Fourier analysis of elastic light scattering spectrum of epithelial cell nuclei

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A new method for simultaneously determining the size and refractive index of epithelial cell nuclei is presented. The function of the modified elastic light scattering spectrum is regarded as a function of wave number factor, $Q=2\lambda^{-1}\sin(\theta/2)$. The modified spectrum has a constant oscillation period with its frequency proportional to the average diameter of cell nuclei. To the same average diameter, the different relative refractive indexes of epithelial cell nuclei only induce the horizontal shift of the spectra. Both the oscillation frequency and the horizontal shift are quantified by the fast Fourier transform on the modified spectra. The average diameter can be figured out through the peak frequency divided by the value of the refractive index of the surrounding medium. The phase angle of the peak frequency has an approximate linear relationship with the relative refractive index of epithelial cell nuclei.

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The epithelium is the tissue composed of cells that line the cavities and surfaces of structures throughout the body. More than 85% of all cancers originate in the epithelium[1,2]. The early form of carcinoma defined by the absence of invasion of surrounding tissues is called carcinoma *in situ* (CIS). The keystone for the early detection of cancer is to diagnose CIS in time. The treatment for CIS is usually simple and completely effective. When treated in the stage of CIS, the patient can make a full recovery. Elastic light-scattering (ELS) spectroscopy shows significant promise as a fast and noninvasive tool for early cancer diagnosis and has attracted considerable attention at present[3−5]. In the measurement of ELS spectroscopy, epithelial cell nuclei can be considered as spheroidal Mie scatterers[6]. The increase of the average size and the relative refractive index value of cell nuclei will indicate the dysplasia of epithelial tissue[7,8].

In the determination of cell configuration with ELS spectroscopy, the inverse method of light scattering spectroscopy plays an important role[9,10]. The inversion of light scattering based on Mie theory is a nonlinear problem related to multiple parameters, which we used to solve the Fredholm integral equation of the first kind. To deal with such a typical ill-posed problem, more recent work is based on assuming a relative refractive index of cell nuclei to retrieve its average size with Tikhonov regularization methods[11,12]. However, these methods are unreliable for ascertaining the relative refractive index of cell nuclei. In this letter, the function of the modified ELS spectrum is regarded as a function of wave number factor. Then the average size and the relative refractive index can be simultaneously determined with the Fourier transformation on the modified spectrum.

Figure 1 illustrates a typical schematic diagram of ELS spectroscopy system to measure the average size of epithelial cell nuclei. The light from a broadband light source arrives at the collimating lens through a fiber-optic cable. Then, the parallel light beam through a broadband polarizer illuminates a circular spot on the surface of epithelial tissue at a certain angle from the normal direction. After impinging on the epithelial tissue, the light scattered normal to the surface passes through an analyzer and is focused on the fiber end face connected with a spectroscope. The polarizer keeps stationary and transmits light polarized parallel to the scattering plane (defined by the directions of the incident light and the scattering light), whereas the collection analyzer can be rotated to make its transmission axis parallel or perpendicular to the transmission axis of the incident polarizer. With the parallel-polarized incident light $I_{\parallel}^{\mathrm{i}}$ illuminating, the scattering light in the normal direction consists of two components. One is the single scattering light from epithelial cell nuclei in the top layer, which preserves its original polarization state, marked as $I_{\parallel,\mathrm{top}}^{\mathrm{s}}$, and the other is the diffusely reflected light $I_{\Sigma,\mathrm{bottom}}^{\mathrm{s}}$, which travels into the sample deeper and re-emits at the surface. As a result of multiple scattering, the diffusely reflected light $I_{\Sigma,\mathrm{bottom}}^{\mathrm{s}}$ is mainly unpolarized. Therefore, the parallel component to the scattering plane of the diffusely reflected light $I_{\parallel,\mathrm{bottom}}^{\mathrm{s}}$ is equal to the perpendicular component $I_{\perp,\mathrm{bottom}}^{\mathrm{s}}$, that is $I_{\parallel,\mathrm{bottom}}^{\mathrm{s}}=I_{\perp,\mathrm{bottom}}^{\mathrm{s}}=0.5I_{\Sigma,\mathrm{bottom}}^{\mathrm{s}}$. In the first measurement when the polarization of the collection analyzer is parallel to the scattering plane, the measured scattering light is $I_{\parallel}^{\mathrm{c}}=I_{\parallel,\mathrm{top}}^{\mathrm{s}}+I_{\parallel,\mathrm{bottom}}^{\mathrm{s}}$. In the second measurement when the polarization of the collection analyzer is perpendicular to the scattering plane, the measured scattering light is $I_{\perp}^{\mathrm{c}}=I_{\perp,\mathrm{bottom}}^{\mathrm{s}}$. By subtracting the second measured light intensity from the first measured light intensity, the single scattering light from epithelial cell nuclei in the top layer is $I_{\parallel,\mathrm{top}}^{\mathrm{s}}=I_{\parallel}^{\mathrm{c}}-I_{\perp}^{\mathrm{c}}$. This equation holds true as long as the intensities of parallel and

