Numerical investigation of two-dimensional light scattering patterns of cervical cell nuclei to map dysplastic changes at different epithelial depths

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Abstract: We use an extensive set of quantitative histopathology data to construct realistic three-dimensional models of normal and dysplastic cervical cell nuclei at different epithelial depths. We then employ the finite-difference time-domain method to numerically simulate the light scattering response of these representative models as a function of the polar and azimuthal scattering angles. The results indicate that intensity and shape metrics computed from two-dimensional scattering patterns can be used to distinguish between different diagnostic categories. Our numerical study also suggests that different epithelial layers and angular ranges need to be considered separately to fully exploit the diagnostic potential of two-dimensional light scattering measurements.

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OCIS codes: (170.0170) Medical optics and biotechnology; (170.1530) Cell analysis; (170.4580) Optical diagnostics for medicine; (170.4730) Optical pathology; (290.0290) Scattering.

References and links


1. Introduction

Analysis of light scattering properties of tissues has always been a major focus of biomedical optics research. Numerous studies have been carried out to quantify the optical scattering...
response of structural fibers, cells, and even individual organelles including nuclei, mitochondria, and lysosomes [1–11]. The main goal of these studies was to establish a link between disease-related modifications in tissue components and the resulting alterations in their respective scattering profiles.

Dysplastic progression in epithelial tissues is known to be associated with changes in internal structure of cells and cell-to-cell spatial organization. It is particularly well established that dysplastic nuclei are larger and more irregularly shaped with increased DNA content and coarse chromatin distribution. As evidenced by prior computational studies, these properties result in elevated scattering that can be used as an optical signature for precancer detection [3,4].

In conventional analysis of light scattering from normal and dysplastic cell nuclei, there is a tendency to compute the scattered intensity only in terms of the polar angle by averaging over the azimuthal direction; the resulting function is commonly referred to as the scattering pattern. It is possible to integrate this one-dimensional pattern in order to quantify the strength of scattering in a particular angular range. If the entire range of polar angles is considered and the integrand is properly normalized, the computed metric gives the scattering cross section that characterizes the overall scattering strength of the nuclei. As these intricate structures are not perfectly spherical and contain refractive index heterogeneities at different length scales, however, their scattering response will exhibit complex azimuthal asymmetry [12–16]. It may thus prove worthwhile to carry out a comprehensive two-dimensional investigation that will enable a comparative assessment of azimuthal dependence in nuclear scattering profiles. This may lead to observation of fine pattern changes that can further be linked to precancer progression.

In this study, we first analyze an extensive set of quantitative histopathology images from cervical biopsies and we obtain detailed information on morphological and structural features of segmented epithelial cell nuclei. We construct realistic three-dimensional computational models of basal, parabasal, intermediate, and superficial nuclei that are representative of four diagnostic categories, namely normal or negative for dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia or carcinoma in situ (CIS). We then employ the finite-difference time-domain (FDTD) method, a popular numerical tool in electromagnetics, to compute the two-dimensional scattering patterns of these representative models. The results are expected to provide useful insights into whether azimuth-resolved measurements can be explored to identify additional optical signatures for diagnostic purposes.

2. Methods

2.1 Construction of nuclear models

Construction of realistic nuclear models necessitates a detailed documentation of the morphological and structural properties of cell nuclei. To this end, we analyzed quantitative histopathology images from a set of 20 cervical biopsies stained with Feulgen-thionin. All the biopsies were obtained and imaged at the British Columbia Cancer Research Center (Vancouver, Canada). Each image spanned about 200 to 300 μm in length and corresponded to the diagnostic area selected by the study pathologist. The specifications of the imaging system were such that the effective pixel area at the sample plane was 0.34 × 0.34 μm² [17]. Since Feulgen-thionin is stoichiometric for DNA, the intensity of a given pixel is directly related to the amount of DNA at that location [17,18]. Hence, these images can be processed to extract information on nuclear size, shape, and chromatin texture. The main stages of the image processing algorithm used in this work are described below and summarized in Fig. 1.
2.1.1 Nuclear segmentation

Nuclear segmentation for each biopsy image was carried out with a special analysis software [17]. All the nuclei were classified into one of four epithelial layers, namely basal, parabasal, intermediate, and superficial. This classification was mainly based on the location of nuclei relative to the entire thickness of the epithelium and on cell examination.

