Characterizing microstructures of cancerous tissues using multispectral transformed Mueller matrix polarization parameters

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Abstract: In this paper, we take the transmission 3 × 3 linear polarization Mueller matrix images of the unstained thin slices of human cervical and thyroid cancer tissues, and analyze their multispectral behavior using the Mueller matrix transformation (MMT) parameters. The experimental results show that for both cervical and thyroid cancerous tissues, the characteristic features of multispectral transmitted MMT parameters can be used to distinguish the normal and abnormal areas. Moreover, Monte Carlo simulations based on the sphere-cylinder birefringence model (SCBM) provide additional information of the relations between the characteristic spectral features of the MMT parameters and the microstructures of the tissues. Comparisons between the experimental and simulated data confirm that the contrast mechanism of the transmission MMT imaging for cancer detection is the breaking down of birefringent normal tissues for cervical cancer, or the formation of birefringent surrounding structures accompanying the inflammatory reaction for thyroid cancer. It is also testified that, the characteristic spectral features of polarization imaging techniques can provide more detailed microstructural information of tissues for diagnosis applications.

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References and links

1. R. S. Gurjar, V. Backman, L. T. Perelman, I. Georgakoudi, K. Badizadegan, I. Itzkan, R. R. Dasari, and M. S. Feld, “Imaging human epithelial properties with polarized light-scattering spectroscopy,” Nat. Med. 7(11), 1245–1248 (2001).
2. B. Kunnen, C. Macdonald, A. Doronin, S. Jacques, M. Eccles, and I. Meglinski, “Application of circularly polarized light for non-invasive diagnosis of cancerous tissues and turbid tissue-like scattering media,” J. Biophotonics 8(4), 317–323 (2015).
3. N. Ghosh and I. A. Vitkin, “Tissue polarimetry: concepts, challenges, applications, and outlook,” J. Biomed. Opt. 16(11), 110801 (2011).
4. S. Alali and A. Vitkin, “Polarized light imaging in biomedicine: emerging Mueller matrix methodologies for bulk tissue assessment,” J. Biomed. Opt. 20(6), 061104 (2015).
5. S. L. Jacques, J. R. Roman, and K. Lee, “Imaging superficial tissues with polarized light,” Lasers Surg. Med. 26(2), 119–129 (2000).
6. S. L. Jacques, J. C. Ramella-Roman, and K. Lee, “Imaging skin pathology with polarized light,” J. Biomed. Opt. 7(3), 329–340 (2002).
7. R. R. Anderson, “Polarized light examination and photography of the skin,” Arch. Dermatol. 127(7), 1000–1005 (1991).
8. J. Qi, M. Ye, M. Singh, N. T. Clancy, and D. S. Elson, “Narrow band 3 × 3 Mueller polarimetric endoscopy,” Biomed. Opt. Express 4(11), 2433–2449 (2013).
34. Y. Guo, N. Zeng, H. He, T. Yun, E. Du, R. Liao, Y. He, and H. Ma, “A study on forward scattering Mueller matrix decomposition in anisotropic medium,” Opt. Express 21(12), 14120–14130 (2013).

33. S. Alali, M. Ahmad, A. Kim, N. Vurgun, M. F. G. Wood, and I. A. Vitkin, “Quantitative correlation between depolarization and transport albedo of various porcine tissues,” J. Biomed. Opt. 19(4), 046020 (2014).

32. C. He, J. Chang, Y. Wang, R. Liao, H. He, N. Zeng, and H. Ma, “Linear polarization optimized Stokes polarimeter based on four-quadrant detector,” Appl. Opt. 54(14), 4458–4463 (2015).

31. V. V. Tuchin, L. V. Wang, and D. A. Zimnyakov, “Tissue Structure and Optical Models” (Springer, New York, 2006), Chapter 2, pp. 7–28.

30. N. Ortega-Quijano, F. Fanjul-Vélez, I. Salas-Garcia, and J. L. Arce-Diego, “Polarized light Monte Carlo analysis of birefringence-induced depolarization in biological tissues,” Proc. SPIE 8803, 88030T (2013).

