Reflective mesoscopic spectroscopy for noninvasive detection of reflective index alternations at nano-scale

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Abstract. Cancer has been one of the most serious threats to human life. However, there is no substantial improvement in overall treatment of cancer patients. One of the key reasons is the unavailability of convenient method to detect cellular alterations in ultra-early stage of carcinogenesis processes, where genetic aberrations at nano-scale have not yet resulted in histological changes. In this paper, we described an optical method based on reflective mesoscopic spectroscopy for ultra-early cancer detection. According to mesoscopic light transport theory, photons propagating in one dimension (1D) within a weakly disordered medium have the non-self-averaging effect. Reflected signal after 1D propagating is sensitive to any length scale of refractive index fluctuations due to multiple interferences of light waves travelling along 1D trajectory. The principle of mesoscopic spectroscopy for perceiving reflective index fluctuations at length scale of nanometers is introduced. A system for the measurement of reflective mesoscopic spectroscopy based on spatial-incoherence broadband source and spectrometer is established. Simulations on light propagation in cell-emulating model with controlled refractive index distribution are done by finite-difference time-domain (FDTD) approach.

1. Introduction

Cancer has been one of the most serious threats to human life. Although a series of new therapy methods were developed, including immunotherapy, endocrine therapy, gene therapy and so on, however, there is no substantial improvement in overall treatment of cancer patients. One of the key reasons is the unavailability of convenient method to detect cellular alterations in ultra-early stage of carcinogenesis processes. Most current cancer detection methods rely on medical imaging, tumor-specific serum markers, and tissue biopsy. The sensitivity and resolution of medical imaging methods such as ultrasound, computed tomography, magnetic resonance imaging and positron emission tomography have serious limitations. Tissue biopsy is the most effective method of tumor diagnosis. Overall, limited by optical resolution, only large-scale structural abnormality information can be detected. There is rare study on the relationship between cell’s nano-scale structures changes and cell’s carcinogenesis process. One possible way is to use transmission electron microscopy [1, 2], which can achieve nano-resolution imaging for thin tissue. But the flaw is thin tissue don’t have the complete information of living cells under natural status. New optical techniques, such as optical coherence tomography, confocal optical

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microscopy, optical diffusion tomography, and imaging technique based on the elastic scattering, fluorescence or Raman spectroscopy [3-8], compared to conventional medical imaging techniques have higher resolution and sensitivity, but they are still limited by the far-field optical resolution (at best, 200nm). So it’s impossible to recognize the earlier stages of carcinogenesis that precede histological changes up to now. Ultra-high resolution optical imaging techniques including near-field and far-field [9-11] have been developed to break through the optical diffraction limitation, but limited by subwavelength contact area, or require additional exogenous nano tags, even for certain specific sample which has nonlinear absorption properties, all of these techniques can not be widely used in non-invasive observation of cells under natural status.

Reflective mesoscopic spectroscopy method is introduced in this paper, which is based on one dimensional light transport property in weakly disorder and weakly scattering medium such as biological cells and it’s sensitivity to nano-scale refractive index fluctuations. It can be used to describe the subtle changes of optical properties in the ultra-early stage of cell’s carcinogenesis process. The proposed method might be useful in probing statistical structural changes at nano-scale which are unperceivable up to now, and hence avoiding the limitation due to optical diffraction in far field.

2. Method

2.1. Theoretical basis

Spectroscopy of elastic scattering has been widely used to probe tissue morphology. However, the traditional scattered light detecting methods are generally record the information after photos propagation in 3D space, due to the Fourier-transform relationship between the scattering signal and the scattering potential [20], the sensitivity to sub-wavelength length scales decreases fast with size. However, according to mesoscopic light transport theory, while photos propagating in 1D are recorded, situations will be completely different [19].

According to 1D mesoscopic light transport theory [24, 25], photons propagating in a one-dimensional weakly disordered refractive-index variable medium have the non-self-averaging effect [21-23]. Reflected signal after 1D propagating is sensitive to any length scale of refractive index fluctuations due to multiple interferences of light waves travelling along 1D trajectory. In other words, the reflected signal is sensitive to any length scale of refractive index fluctuations including those below the wavelength.