2.1.2 Feature extraction

The same analysis software was then used to compute a series of morphometric and photometric features for each segmented nucleus. Morphometric features included the mean radius and eccentricity of the best-fit ellipse. Photometric features included optical density measures that can be linked to texture properties of the nucleus. Two photometric features of interest are the mean optical density and optical density variation. The optical density of an image pixel \((i, j)\) is given by

\[
OD(i, j) = \log I_B - \log I(i, j),
\]

where \(I_B\) is the intensity of the local background and \(I(i, j)\) is the intensity of the pixel. Mean optical density is then defined as

\[
\overline{OD} = \frac{\sum_{(i,j) \in A} OD(i, j)}{c_{\text{norm}} |A|},
\]

where \(A\) denotes the nuclear region and \(|A|\) is the number of pixels belonging to \(A\). Note that mean optical density is normalized by \(c_{\text{norm}}\) determined by the mean intensity of a particular object population such as leukocytes present on the biopsy slide. Inclusion of such a normalization factor is necessary to make this feature comparable across different samples.
Optical density variation, on the other hand, is defined to be the standard deviation of optical density values over \( A \) and is also normalized as in Eq. (2).

It was also necessary to quantify how chromatin was distributed within the nucleus. We visually examined the segmented image of each nucleus and we recorded the number of neighboring pixels that had similar intensity values. This additional feature provided a measure of the extent of chromatin clumping and is hereafter referred to as the texture size.

Next, the features obtained were averaged to generate layer-specific values that collectively characterize a biopsy sample. The averages for the mean radius, eccentricity, and texture size are denoted by \( r, e, \) and \( d \), respectively. Standard deviations were also computed to reveal the variability among nuclei that belong to each one of the four layers in that sample; \( \sigma_f \) denotes the standard deviation of feature \( f \).

As a final step, the averaged photometric features were transformed into refractive index measures that described the dielectric properties of the nuclei. Mean optical density was considered to be a measure of the mean nuclear refractive index denoted by \( n \). In order to establish an absolute scale between mean optical density and nuclear refractive index, the minimum value in the entire image set was assumed to correspond to 1.38 and the maximum value was assumed to correspond to 1.44, which together represent a reasonable refractive index range consistent with prior reports [19–25]. A given mean optical density was then converted to a refractive index value using a linear fit in accordance with a well-substantiated empirical relationship described in [26–28]. The extent of nuclear refractive index variations \( \sigma_n \) was computed in a similar manner based on a given optical density variation.

2.1.3 Model parameters

Each nuclear model representing a layer in a biopsy sample was constructed as an ellipsoid in a voxel-based computational grid. The radius of the ellipsoid in one dimension was set to one standard deviation below the respective mean radius, or \( r - \sigma_r \). The radius in the second dimension was computed as eccentricity \( e \) times the radius in the first dimension. Finally, the radius in the third dimension was adjusted such that the average of the radii in three dimensions was equal to the mean radius \( r \).

All the voxels belonging to the constructed model were first assigned a constant refractive index that was equal to \( n \). Nuclear texture was created by inserting refractive index heterogeneities at different length scales. These heterogeneities were in the form of small ellipsoids placed randomly throughout the nucleus. Their dimensions were sampled from a truncated normal distribution \( N(d, \sigma_d) \); a lower bound of 0.34 \( \mu m \) was imposed to reject values that fell below the pixel size of histopathology images analyzed and an upper bound of \( d + \sigma_d \) was imposed to avoid unrealistically large sizes. The refractive index of each ellipsoid was sampled from \( N(n, \sigma_n) \) truncated between \( n - \sigma_n \) and \( n + \sigma_n \). Our construction algorithm was designed such that non-overlapping ellipsoidal heterogeneities were added one at a time until the entire nuclear volume was filled and there was no more space for addition of another ellipsoid.

