Optical coherence microscopy detects
subwavelength refractive index fluctuations: an
application to cancer diagnosis

Hariharan Subramanian,1 Prabhakar Pradhan,1 Yang Liu,1 Ilker R. Capoglu,2 Jeremy D. Rogers,1
Hemant K. Roy,2 Randall E. Brand,2 and Vadim Backman1,8

1Biomedical Engineering Department, Northwestern University, Evanston, Illinois 60208, USA
2Evanston Northwestern Healthcare, Evanston, Illinois 60208, USA
*Corresponding author: v-backman@northwestern.edu

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Existing optical imaging techniques offer us powerful tools to directly visualize the cellular structure at the
micron-scale; however, their capability of nanoscale sensitivity is restricted by the diffraction-limited reso-

lution. We show that the mesoscopic light transport theory analysis of the spectra of partial waves propa-
gating within a weakly disordered medium, such as biological cells [i.e., partial wave spectroscopy (PWS)]
quantifies refractive index fluctuations at subdiffractional length scales. We validate this nanoscale sensi-
tivity of PWS using experiments with nanostructured models. We also demonstrate the potential of this
technique to detect nanoscale alterations in cells from patients with pancreatic cancer who are otherwise
classified as normal by conventional microscopic histopathology. © 2009 Optical Society of America

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Spectroscopy of elastic scattering is commonly used to
to probe tissue morphology [1]. However, the sensitiv-
ity of a light-scattering signal to refractive index fluctu-
tions is significantly reduced when the size of the
scattering structures falls below the wavelength
(~500 nm). Recently, there has been significant inter-

est in understanding biological systems at the
nanoscale, which requires measurement of subwave-
length refractive index fluctuations. According to the
mesoscopic light transport theory [2–4], for an object
that is weakly disordered and weakly scattering, it is
indeed possible to probe refractive index fluctuations
of any length scale, including those well below the
wavelength [3,4], if one analyzes a signal generated by
the multiple interference of one-dimensional (1D)
propagating waves reflected from the refractive index
fluctuations within the object. The enhanced sensitiv-

ity of 1D propagating waves to subwavelength corre-
lation lengths of refractive index fluctuations $l_c$ (i.e.,
$l_c <$ wavelength $\lambda$) can be understood from the fol-

lowing consideration: while a three-dimensional scat-
ttering coefficient is $-(l_c/\lambda)^3$, and, thus, the contribution from small length scales is weighted down as
$l_c^2$, for 1D waves the scattering coefficient is $-(l_c/\lambda)$. The
1D propagating waves are one of many of a subset of
waves (herein called 1D-partial waves) propagating
within a scattering particle.

Recently, we reported an optical system [5] capable
of isolating 1D-partial waves from different parts of a
homogeneous scattering particle. A detailed descrip-
tion of the partial-wave spectroscopy (PWS) instru-
ment is given elsewhere [5,6]. In brief, a broadband
light with a spatial coherence length of <1 $\mu$m is fo-
cused onto the sample by a low-NA objective (Ed-
mund Optics, NA of objective=0.4, NA of illumina-
tion=0.2, NA of collection=0.4). The illumination-beam diameter (~120 $\mu$m) is much
larger than biological cells (~8 $\mu$m) and is well colli-
mated within a cell located in the waist of the beam.

The resulting backscattered image is projected with a
60× magnification onto the slit of an imaging spec-
trograph (10 $\mu$m slit width) coupled with a CCD cam-
era (Coolsnap HQ, RoperScientific, 1392×1040
6.2 $\mu$m pixels) and mounted onto a motorized linear
scanning stage (Zaber Technologies). The back-
scattering image is acquired by linearly scanning the
slit of the spectrograph with a 10 $\mu$m step. The size of
a pixel in the image plane (image pixel) is 6.2 $\mu$m
×10 $\mu$m, while the size of a pixel in the object plane
(cell pixel) is 100 nm × 165 nm. At each scanning step
$\lambda_1 = 500$ nm to $\lambda_2 = 670$ nm is analyzed owing to the low
illumination and transmission efficacy of the instru-
ment) is recorded for each cell pixel $(x,y)$. All spectra
obtained from each cell pixel are normalized by the
spectra of the incident light using mirror reflection.

For each $(x,y)$, $I(\lambda) = I(\lambda|x,y)$ is further processed
to remove the high-frequency spectral noise using a
sixth-order low-pass Butterworth filter with a normal-
ized cutoff frequency of 0.08, and the variations in the
lamp spectrum using a low-order polynomial
$I_1(\lambda)$ fit to $I(\lambda)$. The normalized cutoff frequency (cut-
off frequency/sampling frequency) was chosen such
that it removes all oscillations below the spectral res-
olution of the spectrometer. The resulting spectrum
$R(\lambda) = I(\lambda) - I_1(\lambda)$ is referred to as the fluctuating part
of the reflection coefficient. It is important to realize
that unlike traditional light scattering experiments,
where a scattering signal is formed by all waves
propagating within a scattering particle and interfer-
ing in the far field, the backscattering spectrum analyzed in PWS is formed by the subset of waves, in particular 1D-partial waves. The properties of both the object and the instrument facilitate the detection of 1D waves. While the low NA of the objective restricts the illumination and collection of light to a narrow cone, the weak refractive index fluctuations and a small radius of curvature of a cell spread on a glass slide reduces the probability of the interference among the adjacent 1D channel, which is further prevented by the low-coherence illumination. Thus, light interaction with a complex weakly disordered medium can be approximated as a combination of several independent parallel 1D channels with \( R(\lambda) \) generated by the multiple interference of photons propagating in these 1D channels.

