 2L\}, \tag{2}$$

where d is the thickness of the sample in the quartz glass cell, L is the extra OPD between the two arms of the interferometer by moving the reflective mirror in one arm away from the zero OPD position without the sample, and $n(\nu)$ is the phase refractive index of the sample. Equation (1) can be Fourier transformed into:

$$\begin{aligned} F[I(\nu)] =& F[I_0(\nu)] \otimes \{[1 + r(\nu)^2]\delta(s) \\ &+ C[s - 2(L - d)] + C^*[s + 2(L - d)]\}, \end{aligned} \tag{3}$$

where C expresses the Fourier transformation of $r(\nu)\exp[\mathrm{j}4\pi\nu dn(\nu)]$, F expresses Fourier transform, and $\otimes$ stands for convolution.

The first-order Fourier spectrum of Eq. (3) can be inversely Fourier transformed to give the information of phase and amplitude as follows:

$$c_1 = I_0(\nu)r(\nu)\exp[\mathrm{j}\phi(\nu)], \tag{4}$$

where c_1 represents the result by inversely Fourier transforming the positive first-order Fourier spectrum. The $r(\nu)$ ratio can be obtained if the light source spectral distribution $I_0(\nu)$ is known. However, another measurement is usually required to obtain $I_0(\nu)$. Here, a new method is advanced to remove the other measurement. The proposed method is described below.

The zero order spectrum could be inversely Fourier transformed into

$$c_0 = I_0(\nu)[1 + r(\nu)^2], \tag{5}$$

then the light source spectral distribution $I_0(\nu)$ can be cancelled out by c_0/c_1. This ratio value could be used to solve the $r(\nu)$ ratio. Furthermore, the absorption $\alpha(\nu)$ of the sample could be derived from the $r(\nu)$ ratio using

$$r(\nu) = \frac{r_s(\nu)\exp[-\alpha(\nu)d]}{r_r(\nu)}, \tag{6}$$

where $r_s(\nu)/r_r(\nu)$ is the ratio of the amplitudes of the two arms of the interferometer, excluding the sample.

The $\phi(\nu)$ phase value in Eq. (2) can be expanded in a Taylor series about $\nu = \nu_0$ as

$$\begin{aligned} \phi(\nu) =& 4\pi\nu_0 dn(\nu_0) + 4\pi\nu_0(L - d) + \left\{4\pi d\left[n(\nu) + \nu\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}\right] + 4\pi(L - d)\right\}\Big|_{\nu=\nu_0}(\nu - \nu_0) \\ &+ 4\pi d\left[2\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu} + \nu\frac{\mathrm{d}^2 n(\nu)}{\mathrm{d}\nu^2}\right]\Big|_{\nu=\nu_0}\frac{(\nu - \nu_0)^2}{2} + 4\pi d\left[3\frac{\mathrm{d}^2 n(\nu)}{\mathrm{d}\nu^2} + \nu\frac{\mathrm{d}^3 n(\nu)}{\mathrm{d}\nu^3}\right]\Big|_{\nu=\nu_0}\frac{(\nu - \nu_0)^3}{6} \\ &+ \cdots. \end{aligned} \tag{7}$$

The obtained $\phi(\nu)$ phase value can then be written by the polynomial fitting process as

$$\phi(\nu) = \phi_0 + \phi_1(\nu - \nu_0) + \phi_2(\nu - \nu_0)^2 + \phi_3(\nu - \nu_0)^3 + \cdots. \tag{8}$$

We obtained the refractive index and group indices of the sample by comparing the coefficients in Eqs. (7) and (8), if the values L and d are known. We also have the group delay $\tau_\mathrm{g} - \tau_0$, and the first- and second-order dispersion coefficients D_1 and D_2 defined as[4]

$$\begin{aligned} \tau_\mathrm{g} - \tau_0 &= \frac{\left[\phi_1 + 2\phi_2(\nu - \nu_0) + 3\phi_3(\nu - \nu_0)^2\right]}{2\pi c} \\ D_1 &= \frac{1}{2d}\frac{\partial\tau_\mathrm{g}}{\partial\lambda} = \frac{1}{2d}\frac{-1}{\lambda^2 2\pi c}\left[2\phi_2 + 6\phi_3(\nu - \nu_0)\right] \\ D_2 &= \frac{\mathrm{d}D_1}{\mathrm{d}\lambda} = \frac{1}{4\pi dc}\left\{\frac{2}{\lambda^3}[2\phi_2 + 6\phi_3(\nu - \nu_0)] + \frac{6\phi_3}{\lambda^4}\right\} \end{aligned}, \tag{9}$$

where c is the light speed in air. All these parameters were feasibly used in this study to differentiate various kinds of tissue cells.