29. T. Yun, N. Zeng, W. Li, D. Li, X. Jiang, and H. Ma, “Monte Carlo simulation of polarized photon scattering in anisotropic media,” Opt. Express 17(19), 16590–16602 (2009).

28. C. He, J. Chang, Y. Wang, R. Liao, H. He, N. Zeng, and H. Ma, “Linear polarization optimized Stokes polarimeter based on four-quadrant detector,” Appl. Opt. 54(14), 4458–4463 (2015).

27. Y. Wang, Y. Guo, N. Zeng, D. Chen, H. He, and H. Ma, “Study on the validity of 3×3 Mueller matrix decomposition,” J. Biomed. Opt. 20(6), 065003 (2015).

26. H. He, M. Sun, N. Zeng, E. Du, Y. Guo, S. Liu, J. Wu, Y. He, and H. Ma, “Mapping local orientation of aligned fibrous scatterers for cancerous tissues using backscattering Mueller matrix imaging,” J. Biomed. Opt. 19(10), 106007 (2014).

25. H. He, N. Zeng, E. Du, Y. Guo, S. Liu, J. Wu, Y. He, and H. Ma, “Characterizing the microstructures of biological tissues using Mueller matrix and transformed polarization parameters,” Biomed. Opt. Express 5(12), 4223–4234 (2014).

24. A. Pierangelo, S. Manhas, A. Benali, C. Fallet, J. L. Totobenazara, M. R. Antonelli, T. Novikova, B. Gayet, P. Validire, and A. De Martino, “Ex vivo photometric and polarimetric multilayer characterization of healthy human colon by multispectral Mueller imaging,” J. Biomed. Opt. 17(6), 066009 (2012).

23. J. Jagtap, S. Chatterjee, A. Pradhan, and N. Ghosh, “Quantitative Mueller matrix fluorescence spectroscopy for precancer detection,” Opt. Lett. 39(2), 243–246 (2014).

22. J. Soni, H. Purwar, H. Chatterjee, C. Banerjee, U. Kumar, and N. Ghosh, “Quantitative fluorescence and elastic scattering tissue polarimetry using an Eigenvalue calibrated spectroscopic Mueller matrix system,” Opt. Express 21(13), 15475–15489 (2013).

21. J. Jagtap, S. Chatterjee, A. Pradhan, and N. Ghosh, “Polarimetric imaging of uterine cervix: a case study,” Opt. Express 21(2), 1582–1593 (2013).

20. M. D. Modell, E. B. Hanlon, I. Itzkan, and L. T. Perelman, “Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett’s esophagus,” Nat. Med. 16(5), 603–606 (2010).

19. J. Jagtap, S. Chatterjee, A. Pradhan, and N. Ghosh, “Quantitative Mueller matrix fluorescence spectroscopy for precancer detection,” Opt. Lett. 39(2), 243–246 (2014).

18. M. Sun, H. He, N. Zeng, E. Du, Y. Guo, S. Liu, J. Wu, Y. He, and H. Ma, “Mapping local orientation of aligned fibrous scatterers for cancerous tissues using backscattering Mueller matrix imaging,” J. Biomed. Opt. 19(10), 106007 (2014).

17. H. He, M. Sun, N. Zeng, E. Du, Y. Guo, S. Liu, J. Wu, Y. He, and H. Ma, “A possible quantitative Mueller matrix transformation technique for anisotropic scattering media,” Photonics Lasers Med. 2(2), 129–137 (2013).

16. H. He, N. Zeng, E. Du, Y. Guo, D. Li, R. Liao, and H. Ma, “A possible quantitative Mueller matrix transformation technique for anisotropic scattering media,” Photonics Lasers Med. 2(2), 129–137 (2013).

15. L. Qiu, D. K. Pleskow, R. Chuttani, E. Vitkin, J. Leyden, N. Ozden, S. Itani, L. Guo, A. Sacks, J. D. Goldsmith, M. D. Modell, E. B. Hanlon, I. Itzkan, and L. T. Perelman, “Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett’s esophagus,” Nat. Med. 16(5), 603–606 (2010).