Since four different epithelial layers were considered, the features extracted from 20 biopsies would be expected to render information on 80 nuclear models. For some biopsies, however, it was not possible to identify all four layers and thus only a total of 66 nuclear models were created. Table 1 shows the breakdown of these models by epithelial layer and diagnostic category. Note that the numbers in parentheses indicate the number of nuclei analyzed to create each model.
Table 1. Breakdown of 66 nuclear models constructed for electromagnetic simulations. The numbers in parentheses indicate the number of nuclei analyzed to construct each model.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Negative for dysplasia</th>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia / CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>(16,3,14,13,15)</td>
</tr>
<tr>
<td></td>
<td>(12,18,9,10,1)</td>
<td>(2,3,7,11,17)</td>
<td>(6,7,1,4)</td>
<td></td>
</tr>
<tr>
<td>Parabasal</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>(30,37,19,19,29)</td>
</tr>
<tr>
<td></td>
<td>(12,45,8,47)</td>
<td>(48,43)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>(14,14,19,30,31)</td>
</tr>
<tr>
<td></td>
<td>(15,42,12,83,6)</td>
<td>(49,32,6,48,100)</td>
<td>(77,109,83,44,51)</td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>(8,7,6,15,8)</td>
</tr>
<tr>
<td></td>
<td>(5,20,4,5,7)</td>
<td>(6,17,3,12)</td>
<td>(9)</td>
<td></td>
</tr>
</tbody>
</table>

2.2 Finite-difference time-domain simulations

Extensive details regarding the FDTD method are available in the literature [29]. Briefly, this is a grid-based numerical technique that can be used to solve discretized versions of Maxwell’s curl equations for electric and magnetic fields. These equations are time-marched until steady-state field values are observed at all points within the scatterer. The total-field/scattered-field formulation provides a simple approach to introduce an impinging source wave into the computational grid. Yet, special truncation conditions must be applied at the edges of the grid to suppress artificial reflections and to simulate propagation in an unbounded medium. The perfectly matched layer (PML) boundary condition minimizes artificial reflections by creating a fictitious absorbing layer that surrounds the grid and is currently considered to be one of the most efficient truncation techniques. Time-marching equations produce the near fields in the immediate vicinity of the scatterer. An algorithm based on the equivalence principle can be invoked to perform a near-to-far-field transformation and to obtain the distant or far fields that are generally of primary interest.

The three-dimensional FDTD code employed for the study presented here was implemented in C/C++ and has been previously described [4,6]. As detailed further in [30], special efforts were made to fine-tune the PML parameters for biologically relevant scatterers and to reduce characteristic backscattering errors down to acceptable levels. All the simulations were carried out at 1.064 $\mu$m, which is a common operating wavelength for scattering-based imaging systems. The nuclear models constructed were assumed to be embedded in a cytoplasmic medium with a refractive index of 1.36 [19]. The voxel size of the computational grid was set to 1/20 of the wavelength inside the cytoplasmic medium; this voxel size has been empirically proven to maintain numerical stability during time-marching of field equations.