The previous research results have confirmed that nano-scale structural abnormalities are the biology basis of ultra-early detection of carcinogenesis cell [12-14], while they are accompanied by refractive index changes which is the most significant changes. In order to describe the disorder degree of refractive index fluctuations in a 1D channel, the statistical parameter disorder strength can be introduced [18, 19]:

\[ L_d = \langle \Delta n^2 \rangle \ell_c \]  

\( \langle \Delta n^2 \rangle \) is the variance of refractive index fluctuations in the channel and \( \ell_c \) is the spatial correlation length of these refractive index fluctuations. The disorder strength quantifies the spatial variability of refractive index and, thus, the local concentration of intracellular material. At a given 1D channel, \( \langle \Delta n^2 \rangle \) is proportional to the local concentration of intracellular solids, whereas \( \ell_c \) can be viewed as the characteristic size of the intracellular ultra-structure of a cell. Although the disorder strength do not directly related to structural changes within the channel at certain specific location, increased disorder strength represents 1D channel toward a more disordered state, so it is feasible to regard disorder strength as optical fingerprint of cell’s carcinogenesis.

The next step is to analysis the association between disorder strength and reflective mesoscopic spectrum from a given 1D channel. Because fluctuating part of reflective mesoscopic spectrum \( R(\lambda) \) is formed primarily by 1D back-scattered photos, \( R(\lambda) \) can be analyzed by means of 1D mesoscopic light
transport theory in disorder medium [24, 25]. If \( kL < 1 \) and \( 4k^2L/L_n < 1 \), where \( k \) is the center wave number, \( L \) is channel’s length, \( n_o \) is the mean refractive index in the channel (These three parameters are all known or can be estimated by reference to typical values), with experimentally acquired spectrum \( R(\lambda) \), disorder strength \( L_d \) can be calculated as [15, 16, 18, 19]:

\[
L_d = A(n_o^2/2k^2) < R > (L/L)
\]

(2)

where \( A \) is the calibration constant, \( < R > \) means the root mean square of fluctuation part of 1D reflective spectrum. The equation (2) shows that the disorder strength is linearly depend on the root mean square of \( R(\lambda) \).

2.2. System description

The design of the reflective mesoscopic spectroscopy instrument is shown in figure 1[17]. The broadband white light from a Xe lamp is collimated by a 4f system (L1-A1-L2) and is focused onto a sample by a low-numerical-aperture objective (NA of objective=0.4, NA of illumination=0.2, NA of collection=0.4, Nikon CFI Plan). The spatial coherence length of the illumination beam is in the order of the diffraction limitation, \( \approx 700 \) nm. The illumination beam diameter is 120\( \mu \)m (the diameter of sample is \( \approx 10 \mu \)m), which ensured that plane-wave illumination can be assumed for analyzing light scattering signatures. The backscattered light is collected by the same objective and focused by a tube lens to form a magnified image. The resulting backscattered image is projected with a 40\( \times \) magnification onto the slit of an imaging spectrograph (20\( \mu \)m slit width, PI SP 2156) coupled with a CCD (PI PIXIS 400BR) camera. The flipper mirror directed the image onto a digital camera for quick visualization of the image while adjusting the distance between the objective lens and the sample. The sample is mounted on a scanning stage (KOHZU).

![Figure 1. Schematic of the reflective mesoscopic spectroscopy system.](image)

Assume that sample such as a single cell was placed flat on a slide. In the sample space, the spatial coherence length is in the order of the diffraction limited lateral resolution(\( \approx 700 \)nm in this paper). As shown in figure 2, the whole cell (Figure 2 (a)) was virtually divided into several parallel 1D channels (Figure 2 (b)), each with a diffraction limited lateral size. Compared to the typical diameter of biological cells (such as 10\( \mu \)m), refractive index distribution in the transverse direction within a single
channel can be considered constant. So the photos can be approximately regarded as one-dimensional transmission along a channel while there is only one dimension of refractive index fluctuations.

**Figure 2.** Single cell was virtually divided into several parallel 1D channels

This instrument can simultaneously obtain a diffraction-limited resolution microscopic image of sample and record the spectrum from each pixel (each 1D channel). The back-scattering image is acquired by linearly scanning the sample with a 0.5μm step. The size of a pixel in the image plane (image pixel) is 20μm×20μm, while the size of a pixel in the object plane (sample pixel) is 500nm×500nm. At each scanning step x, the CCD camera records a matrix with one axis corresponding to y and the other to the spatial position of the image y, resulting in a data cube \( I(x,y;\lambda) \).

This also means that each spectrum \( I(x,y;\lambda) \) is from a particular one-dimensional channel. So the spectrum of the back-scattered light intensity \( I(x,y;\lambda) \) which is formed by interference of photons reflected from refractive index fluctuations within a channel is recorded. Experiments on nano-structured models and biological samples are on schedule.

3. Simulation

**Figure 3.** Simulation layout, refractive index profile and its autocorrelation function

We have confirmed the dependence of \( L_d \) on nano-scale refractive index fluctuations by conducting rigorous numerical experiments using finite-difference time-domain (FDTD) approach which provides an accurate solution for the propagation of light in essentially arbitrary media. All the simulation parameters settings were refer to reported conclusions [15-19].