14. W. Wang, L. G. Lim, S. Srivastava, J. S. Yan, A. Shabbir, and Q. Liu, “Roles of linear and circular polarization in depolarization and effect of wavelength choice on differentiation between ex vivo normal and cancerous gastric samples,” J. Biomed. Opt. 19(4), 046020 (2014).

13. P. Shukla and A. Pradhan, “Mueller decomposition images for cervical tissue: Potential for discriminating normal and dysplastic states,” Opt. Express 17(3), 1600–1609 (2009).

12. E. Du, H. He, N. Zeng, H. Ma, “Mueller matrix polarimetry for differentiating characteristic features of cancerous tissues,” J. Biomed. Opt. 19(7), 076013 (2014).

11. A. Pierangelo, S. Manhas, A. Benali, C. Fallet, J. L. Totobenazara, M. R. Antonelli, T. Novikova, B. Gayet, and A. De Martino, “Ex vivo characterization of human colon cancer by Mueller polarimetric imaging,” Opt. Express 19(2), 1582–1593 (2013).

10. A. Pierangelo, A. Benali, M. R. Antonelli, T. Novikova, P. Validire, B. Gayet, and A. De Martino, “Ex vivo characterization of human colon cancer by Mueller polarimetric imaging,” Opt. Express 19(2), 1382–1395 (2013).

9. N. T. Clancy, S. Arya, J. Qi, D. Stoyanov, G. B. Hanna, and D. S. Elson, “Polarised stereo endoscope and narrowband detection for minimal access surgery,” Biomed. Opt. Express 4(12), 4108–4117 (2014).

8. A. Pierangelo, A. Nazae, A. Benali, P. Validire, H. Cohen, T. Novikova, B. H. Ibrahim, S. Manhas, C. Fallet, M. R. Antonelli, and A. D. Martino, “Polarimetric imaging of uterine cervix: a case study,” Opt. Express 21(12), 14120–14130 (2013).

7. S. Alali, M. Ahmad, A. Kim, N. Vurgun, M. F. G. Wood, and I. A. Vitkin, “Quantitative correlation between depolarization and transport albedo of various porcine tissues,” J. Biomed. Opt. 17(4), 045004 (2012).

6. E. Du, H. He, N. Zeng, M. Sun, Y. Guo, J. Wu, S. Liu, and H. Ma, “Mueller matrix polarimetry for differentiating characteristic features of cancerous tissues,” J. Biomed. Opt. 19(7), 076013 (2014).

5. V. B. Backman, M. B. Wallace, L. T. Perelman, J. T. Arendt, R. Gurjar, M. G. Müller, Q. Zhang, G. Zonios, E. Kline, J. A. McGilligan, S. Shapshay, T. Valdez, K. Badizadegan, J. M. Crawford, M. Fitzmaurice, S. Kabani, H. S. Levin, M. Seiler, R. R. Dasari, I. Itzkan, J. Van Dam, and M. S. Feld, “Detection of preinvasive cancer cells,” Nature 406(6791), 35–36 (2000).

4. E. Du, H. He, N. Zeng, H. Ma, “Mueller matrix polarimetry for differentiating characteristic features of cancerous tissues,” J. Biomed. Opt. 13(5), 1106–1113 (1996).

3. M. Sun, H. He, N. Zeng, E. Du, Y. Guo, C. Peng, Y. He, and H. Ma, “Probing microstructural information of anisotropic scattering media using rotation-independent polarization parameters,” Appl. Opt. 53(14), 2949–2955 (2014).

2. A. Pierangelo, M. R. Antonelli, T. Novikova, B. Gayet, and A. De Martino, “Multispectral Mueller polarimetric imaging detecting residual cancer and cancer regression after neoadjuvant treatment for colorectal carcinomas,” J. Biomed. Opt. 18(4), 046014 (2013).