The output of each simulation contains the intensity of scattered light at a set of directions denoted by $(\theta, \phi)$. As depicted in Fig. 2, $\theta \in \{0, 1, \ldots, 180\}$ is the polar scattering angle and $\phi \in \{0, 1, \ldots, 360\}$ is the azimuthal scattering angle, both in degrees.
3. Results

3.1 Nuclear features

Figure 3 shows sample images of basal, parabasal, intermediate, and superficial nuclei in four diagnostic categories. It is obvious that each layer exhibits different characteristics; basal, parabasal, and intermediate nuclei are generally larger and rounder, whereas superficial nuclei are smaller with a denser internal composition. The images shown also illustrate typical morphological and structural changes associated with dysplastic progression. Most prominent changes include an increase in nuclear size, a more elliptical shape, and a more heterogeneous chromatin texture. Even though our modeling strategy relies on simulation parameters extracted on a per-biopsy basis, Table 2 lists the nuclear features averaged over the entire image set and provides a general quantitative overview of layer-specific trends observed as dysplasia progresses.

3.2 Two-dimensional scattering patterns

Figure 4 shows a set of sample scattering patterns for different epithelial layers and diagnostic categories. Note that the intensity values obtained from FDTD simulations are plotted on a log scale and the results have been normalized such that the maximum value for each pattern is zero. As illustrated in this set of plots, two-dimensional scattering patterns exhibit characteristic intensity ridges that appear to change shape with progression of dysplasia; most notably, the patterns generally get compressed along the $\phi$ direction and the separation between successive ridges decreases. There are also layer-dependent changes in these scattering profiles. For example, while the ridges for basal, parabasal, and intermediate nuclei are deep with well-defined edges, those for superficial nuclei are observed to be ragged and blurry.
Fig. 3. Sample images of basal, parabasal, intermediate, and superficial nuclei that are representative of four diagnostic categories, namely normal or negative for dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia or CIS. Each pixel shown is $0.34 \times 0.34 \mu m^2$.

Table 2. Nuclear features averaged over the entire image set.

<table>
<thead>
<tr>
<th></th>
<th>Negative for dysplasia</th>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia / CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$ ($\mu$m)</td>
<td>$\sigma_r$ ($\mu$m)</td>
<td>$e$</td>
<td>$d$ ($\mu$m)</td>
</tr>
<tr>
<td>Basal</td>
<td>3.05</td>
<td>0.80</td>
<td>2.10</td>
<td>1.11</td>
</tr>
<tr>
<td>Parabasal</td>
<td>3.19</td>
<td>0.67</td>
<td>1.79</td>
<td>1.11</td>
</tr>
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<td>Intermediate</td>
<td>3.25</td>
<td>0.48</td>
<td>1.44</td>
<td>1.18</td>
</tr>
<tr>
<td>Superficial</td>
<td>2.83</td>
<td>0.92</td>
<td>2.67</td>
<td>1.02</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.35</td>
<td>0.87</td>
<td>2.11</td>
<td>1.25</td>
</tr>
<tr>
<td>Parabasal</td>
<td>3.60</td>
<td>0.77</td>
<td>1.80</td>
<td>1.22</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3.57</td>
<td>0.53</td>
<td>1.45</td>
<td>1.24</td>
</tr>
<tr>
<td>Superficial</td>
<td>2.75</td>
<td>0.66</td>
<td>1.99</td>
<td>1.35</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>4.21</td>
<td>0.98</td>
<td>2.01</td>
<td>1.33</td>
</tr>
<tr>
<td>Parabasal</td>
<td>4.33</td>
<td>1.00</td>
<td>1.90</td>
<td>1.33</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4.55</td>
<td>0.85</td>
<td>1.70</td>
<td>1.36</td>
</tr>
<tr>
<td>Superficial</td>
<td>3.30</td>
<td>0.75</td>
<td>1.80</td>
<td>1.40</td>
</tr>
<tr>
<td>Severe dysplasia / CIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>4.08</td>
<td>1.02</td>
<td>2.02</td>
<td>1.74</td>
</tr>
<tr>
<td>Parabasal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3.72</td>
<td>0.80</td>
<td>1.74</td>
<td>1.49</td>
</tr>
<tr>
<td>Superficial</td>
<td>2.74</td>
<td>1.01</td>
<td>2.96</td>
<td>1.29</td>
</tr>
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</table>
Fig. 4. Sample two-dimensional scattering patterns of basal, parabasal, intermediate, and superficial nuclei that are representative of four diagnostic categories, namely normal or negative for dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia or CIS. Simulation results are plotted on a log scale where the maximum value is normalized to zero for each layer and diagnostic category.