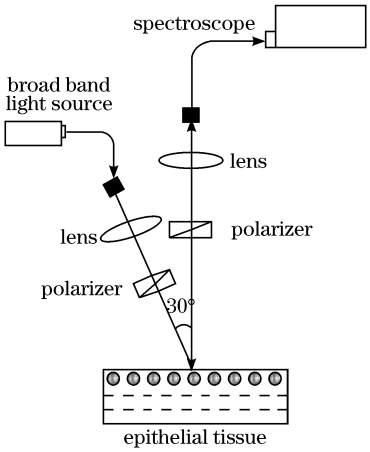

Fig. 1. Schematic diagram of ELS spectroscopy system.

perpendicular incident light are equal and the multiple scattering light from the deep substrate is totally unpolarized.

Only the single scattering light from epithelial cell nuclei is utilized in the measurement. The multiple scattering background is subtracted because the mathematical modeling for multiple scattering is intractable and the multiple scattering light loses the information about epithelial cell nuclei with the long walking path in biologic tissue. Mie theory provides an exact solution for calculating the intensity of single scattering polarized light from a micro sphere with a diameter comparable to the incident wavelength λ[13]:

$$I^{\mathrm{s}}_{\parallel,\mathrm{top}} = cI_0 \left|S_2(\theta, m, d, \lambda)\right|^2, \tag{1}$$

where c is the system constant, I_0 is the intensity of the incident light, θ is the scattering angle, m is the relative refractive index of the micro sphere to the surrounding medium, d is the average diameter of epithelial cell nuclei, and S_2 can be expressed in a series of spherical harmonic forms. The algorithms for S_2 were given in Ref. [14].

The average diameter range of epithelial cell nuclei is typically 5−15 μm and the relative refractive index range is 1.01−1.1[15]. Figure 2 shows a typical light scattering spectrum calculated by Eq. (1) for epithelial tissue, where d=5 μm, m=1.01, θ=150°, the refractive index of the surrounding medium is 1.33, and the incident wavelength ranges from 0.4 to 0.8 μm. As shown in Fig. 2, the scattering is composed of a series of maxima and minima. The whole spectrum displays an approximate period oscillation with the period increasing when the wavelength increases.

In order to explore the relationship between the oscillation frequency of the scattering spectrum and cell nuclei, the constant oscillation period is favorable. To the scatterers with a lower value of the relative refractive index $[(m-1)d \ll \lambda]$, the scattering light can be approximated using the Rayleigh–Gans formula[16,17]:

$$I(u) \sim \left[3(\sin u - u\cos u)/u^3\right]^2, \tag{2}$$

where $u = 2\pi\lambda^{-1}\sin(\theta/2)m_{\mathrm{medium}}d = \pi Q m_{\mathrm{medium}}d$, m_{medium} denotes the refractive index of the surrounding medium. The period of Eq. (2) is obviously