1. A. Pierangelo, S. Manhas, A. Benali, C. Fallet, J. L. Totobenazara, M. R. Antonelli, T. Novikova, B. Gayet, A. De Martino, and P. Validire, “Multispectral Mueller polarimetric imaging detecting residual cancer and cancer regression after neoadjuvant treatment for colorectal carcinomas,” J. Biomed. Opt. 18(4), 046014 (2013).
1. Introduction

Polarization imaging techniques are recognized as potentially powerful methods to detect the pathological changes of biological tissues [1–4]. In the past decades many attempts have been made for clinical diagnosis applications using simple polarization parameters such as the degree of polarization (DOP) [5,6] and difference polarization (DP) [7]. Some of the linear polarization imaging methods have even been combined with endoscopes for in vivo diagnostic purposes [8,9]. Since a Mueller matrix provides a comprehensive characterization of the polarization properties and contains abundant microstructural and optical information of the sample, imaging methods based on Mueller matrix polarimetry are becoming increasingly attractive for differentiating the pathological structural features of biological samples, such as different types of cancers at different stages [10–15].

To disentangle the information encoded in the 16 Mueller matrix elements, different techniques have been proposed to derive new sets of polarization parameters, which are functions of the Mueller matrix elements but connect more explicitly to the optical properties or microstructural features of the sample. The Mueller matrix polar decomposition (MMPD) method decomposes the complicated interactions between the polarized light and the sample into a series of polarization sensitive processes, and derives the corresponding polarization optical parameters such as depolarization ($\Delta$), dichroism ($D$) and retardance ($\delta$) [16]. Alternatively the Mueller matrix transformation (MMT) method provides new polarization parameters which are sensitive to specific structure features, such as the anisotropy ($A$) and orientation angle ($\chi$) of the aligned fibers or the density of sub wavelength “small” particles ($b$) [17]. An additional advantage of using these transformed Mueller matrix parameters, from both MMPD and MMT, is that the new parameters are either explicitly related or not sensitive to the azimuth angle of the sample orientation [18]. Apart from polarization, spectral features of the scattered light also carry information on the microstructure of biological samples, therefore can be applied in diagnosis of cancerous tissues [19]. Studies have shown that combining polarization and spectral techniques can provide more powerful tools for cancer diagnosis [20–24].

In this paper, we take the forward scattering 3 × 3 linear polarization Mueller matrix of human cancer tissues at different wavelengths. The MMT parameters $A$ and $b$ within the normal and cancerous regions are calculated and their spectral behaviors are compared with Monte Carlo (MC) simulations based on the sphere-cylinder birefringence model (SCBM), which approximates the complicated biological tissues to a mixture of spheres and well aligned cylinders imbedded in birefringent interstitial medium. Experimental and MC simulated results indicate that the multispectral MMT parameters can provide the detailed microstructural information to differentiate the cancerous and healthy tissues. We believe that the transmission 3 × 3 linear Mueller polarimetric imaging technique has the potential to be applied to polarization microscopy for diagnosis of unstained frozen tissue sections. Compared to the standard H-E staining method, the unstained Mueller microscopy can be used as a rapid pathological diagnosis technique in surgeries. Besides, the 3 × 3 Mueller polarimetric technique may also be applied to quick screening of samples such as blood cell smears.

2. Methods and materials

2.1 Experimental setup

We use a forward scattering configuration for taking the multispectral polarization images of thin tissue slices. The schematic of the experimental setup is shown in Fig. 1. The light from the incoherent white light source halogen lamp (Daheng Optic, Beijing, China) passes through a liquid crystal transmission filter (LCTF) (VariSpec, Cri, USA) which can vary the wavelength from 500 nm to 680 nm in steps of 30 nm with 10 nm bandwidth. The light then is modulated by an achromatic quarter wave plate (R, Thorlabs, USA) and a polarizer (P1, Thorlabs, USA). The photons forward scattered from the sample pass through the analyzing polarizer (P2, Thorlabs, USA) and recorded by a CCD camera (CCD, QImaging, 32-0122A,
Since the light passing through the LCTF becomes horizontally linear polarized, during the measurements, the fast axis of the wave plate (R) is fixed in $45^\circ$ direction, and the polarizers (P1, P2) rotate to $0^\circ$, $45^\circ$, $90^\circ$, $135^\circ$ directions to measure the MMT parameters of the samples. The errors due to the retarder and polarizers are compensated by measuring the Mueller matrices of air and quarter wave plate. The maximum errors for all the Mueller matrix elements are less than 1.5%.