As shown in figure 3 (a), we used 1D slab model to simulate the 1D channel in real sample. The lateral size (x axis) was set to 600nm which was in the order of the diffraction limited lateral resolution. The simulated channel’s length was set to 5μm which was the height when a cell was
placed flat on a slide. Refractive index fluctuations exist in only one direction (z axis) and obey the
gaussian white noise distribution. The spatial distribution was ladder-like with a uniform spatial step
(50nm in Figure 3). Figure 3 (b) is the refractive index profile of 1D channel as shown in Figure 3 (a),
the variance of refractive index fluctuations is $<\Delta n^2> = 1 \times 10^{-4}$, while Figure 3(c) is the
autocorrelation function of refractive index fluctuations. The spatial correlation length of refractive
index fluctuations was approximately equal to ladder step. Both standard deviation and spatial
correlation length of the refractive index fluctuations along the one-dimensional channel were under
precise control.

In the FDTD simulation layout, the grid was terminated using a Berenger perfectly matched layer
(PML) absorbing boundary condition, the shadow area in Figure 3 (a). A modulated Gaussian pulse as
the time-domain source waveform which accommodates the complete frequency range of visible light
was employed to source an x-polarized plane wave propagating in the FDTD grid. The back-scattered
frequency response is extracted via a discrete Fourier transform (DFT) of the time-domain data
recorded on the backward surface and normalized by the spectrum of the source pulse. Then the wave
number was converted to wavelength, the final reflective mesoscopic spectrum along 1D channel was
acquired.

![Figure 4. Simulated 1D mesoscopic reflective spectrum versus standard deviation](image)

![Figure 5. Simulated 1D mesoscopic reflective spectrum versus spatial correlation length](image)

If the spatial correlation length was constant (lc = 50nm), while the standard deviation of the
refractive index fluctuations was varied, simulated 1D mesoscopic reflective spectrums were shown as
figure 4. In turn, if the standard deviation of the refractive index fluctuations was constant
($\Delta n = 0.015$), while the spatial correlation length was varied, simulated 1D mesoscopic reflective
spectrums were shown as figure 5. Known from figure 4 and figure 5, the reflective spectrums were
highly sensitive to subtle changes of standard deviation $\Delta n$ and spatial correlation length $l_c$ of refractive
index fluctuations. In other word, the 1D reflective mesoscopic spectrums were also highly sensitive to
subtle changes of disorder strength.
Through precise control of the standard deviation and the spatial correlation length of refractive index fluctuations along a one-dimensional channel, calculated disorder strength (calculated by equation (2)) and theoretical disorder strength (calculated by equation (1)) were quantitatively analyzed. The simulation results were illustrated in figure 6.

Figure 6 (a) showed situations of the spatial correlation length was constant ($l_c = 50 \text{ nm}$), while the standard deviation of refractive index fluctuations $\Delta n$ varied from 0 to 0.02. Figure 6 (b) showed situations of the standard deviation of refractive index fluctuations was constant ($\Delta n = 0.015$), while the spatial correlation length $l_c$ varied from 10nm to 100nm. In the areas of marked with red solid lines in both figure 6 (a) and figure 6 (b), the linear correlation of theoretical disorder strength and calculated disorder strength were as high as $r = 0.9968$ and $r = 0.9904$ respectively. So calculated disorder strength was essentially equal to theoretical disorder strength. However, in the area of without marked with red solid lines in figure 6 (b), calculated disorder strength was generally smaller than theoretical disorder strength. The main reason was that the approximation condition which was used in deriving calculated disorder strength formula was not fully met.

4. Conclusion
The mesoscopic theory was applied to analysis the properties and optical information of the photon transmission in 1D medium which has nanometer scale refractive index fluctuations. One-dimensional photonic transmission channel model of weakly disordered and weakly scattering biological cell was established. The root mean square of the fluctuating part of reflective spectrum from 1D channel was linearly depends on the disorder strength. Although the disorder strength do not directly related to structural changes within the channel at certain specific location, increased disorder strength represents 1D channel toward a more disordered state, so regarding disordered strength as optical fingerprint of cell’s carcinogenesis state is feasible. The FDTD simulation results show that reflective spectrum is highly sensitive to disorder strength. Under certain approximating conditions, disorder strength is proportionally enlarged with increasing variance and spatial correlation length of the refractive index fluctuations. An experimental instrument has been established, which can simultaneously obtain a diffraction-limited resolution microscopic image of sample and record the spectrum from each pixel (each 1D channel). Experiments on nano-structured models and biological samples are on schedule.

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