We first compare and contrast two generic metrics based on integration of scattered intensity values. The first metric was obtained by directly integrating the FDTD simulation results with no normalization. Integration was performed over various angular ranges to reveal direction-dependent scattering properties of cell nuclei. Figure 5 shows the mean integrated absolute intensity for different epithelial layers and diagnostic categories. The markers for different diagnostic categories are slightly offset to maintain clarity; transition from blue to red color signifies progression to higher grades of dysplasia and the standard error bars point to the extent of variability over all the nuclear models constructed for a given group. The results of integration over the entire range defined by $\theta = 0^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$ represent the overall scattering strength of the cell nuclei [Fig. 5(a)]. Forward scattering strength, side scattering strength, and backscattering strength correspond to scattered intensity integrated over $\theta = 0^\circ$-$40^\circ$ and $\phi = 0^\circ$-$360^\circ$ [Fig. 5(b)], $\theta = 40^\circ$-$140^\circ$ and $\phi = 0^\circ$-$360^\circ$ [Fig. 5(c)], and $\theta = 140^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$ [Fig. 5(d)], respectively. Nuclei from moderately dysplastic biopsies are observed to be the strongest scatterers for all four layers, but this diagnostic category is characterized by a high degree of variability. It is also evident that scattering from severely dysplastic or CIS nuclei is generally stronger compared to scattering from normal and mildly dysplastic nuclei. The results also illustrate that there can be intensity level variations among different epithelial layers; backscattering strength appears to decrease in going from the basal layer to the parabasal and intermediate layers, but then increases in the superficial layer for all four diagnostic categories.
Fig. 5. Integrated absolute intensity computed from two-dimensional scattering patterns of cervical cell nuclei. Four angular ranges are considered separately: (a) $\theta = 0^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$, (b) $\theta = 0^\circ$-$40^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to forward scattering, (c) $\theta = 40^\circ$-$140^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to side scattering, and (d) $\theta = 140^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to backscattering.

The second intensity metric was obtained by integrating the FDTD results after normalizing each pattern to a maximum value of one (cf. Fig. 4). Figure 6 is analogous to Fig. 5, but it rather depicts the mean and standard error of integrated normalized intensity for different groups. Here, we see a reverse trend; normal and mildly dysplastic nuclei tend to have a higher integrated normalized intensity compared to moderately and severely dysplastic or CIS nuclei in basal, parabasal, and intermediate layers [Figs. 6(a) through 6(d)]. This metric has a lower variability and provides a better separation between different diagnostic categories except for the backscattering range. Also note that superficial nuclei are always associated with a higher integrated normalized intensity compared to the nuclei in other layers, especially when side scattering or backscattering is considered.

In order to obtain a quantitative measure of shape differences observed in two-dimensional scattering profiles of cell nuclei, we processed each pattern and identified the locations of the regional maxima. Note that regional maxima as defined in this work correspond to intensity entries whose eight nearest neighbors all have a lower value. Figure 7 shows the mean number of regional maxima identified for different epithelial layers and diagnostic categories. Note that various angular ranges are again displayed separately to reveal any direction-dependent trends; the results for the number of regional maxima over the entire range ($\theta = 0^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$) are shown first [Fig. 7(a)], followed by the results for forward scattering ($\theta = 0^\circ$-$40^\circ$ and $\phi = 0^\circ$-$360^\circ$) [Fig. 7(b)], side scattering ($\theta = 40^\circ$-$140^\circ$ and $\phi = 0^\circ$-$360^\circ$) [Fig. 7(c)], and backscattering ($\theta = 140^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$) [Fig. 7(d)]. It appears that this metric performs reasonably well in separating moderately dysplastic and severely dysplastic or CIS nuclei from normal and mildly dysplastic nuclei, especially when side scattering is considered. There is no particular trend observed for backscattering since significant overlap exists between different diagnostic categories.
Fig. 6. Integrated normalized intensity computed from two-dimensional scattering patterns of cervical cell nuclei. Four angular ranges are considered separately: (a) $\theta = 0^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$, (b) $\theta = 0^\circ$-$40^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to forward scattering, (c) $\theta = 40^\circ$-$140^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to side scattering, and (d) $\theta = 140^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to backscattering.