![Fig. 1. Schematic of the forward MMT experimental setup. L1, L2: lens; VariSpec: liquid crystal transmission filter; R: quarter wave plate; P1, P2: polarizer; The light source (halogen lamp) is about 40 cm away from the sample. The diameter of the illumination area is about 1.5 cm. The field of view and magnification of the imaging system are 0.5 cm × 0.5 cm and 0.4, respectively.]

### 2.2 Cancerous tissue samples

Backscattering Mueller matrix imaging technique has been used to distinguish cervical and papillary thyroid carcinoma tissues [12,25]. Experimental and MC simulated results have shown that the healthy cervix tissues are highly anisotropic [10,25]. However, anisotropy, as represented by retardance $\delta$ or the MMT parameter $A$, reduces significantly in the cancerous regions. Such cancer induced changes are explained by the reduction of birefringence in the interstitial medium [10] or the breaking down of the well-ordered fibrous structure [25]. For the papillary thyroid carcinoma tissues however, the development for the cancer cells is accompanied by inflammatory reactions and fibrosis formations in the surrounding tissues [12,26], leading to an increase in the anisotropy parameter $A$ from MMT. Since transmission microscopic images of thin tissue slices are often used in pathologic diagnosis, in this paper we adopt the forward scattering configuration for easier comparisons between the polarization images and the colored transmission images of the stained pathologic slides. Figure 2 shows the slices of human cancerous tissues used in this study, which are provided and prepared by Shenzhen Sixth People’s (Nanshan) Hospital. The tissues are cut, fixed, dehydrated, and embedded in paraffin after surgery. Figure 2(a) and 2(b) show the imaging samples, which are the unstained 28 $\mu$m thick slices of tissues cut from the dehydrated paraffin. The tissue slides were cut transversely with respect to the tissue surface. For histological comparisons, the corresponding hematoxylin-eosin (H-E) stained 4 $\mu$m thick slices are also prepared. The microscope images shown in Fig. 2(c), 2(d) and Fig. 2(e), 2(f) confirm that the cancerous cells are with a darker stained color than the healthy cells.
Fig. 2. (a), (b) Photograph of the 28 μm thick slices of unstained human cervix and papillary thyroid carcinoma tissues, the red squares indicate the imaging regions and the black squares indicate the testing regions used for Fig. 3, (c), (d) microscopic images of the corresponding H-E stained slices of cancerous and healthy cervical tissues, (e), (f) microscopic images of the corresponding H-E stained slices of cancerous and healthy thyroid tissues.

2.3 Mueller matrix transformation (MMT) parameters

Mueller matrix elements contain rich structural and optical information of samples. However, individual Mueller matrix elements are often lack of explicit connection to the characteristic microstructure or optical properties of the sample. Also they are often seriously affected by the orientations of anisotropic structures [18]. In previous studies, we proposed the Mueller matrix transformation (MMT) technique to extract a group of new parameters shown as Eq. (1) [17].

\[
\begin{align*}
A &= \frac{2b \cdot t}{b^2 + t^2} \in [0,1] \\
b &= \frac{m_{22} + m_{33}}{2} \\
t &= \frac{\sqrt{(m_{22} - m_{33})^2 + (m_{23} + m_{32})^2}}{2}
\end{align*}
\] (1)

Both parameters \(A\) and \(b\) represent specific structural features but are not sensitive to the azimuth angle of the sample [18]. It was also found that the parameters from the Lu-Chipman Mueller matrix polar decomposition (MMPD) method, i.e, diattenuation \(D\), retardance \(R\) (containing linear retardance \(\delta\), and circular retardance \(\Psi\)), depolarization power \(\Lambda\), also represent the polarization optical properties and are not sensitive to the azimuth orientation of the sample [16]. These MMPD parameters have been widely applied to the studies of cancerous tissues diagnosis [13–15]. However, it is not trivial to take the \(4 \times 4\) Mueller matrices at different wavelengths using the current experimental setup. Also, decomposition of the \(3 \times 3\) Mueller matrix results shows large discrepancies in the set of parameters [27].
Therefore, we use the MMT parameters $A$ and $b$, which are only linked to the linear polarization Mueller matrix elements, i.e. $m_{22}$, $m_{33}$, $m_{23}$ and $m_{32}$ in this study. In fact, most biomedical applications of polarization imaging evolve only linear polarizations [8,28].