Typical \( I(\lambda) \) and \( I_p(\lambda) \) obtained from a particular cell pixel (in this case, a cell isolated from human pancreatic epithelium) spread on a glass slide is shown in Fig. 1(a). Figure 1(b) shows the corresponding \( R(\lambda) \). To show the noise level, the spectrum is compared with the one obtained from a glass slide. As seen, the intensity fluctuations seen in the spectrum from the cell are above the noise floor. Absorption does not play a significant role in these spectral fluctuations, as the absorption coefficient \( \mu_a \) is \( \sim 1.5 \text{ cm}^{-1} \) [7] and the thickness of the cell is \( \sim 4 \mu \text{ m} \). Similarly, the glass slide behind the cell does not contribute to the observed spectral fluctuations, as the spectrum of the reflection from the slide is expected to be flat. This brings us to the question of how \( R(\lambda) \) is related to the properties of the object: thickness \( L \), average refractive index \( n_0 \), the variance and the correlation length of refractive index fluctuations \( \langle \Delta n^2 \rangle \) and \( \ell_c \).

In the regime where the approximation of 1D independent channels is valid, \( R(\lambda) \) (where \( k = 2\pi/\lambda \) is the wavenumber) can be characterized using the 1D mesoscopic light transport theory [2–4]. Accordingly, the rms average of \( R(\lambda) \) can be written as \( \langle R \rangle = L \xi^{-1} \), where \( \xi^{-1} \) is the scattering coefficient of a 1D channel. Although a complicated function of \( \langle \Delta n^2 \rangle \) and \( \ell_c \), \( \xi^{-1} \) can be simplified for \( kl_c < 1 \): \( \xi^{-1} \approx 2k^2Ld/n_0^2 \) with \( L_d = \langle \Delta n^2 \rangle / \ell_c \). Following terminology used in condensed matter physics, \( L_d \) is referred to as the disordered strength. In the following discussion we consider the case of \( kl_c < 1 \) without the loss of generality [8]. If \( L \) and \( n_0 \) are known, \( L_d \) can be determined from \( \langle R \rangle = 2k^2Ld/L/n_0^2 \) for each cell pixel. In turn, \( L \) can be estimated from the autocorrelation function \( C(\Delta k) \)

\[
C(\Delta k) = \langle (R(k)L + \Delta k)/R(k)L(k) \rangle [3, 4] \quad \text{In}(C(\Delta k)) = - (\Delta k)^2 / 2LdAL^2, \quad \text{where} \quad A = \text{const} \quad (\text{in units of length}) [4].
\]

Function \( f \) and parameter \( a \) are numerically derived using finite-difference time-domain (FDTD) simulations. In a weakly disordered medium and in the absence of strong localization, \( f \) is a slowly varying function of \( L_d \) \((r^2 = 0.04)\) and is approximated as a constant (i.e., \( f \sim 1 \)); \( a \) arises owing to the finite spectral bandwidth \( (\lambda_2 - \lambda_1) \) of the spectrum that is being used to calculate \( C(\Delta k) \) (in the limit of a very large bandwidth, \( L(\lambda_2 - \lambda_1)/\lambda^2 \gg 1 \), \( a = 2 \); for the bandwidth in our system \( \alpha \sim 1 \)). \( C(\Delta k) \) is calculated for the entire spectrum with a center wavelength \( \lambda_{\text{mean}} = 585 \text{ nm} \) for each cell pixel. Therefore, knowing the experimentally obtained quantities \( \langle R \rangle \) and \( C(\Delta k) \) for a given cell pixel \((x, y)\) and \( n_0 \) (assumed to be \( \sim 1.38 \)), we can calculate \( L_d \):

\[
L_d = B \frac{n_0^2}{2k^2} \left( \frac{\langle \Delta k \rangle^2}{-\ln(C(\Delta k))} \right),
\]

where \( B \) is the calibration constant and \( \ln(C(\Delta k))/\langle \Delta k \rangle^2 \) is obtained by fitting a linear slope to \( -\ln(C(\Delta k)) \) versus \( \langle \Delta k \rangle^2 \).