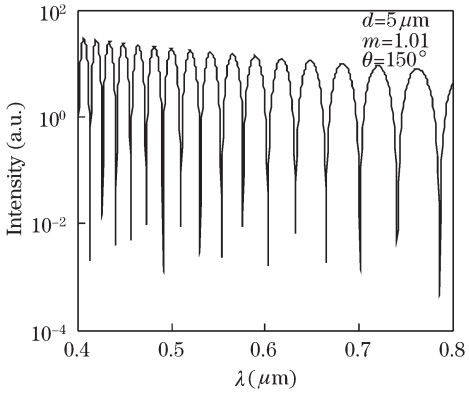

Fig. 2. Typical light scattering spectrum for epithelial tissue.

π with respect to the variable u; hence $1/(m_{\mathrm{medium}}d)$ is with respect to the variable Q. Therefore the frequency of Eq. (2) is $\nu_0 = m_{\mathrm{medium}}d$ with respect to the variable Q, which inspires us to select the inverse of wavelength (wave number) as the independent variable. Figure 3 shows the modified ELS spectra with Mie theory as a function of wave number factor, Q=$2\lambda^{-1}\sin(\theta/2)$. Figure 3 presents the stable oscillation periods of the scattering spectra. Another important point is that for the same average diameter of cell nuclei, the different relative refractive indexes of cell nuclei only induce the horizontal shift of the spectra. Carefully comparing Figs. 3(a) and (b), we find that the larger the cell nuclei, the greater the oscillation frequency, which provides possibility to determine the parameters of cell nuclei.

The corresponding power spectra of the scattering spectra in Fig. 3 can be obtained from the fast Fourier transform (FFT)[18]. Figure 4(a) presents the power spectra when the relative refractive index of cell nuclei is

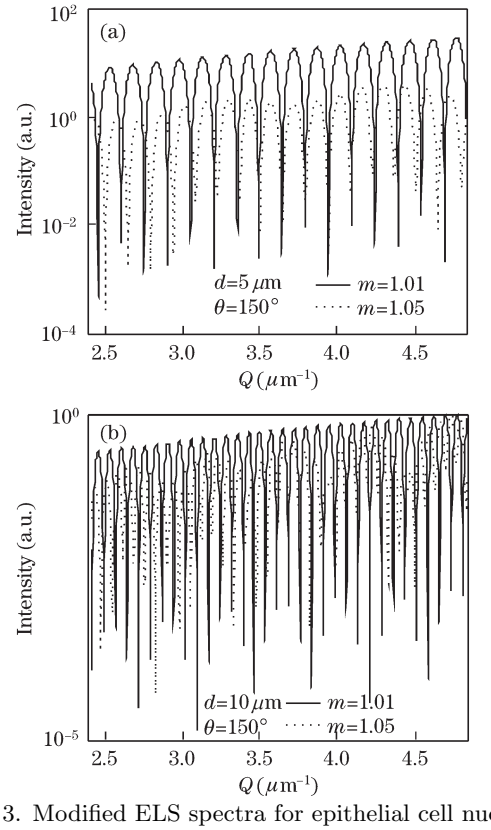

Fig. 3. Modified ELS spectra for epithelial cell nuclei.