Previous studies also testified that the MMT parameters have explicit relations with certain microstructures. Parameter $A$ is related to the anisotropy degree or the order of alignment of the fibrous structures: $A$ is close to 1 for well-aligned fibers, but close to 0 for isotropic media. Parameter $b$ is related to the diagonal elements $m_{22}$ and $m_{33}$. It is strongly correlated to the depolarization property: for highly depolarizing media, the value of parameter $b$ is close to 0. As the depolarization decreases, the value of parameter $b$ will increase [17]. Figure 3 shows the comparison between the MMT parameters $A$, $b$ and the MMPD parameters $\delta$ and $\Delta$. The imaging regions are indicated with the black squares in Fig. 2(a) and 2(b). We calculate both the MMT and MMPD parameters by measuring the $4 \times 4$ Mueller matrices of the testing regions under the incident wavelength of 630 nm. It can be observed in Fig. 3 that the retardation parameter $\delta$ and the MMT parameter $A$ are correlated positively, while the depolarization parameter $\Delta$ and the MMT parameter $b$ have a negative correlation. Although more systematic studies are still needed, the semi-quantitative comparisons shown in [25] and [12] indicate a good match between the $3 \times 3$ MMT and $4 \times 4$ MMPD parameters.

![Fig. 3. Pseudo-color images of the MMPD and MMT parameters of (a) cervical carcinoma tissue and (b) papillary thyroid carcinoma tissue. The wavelength of incident light is 630 nm. The cancerous and healthy regions of the tissues are distinguished by the white and black dotted lines.](image)

### 2.4 Monte Carlo simulation

We use the sphere-cylinder birefringence model (SCBM) to mimic biological tissues and the Monte Carlo (MC) simulation program to track the trajectories and polarization states of scattered photons as they propagate in the medium [29,30]. The SCBM contains three key components: spherical scatterers, infinitely long cylindrical scatterers, and a birefringent interstitial medium, representing different microscopic structures and optical properties of turbid media. For biological tissues the fibrous structures such as collagen, elastin, and muscle fibers can be approximated as cylindrical scatterers, the cell nuclei and organelles can be represented by spherical scatterers with different sizes. In this work, the parameters of the MC simulations are set according to the characteristic features of the cervical and thyroid cancerous tissue samples. The variables include the sizes, refractive indices, and scattering coefficients of the scatterers, the value and fast axis of the birefringence, the refractive index
of the interstitial medium, and the wavelengths of the incident light. The MC simulated results and detailed parameters will be introduced in the following sections.

3. Results and discussion

3.1 Cervix carcinoma tissue

Firstly, we measure the transmitted MMT parameters of the unstained slice of human cervix carcinoma tissue at different wavelengths varied from 500 to 680 nm in steps of 30 nm (a total of seven illumination wavelengths). The imaging region is indicated by the red square in Fig. 2(a). Figure 4 shows the pseudo-color images of the MMT parameters $A$ and $b$ at 500 nm, 590 nm, and 680 nm. In the first images of Fig. 4, the cancerous and healthy areas are marked by the white and black dotted lines. In previous studies, we have found that the healthy cervical tissues are with well-ordered structures, which will be destructed and broken down during the neoplasia process [25]. Therefore, when measuring the backscattering MMT and MMPD parameters, the cancerous cervical tissues are always of smaller values of $A$ and retardance $\delta$ than the healthy cervical tissues. On the other hand, we have also observed that compared with the healthy human tissues, the cancerous tissues contain denser small organelles, resulting a larger value of parameter $b$ [12]. It can be seen from Fig. 4 that for the forward scattering MMT images, the healthy tissues (upper left parts) have larger values of parameter $A$ and smaller values of parameter $b$ than the cancerous tissues (lower right parts), which are consistent with the backscattering measurement results. Moreover, Fig. 4 also shows that as the incident wavelength increases, the values of parameters $A$ and $b$ represent different characteristic variations. To analyze the relations between the MMT parameters and incident wavelength quantitatively, we choose randomly three squares of 133 $\mu$m sizes in both the cancerous and the healthy areas, then calculate the average values and standard deviations of parameters $A$ and $b$, respectively. As shown in Fig. 5, it comes to the following conclusions: (a) for the MMT parameter $A$, the normal areas of cervical tissue have higher values than the abnormal ones. As the incident wavelength varies from 500 nm to 680 nm, the value of the normal area decreases, whereas the value of the abnormal area almost remains unchanged. (b) For the MMT parameter $b$, the normal areas of cervical tissue have lower values than the abnormal ones. As the incident wavelength varies from 500 nm to 680 nm, the values of both the healthy and cancerous areas increase, however, the normal area has a higher growth rate.