To confirm the hypothesis that the PWS scheme enables collecting 1D propagating waves and Eq. (1) is valid, we performed experiments on a series of nanostructured model media comprised of aggregated polystyrene nanospheres. The fabrication protocol is described in detail in [9]. In brief, the aqueous suspension of monodispersed polystyrene nanospheres (Duke Scientific, Inc.) of volume \( \sim 50 \mu \text{ l} \) was uniformly smeared on a glass slide. The self-assembled lattice formed after 15 min of evaporation. We used models with \( L \) varying from 0.3 to 13 \( \mu \text{ m} \) and nanosphere sizes 20, 40, 60, 80, 100, and 125 nm (standard deviations of sizes \( \sim 10\% \)). PWS measurements were obtained from 30 different combinations of \( L \) and nanosphere sizes. Since both \( L_c \) and \( \Delta n \) are known \textit{a priori} in this model, i.e., \( L_c \) is approximately the size of a nanosphere and \( \Delta n \) is approximately the refractive index of a polystyrene, we were able to compare the actual \( L_d \) of the model with the \( L_d \) found from the experimental PWS data obtained using Eq. (1). As shown in Fig. 2, there was a good agreement \((r^2 = 0.97)\) between the experimentally observed and the actual values of \( L_d \). These experiments were also used to determine constant \( B \) in Eq. (1). Finally, this experiment demonstrated that the minimal length scale of refractive index fluctuations to which PWS is sensitive is to below 20 nm.

The nanoscale sensitivity of PWS could be critical to cell microscopy, where cells are regularly imaged to understand disease processes. Conventional cytology is not sensitive to changes in cell nanoarchitecture (e.g., ribosomes, membranes, nucleosomes, just to name a few cell structures with subdiffractional dimensions). At the same time, these are some of the

![Fig. 1. (Color online) (a) Normalized backscattering spectrum \( I(\lambda) \) from a single pixel of a biological cell (solid blue) and a pure glass slide (dashed red) with the \( I_p(\lambda) \) (black). (b) The corresponding spectrum \( R(\lambda) \) obtained from a biological cell (solid blue) and from a pure glass slide (dashed red).](image-url)
most fundamental building blocks of the cell. We hypothesized that PWS can detect nanoarchitectural alterations in cells that are undetectable by cytology. We considered pancreatic cancer as a case in point. Pancreatic cancer is the fourth leading cause of cancer deaths in the U.S. with an overall five-year survival rate of <5%. For diagnosis, pancreatic cells are extracted using fine needle aspirations and subjected to a cytopathological analysis. However, the sensitivity of cytology is low for mass lesions in symptomatic patients (~70%) and much lower for early lesions due in part to the relative rarity of frankly malignant-appearing cells that can be identified by cytology. We performed a pilot study on archival pancreatic cells (fixed with alcohol) obtained from 16 patients (seven normal and nine malignant). Six cases from these nine adenocarcinomas were cytologically diagnosed as cytologically normal. (We note that although fixation modifies the internal refractive index distribution compared to that of live cells, the intracellular morphology is expected to be maintained.) PWS measurements were obtained from three different cohorts of cells: cytologically normal cells from normal patients (N), cytologically malignant cells from cancer patients (C), and cytologically normal cells from cancer patients (CN). For each patient, ~40 cells were chosen at random. Typical bright field and PWS images (i.e., $L_d(x,y)$) obtained for these three cell types are shown in Figs. 3(a) and 3(b). As seen, the $L_d$ image shows a clear difference between N and C cells. More importantly, $L_d$ images are different between N and CN cells also. Further statistical analysis was performed using the two parameters that are obtained from the $L_d$ maps, the mean and the standard deviation of the intracellular disorder strength $\langle L_d^{(c)} \rangle$ and $\langle \sigma^{(c)} \rangle$. As shown in Figs. 3(c) and 3(d) both the $\langle L_d^{(c)} \rangle$ and $\langle \sigma^{(c)} \rangle$ ($\langle \rangle$ indicates the average taken over all the cells within a patient cohort) are highly significantly elevated in cancer patients compared to the control group ($P<0.001$). A prediction rule developed using a linear regression model yielded 100% sensitivity and 100% specificity for cytologically normal patients versus cancer patients. Interestingly, the cytologically normal cells from cancer patients also had significantly elevated $\langle L_d^{(c)} \rangle$ and $\langle \sigma^{(c)} \rangle$ ($P<0.001$) with 83% sensitivity and 100% specificity. We note that both $\langle L_d^{(c)} \rangle$ and $\langle \sigma^{(c)} \rangle$ vary within a patient cohort as indicated by the error bars in Figs. 3(c) and 3(d). However this variation (~50% in normal population) is much smaller than the difference between the patient cohorts (which is >100%). A higher $L_d$ in cancer patients may be due to the increase in $\langle \Delta n^2 \rangle$ and/or $l_d$. Higher $\langle \Delta n^2 \rangle$ can be associated with the increased density of intracellular macromolecular complexes, while the change in $l_d$ may be due to the macromolecular aggregation.

In summary, the backscattering spectrum from a weakly disordered medium contains spectral fluctuations that can be used to measure the disorder strength of the refractive index fluctuations within the scattering object. The disorder strength is sensitive to subwavelength nanoscale refractive index fluctuations. As an illustration of the potential capabilities of PWS, we showed that this technique may identify cancer cells by sensing microscopically undetectable alterations in cell architecture.

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