Fig. 7. Number of regional maxima in two-dimensional scattering patterns of cervical cell nuclei. Four angular ranges are considered separately: (a) $\theta = 0^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$, (b) $\theta = 0^\circ$-$40^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to forward scattering, (c) $\theta = 40^\circ$-$140^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to side scattering, and (d) $\theta = 140^\circ$-$180^\circ$ and $\phi = 0^\circ$-$360^\circ$ corresponding to backscattering.
Another metric was obtained by computing the correlation matrix for each pattern. These matrices contain pairwise correlation of $\theta$-dependent intensity functions at different $\phi$. When the correlation values are averaged over the entire matrix, the result can be considered to be a measure of the overall azimuthal symmetry associated with each pattern. Figure 8 displays the mean and standard error of average correlation for all the nuclear models constructed. As in Figs. 5 through 7, parts (a) through (d) correspond to different angular ranges. When forward scattering is the dominant contributor, the patterns exhibit a high degree of symmetry evidenced by high average correlation values [Figs. 8(a) and 8(b)]. Side scattering is characterized by lower average correlation values that are comparable across different epithelial layers and diagnostic categories [Fig. 8(c)]. Interestingly, backscattering is associated with average correlation values that tend to decrease with dysplastic progression. In particular, the patterns for severely dysplastic or CIS nuclei possess a greater azimuthal asymmetry compared to other diagnostic categories [Fig. 8(d)]. It is also important to note that the correlation metric computed over this angular range may have a layer-dependent trend; in most cases, the patterns for basal and superficial nuclei appear to be azimuthally more asymmetric compared to those for parabasal and intermediate nuclei.

![Fig. 8](image)

**Fig. 8.** Average correlation for two-dimensional scattering patterns of cervical cell nuclei. Four angular ranges are considered separately: (a) $\theta = 0^\circ-180^\circ$ and $\phi = 0^\circ-360^\circ$, (b) $\theta = 0^\circ-40^\circ$ and $\phi = 0^\circ-360^\circ$ corresponding to forward scattering, (c) $\theta = 40^\circ-140^\circ$ and $\phi = 0^\circ-360^\circ$ corresponding to side scattering, and (d) $\theta = 140^\circ-180^\circ$ and $\phi = 0^\circ-360^\circ$ corresponding to backscattering.

4. Discussion

The results presented in this work offer significant insights into the diagnostic potential of two-dimensional light scattering measurements. The intensity and shape metrics computed from FDTD patterns point to the possibility of monitoring dysplastic progression at different epithelial depths. It is important to note, however, that a given metric can provide diagnostically useful information only when an optimal angular range is selected for its computation.

Elevated cellular scattering has previously been reported to be an optical signature of dysplastic progression [3,4,7]. Our results in Fig. 5 for the first metric computed from
absolute intensity values are in line with these prior reports. Yet, it is necessary to point out that the use of this metric as a diagnostic indicator requires a proper intensity calibration so that measurements can be interpreted on a comparative basis. In order to avoid this limitation, we propose a second metric that is computed based on normalized intensity values. Here, normalization of each scattering pattern is self-independent and hence there is no need for calibration across measurements. The results in Fig. 6 indicate that this second metric can act as a diagnostic indicator for dysplasia, especially when forward scattering or side scattering is considered. Lower metric values associated with moderately and severely dysplastic or CIS nuclei are due to the fact that these scattering patterns span a greater intensity range. As such, relatively higher metric values obtained for side scattering or backscattering from superficial nuclei are consistent with shallow and blurry ridges observed in Fig. 4.