![Fig. 4. Pseudo-color images of the MMT parameter $A$ (upper row) and parameter $b$ (lower row) for the unstained human cervix carcinoma tissue slice with the incident wavelengths of 500 nm, 590 nm, 680 nm. The imaging region is indicated by the red square in Fig. 2(a). The white and black dotted lines represent the border of the cancerous tissue and the healthy tissue approximately, and the squares represent the random sample areas used for quantitative analysis.](image-url)

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3.2 Thyroid carcinoma tissue

We also apply the multispectral transmitted MMT parameters to the human papillary thyroid carcinoma tissues. Figure 6 shows the pseudo-color images of the parameter $A$ and $b$ at wavelength of 500 nm, 590 nm, and 680 nm. The imaging region is indicated by the red square in Fig. 2(b). The white and black dotted lines represent the approximated border between the normal and abnormal regions. In previous studies, we have measured the backscattering Mueller matrix and MMT parameters of the human papillary thyroid carcinoma tissues, and found that the inflammatory reactions can result in fibrosis formations surrounding the cancerous cells. Therefore, there are always normal tissues with high values of parameter $A$ near the cancerous tissues [12,26]. It can be observed from Fig. 6 that, for the forward scattering MMT parameters, the normal tissues (left part) are with larger values of parameter $A$ than the cancerous tissues (right part), indicating the existence of fibrous structures near the cancer cells. To study the relations between the parameters and incident wavelengths, we also choose three squared areas in both the cancerous and healthy areas (shown as Fig. 6), then calculate the average values and standard deviations of parameter $A$ and $b$. From Fig. 7, we can observe that: (a) for the MMT parameter $A$, the normal areas of human papillary thyroid carcinoma tissues also have higher values than the abnormal ones. When the incident wavelength varies from 500 nm to 680 nm, $A$ decreases in the normal area, and remains almost unchanged in the abnormal area. (b) For the parameter $b$, the normal areas have lower values than the abnormal ones. When the incident wavelength increases, $b$ of both areas rise, and the normal area shows a higher growth rate. The experimental results show that the multispectral MMT parameters $A$ and $b$ of human cervical carcinoma and human papillary thyroid carcinoma tissues have characteristic features, which can be used as indicators for the discrimination of normal and abnormal areas.
Fig. 6. Pseudo-color images of the MMT parameter $A$ (upper row) and parameter $b$ (lower row) for the unstained slice of human papillary thyroid carcinoma tissue with the incident wavelengths of 500 nm, 590 nm, 680 nm. The imaging region is indicated by the red square in Fig. 2(b). The white and black dotted lines represent the border of the cancerous tissue and the healthy tissue approximately, and the squares represent the random sample areas used for quantitative analysis.

Fig. 7. Values of parameter $A$ (a) and $b$ (b) of the normal and human papillary thyroid carcinoma tissues at different wavelengths.