In our experiment, the nominal thickness of the quartz glass cell was set at $d = 1$ mm, the center frequency ν_0 was located at about $\lambda_0 = 1.3$ μm, and the phase and the ratio $r(\nu)$ were taken as the mean values from five interferograms for each sample. The measured values of $\mathrm{d}^2 n/\mathrm{d}\lambda^2$ of the distilled water are given in Fig. 1(a). In comparison with the published data (see Table 1 in Ref. [13]), our measured values of $\mathrm{d}^2 n/\mathrm{d}\lambda^2$ at 1.3 and 1.25 μm are -0.0278 and -0.0232 $\mu\mathrm{m}^{-2}$, respectively. The values at 1.3 μm agree well with each other, although there is a value difference of 0.0041 $\mu\mathrm{m}^{-2}$ at 1.25 μm. The reason for this may be due to the worse fringe visibility at 1.25 μm. The absorption of the distilled water shown in Fig. 1(b), as calculated by Eq. (6), failed compared with the data in Ref. [14]. Nevertheless, some absorption peaks can be seen in the curves at about 1.33, 1.36, and 1.37 μm; these seem to correspond with the data in Ref. [14] with the system errors. The failure could be explained by the fact that first, the calibration process is needed for the measurement of wavelength and absorption. The $r_s(\nu)/r_r(\nu)$ ratio of the amplitudes of the two arms of the interferometer, excluding the sample, is not measured correctly because the two measurements at different times are necessary to determine $r_s(\nu)$ and $r_r(\nu)$ at present, and the light ray of the sample arm of the interferometer is deviated by the sample due to misalignment. Despite these, the $r(\nu)$ ratios are still given in Fig. 2(d) with the system errors. The $r(\nu)$ ratios of the distilled water and the culture medium are similar, but the culture medium has lower values when the wavelength is larger than about 1.4 μm. The $r(\nu)$ ratio of the living HeLa in the culture medium is generally higher than those of the distilled water and the culture medium, indicating that the absorption of the living HeLa is lower than those of the culture medium and the distilled water. On the other hand, the values of $\mathrm{d}^2 n/\mathrm{d}\lambda^2$, and the first- and second-order dispersions in Figs. 2(a), (b), and (c), show that the culture medium is drastically different from the distilled water and the living HeLa in the culture

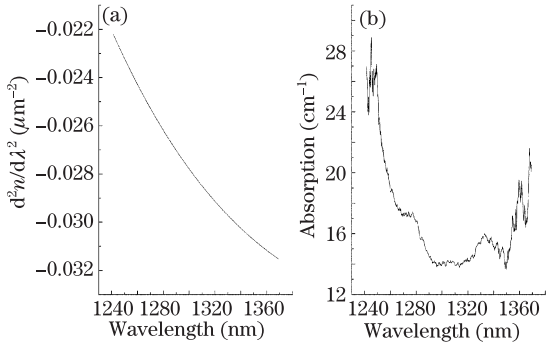

Fig. 1. (a) Second derivative of the index of refraction $d^2n/d\lambda^2$ and (b) absorption of the distilled water.

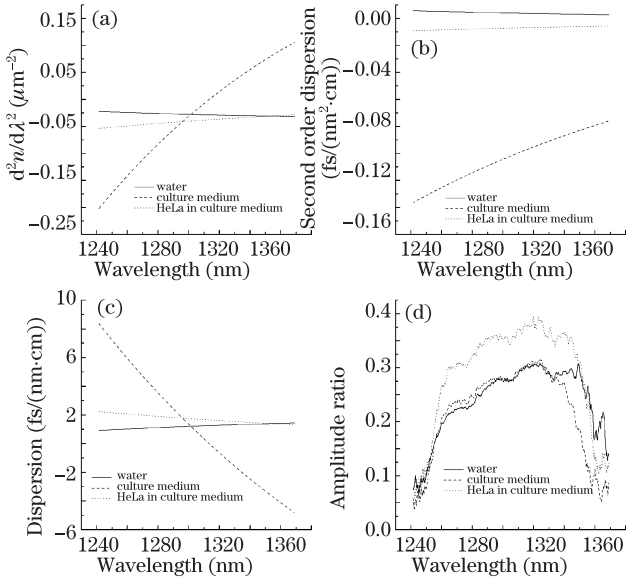

Fig. 2. (a) Second derivatives of the index of refraction $d^2n/d\lambda^2$, (b) second order dispersions, (c) first order dispersions, and (d) amplitude ratios $r(\nu)$ of the distilled water, the culture medium, and the HeLa in the culture medium.

medium in this aspect of phase change. The distilled water and the living HeLa in the culture medium also show apparent differences at this point.

In conclusion, spectral interferometry can be applied in the characterization of the living cells in culture medium, which requires just a single measurement. The phase and amplitude can be derived directly from the spectrally resolved interferogram at the same time. With changed phase sensitivity, the derived phase values and the relevant parameters, such as $d^2n/d\lambda^2$, the first- and second-order dispersion, etc., should be able to characterize and differentiate living cells in real time and with high sensitivity. Although the zero order of the Fourier transform of the interferogram has been used to extract the source spectrum[15], we introduce a new method to focus on the measurement of the amplitude ratio $r(\nu)$ and the absorption of the sample. Spectral interferometry should have much wider applications in biomedical instruments. It has many advantages, including the use of just a single measurement, full information (phase and amplitude), many parameters, and full wavelength range.

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