Shape metrics are also intensity-invariant and can thus be computed in a self-independent manner with no need for calibration. As evidenced in Fig. 7, dysplastic changes in scattering patterns are well captured with an analysis of regional intensity maxima. In particular, an increase in the number of regional maxima with progression of dysplasia is a direct consequence of smaller separation between successive ridges; these more frequent intensity transitions are most pronounced for side scattering. The average correlation values displayed in Fig. 8, on the other hand, are quite informative of the degree of azimuthal symmetry characterizing the nuclear scattering profiles. Note that effective polarization at around $\theta = 90^\circ$ accounts for lower correlation values associated with side scattering. Most importantly, it is obvious that azimuthally asymmetric backscattering is indicative of dysplastic progression; increased asymmetry for severely dysplastic or CIS nuclei is manifested in significantly lower correlation values and can be traced back to a more heterogeneous nuclear texture depicted in Table 2. This finding agrees well with prior observations on azimuth-resolved backscattering measurements [13].

Overall, the results presented corroborate the fact that a depth-specific analysis is necessary to fully map the scattering properties of epithelial tissues [4,31,32]. While previous studies have revealed a layer-to-layer variation in scattering strength, a similar trend is also evident here for other intensity and shape metrics computed from two-dimensional patterns. This needs to be taken into consideration when devising imaging schemes or interpreting scattering-based optical measurements.

As a final remark, we point out that the use of realistic model input is of utmost importance for numerical investigation of scattering trends. Even though quantitative histopathology images have provided extensive details on nuclear features and have guided the construction of nuclear models employed in our simulations, it may be worth discussing a few limitations associated with these images. First, the influence of staining on morphological and structural properties of cell nuclei is largely unknown and is not considered in the current study. On a related matter, each stained biopsy section has a thickness of about 4 $\mu$m and the intensity of each pixel corresponds to an integral over the axial direction. Nevertheless, our model parameters are based on analysis of many nuclei in a given section; averaged features are expected to retain information on relative differences between tissue samples from different diagnostic categories. As long as there is no directional preference in nuclear architecture, these issues are not likely to hinder depiction of dysplastic changes. Second, the effective lateral resolution of the imaging system enables quantification of refractive index variations down to a size of 0.34 $\mu$m. Recent studies indicate that scattering from tissue constituents can be sensitive to index variations at smaller scales [33,34]. This may need to be taken into account when simulations are to be carried out at smaller wavelengths. After all, the FDTD method is capable of delineating the influence of finer texture that is likely to have a greater impact on backscattering predictions. Third, quantitative histopathology images can be used to extract morphological and structural information in two dimensions; extrapolation to three dimensions is hence necessary for setting up the simulations. Note, however, that high-resolution imaging techniques such as quantitative phase microscopy or holographic tomography can be employed to generate direct three-dimensional input for FDTD simulations and to overcome the limitations described [21,25]. An alternative approach is to
couple information obtained from high-resolution imaging techniques with stochastic models of refractive index distribution [23,35].

5. Conclusions

In summary, the research described here provides a detailed account of two-dimensional light scattering properties of cervical cell nuclei. The results suggest that assessment of azimuthal dependence in scattering patterns of normal and dysplastic nuclei can lead to identification of well-performing diagnostic metrics. A possible extension of this study to simulate clusters of cell nuclei is likely to prove useful for prediction of scattering trends at the tissue level.

Acknowledgment

This work was supported by the National Cancer Institute of the National Institutes of Health (grant 2P01CA082710-09A2).