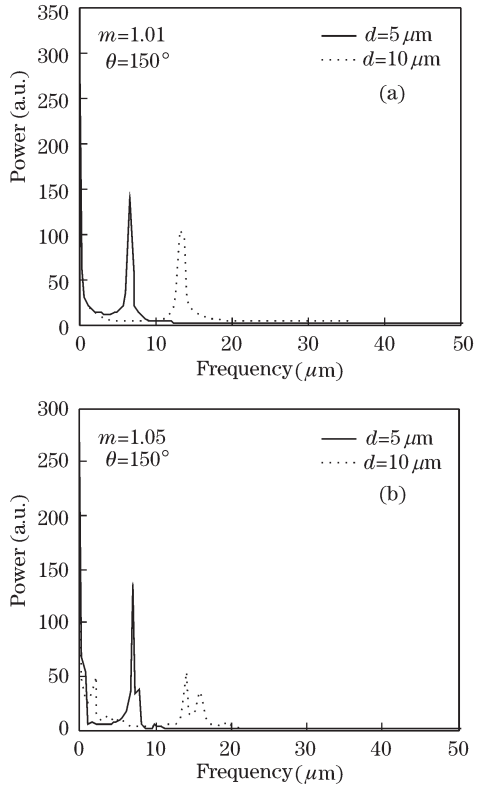

Fig. 4. Power spectra of Fig. 3 through FFT.

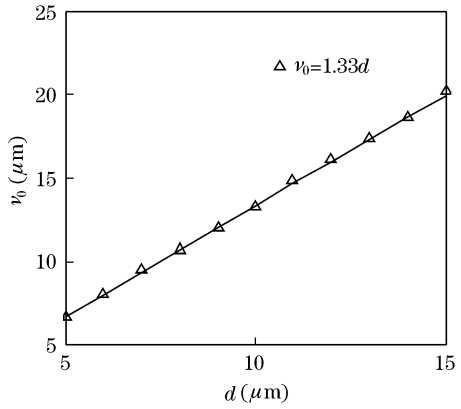

Fig. 5. Peak frequency versus average diameter.

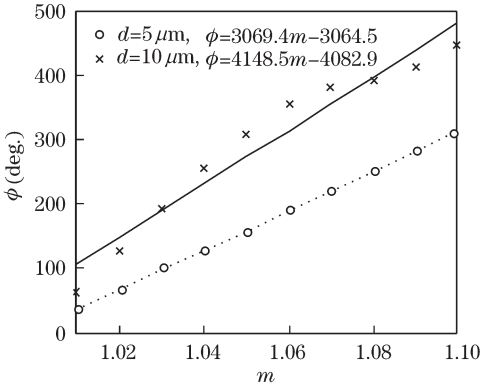

Fig. 6. Phase angle versus the relative refractive index of epithelial cell nuclei for two different diameters.

1.01 and the average diameters are 5 and 10 μm, respectively. Except the direct component, the power for 5-μm diameter has a peak value at the frequency ν_0=6.619 μm; the power for 10-μm diameter has a peak value at the frequency ν_0=13.238 μm. Figure 4(b) presents the power spectra when the relative refractive index of cell nuclei is 1.05 and the average diameters are 5 and 10 μm, respectively. There is no distinct difference between Figs. 4(a) and (b) except that the curves in Fig. 4(a) are smoother than those in Fig. 4 (b). Furthermore, we display the peak frequency (i.e., the frequency corresponding to the peak value of the power spectrum) versus the average diameter in Fig. 5. It is clear that the slope of the curve comes up to 1.33 (the refractive index of the surrounding medium).

When the average diameter is determined, the different relative refractive indexes of cell nuclei will change the initial phase of the spectra, which can be described by the phase angle through FFT. Figure 6 is the phase angle versus the relative refractive index with average diameters of 5 and 10 μm. It can be seen that there is an approximate linear relationship between the phase angle and the relative refractive index of cell nuclei. Thus the relative refractive index is easily obtained when the average diameter of cell nuclei and the phase angle are known.

In conclusion, we put forward a new method to simultaneously determine the average size and the refractive index of epithelial cell nuclei. The function of the modified ELS spectrum is regarded as a function of wave number factor, $Q=2\lambda^{-1}\sin(\theta/2)$. Based on the peak frequency of the power spectrum through FFT of the modified ELS spectrum, the average diameter can be determined with $d \approx \nu_0/m_{\text{medium}}$. The relative refractive index of the surrounding medium can also be obtained according to an approximate linear relationship between the phase angle and the relative refractive index of cell nuclei. The linear coefficients are not identical for different average diameters (the slope of curve increases with increasing the average diameter).

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