3.3 Monte Carlo simulation results

For a better understanding of the experimental results, we carry on MC simulations using the SCBM to analyze the multispectral characteristic features of the transmitted MMT parameters $A$ and $b$. In previous studies we have found that, the anisotropy of tissues can be resulted from both aligned cylindrical scatterers and birefringent interstitial medium. The MMPD results shown in Fig. 3 testify that the anisotropy of cervical and thyroid tissues used in this study have large values of birefringence. Therefore we first choose the sphere-birefringence model (SBM) for the simulations and set the parameters as follows: the diameters of the spherical scatterers are set to be 0.5 $\mu$m and 8 $\mu$m to mimic the cell organelles and nuclei, respectively [12]. The refractive index of the scatterers are 1.45, the scattering coefficients of small and large scatterers are 50 cm$^{-1}$ and 150 cm$^{-1}$, the thickness of the medium is 28 $\mu$m, and the refractive index of the interstitial medium is 1.33 [12]. It should be noted that the scattering cross-sections of the scatterers change with the wavelength. However, in the simulations we
keep the scattering coefficients constant approximately to obtain the main characteristic variations of parameters $A$ and $b$, since the scattering cross-sections of spheres, especially of the 8 μm diameter spheres, are not very sensitive to the changes in wavelengths. Also, for the thin slices of 28 μm thickness, birefringence plays the dominant role in the Mueller matrix measurements compared to the scattering. To simulate the destruction of birefringent structures due to the cancerous process, we change the value of the birefringence $\Delta n$ from 0.001 to 0.002 and 0.003 [30,31], the optical axis is in the X-Y plane, along the x-axis (0-degree) direction. As shown in Fig. 8, the simulated results show that for the SBM medium and forward scattering scheme, the MMT parameter $A$ decreases and $b$ increases when the incident wavelength increases, which are consistent with the experimental observations. It can also be observed in Fig. 8(b) that as the value of birefringence increases, the baseline of the spectra of the parameter $b$ decreases. This is because that increasing birefringence can induce stronger depolarization effectively [32,33], resulting in a smaller value of the parameter $b$ which is negatively correlated with the depolarization.

![Fig. 8. Multispectral Monte Carlo simulation results of the parameters $A$ (a) and $b$ (b) using the sphere-birefringence model with 0.001, 0.002 and 0.003 birefringence $\Delta n$ values.](image)

### 3.4 Discussions

As discussed above, using the properly selected scattering model, SCBM and its variant SBM, we can simulate the polarized photons propagating in cancerous tissues with different characteristic microstructures. In order to analyze the contrast mechanism of the multispectral MMT imaging results, also for a better understanding of the detailed structural differences between the normal and abnormal tissues, in this section, we conduct more MC simulations. In previous studies, we have found that the contrast mechanism of Mueller matrix imaging for cancer detection can be resulted from the following structural variations: the breaking down or formation of well aligned fibrous structures [25,26], variation of sub-wavelength small scatterers such as the size and density of intra-cellular organelles [12]. In Fig. 8, we can observe that the difference of birefringence between the normal and abnormal tissues is also a possible explanation for the experimental results shown in Figs. 5 and 7. However, since the birefringence of tissues can also be originated from the well aligned cylindrical scatterers [34], we carry on further simulations using the sphere-cylinder scattering model (SCSM) which does not include the birefringent interstitial medium.
Fig. 9. Monte Carlo simulation results of: (a), (b) Values of parameter $A$ and $b$ of the sphere-cylinder scattering model. The diameters and the scattering coefficients of the cylindrical scatterers are set to be 1.5 μm and 200 cm$^{-1}$, 0.2 μm and 200 cm$^{-1}$, 0.2 μm and 400 cm$^{-1}$, respectively. The parameters of the spherical scatterers remain the same as Fig. 8. (c), (d) Values of parameter $A$ and $b$ of the sphere-birefringence model. The diameter of the small spherical scatterers is set to be 0.2 μm, 0.5 μm, and 0.8 μm. The other parameters are the same as Fig. 8. (e), (f) Values of parameter $A$ and $b$ of the sphere-birefringence model. The ratio between the scattering coefficients of the small and large spheres is changed from 50:150 to 100:100 and 150:50.

For the MC simulated results of SCSM shown in Fig. 9(a) and 9(b), the diameters and the scattering coefficients of the cylindrical scatterers are varied, while the parameters of the spherical scatterers remain the same as in Fig. 8. It can be observed from Fig. 9(a) and 9(b) that, for the SCSM medium, when the incident wavelength increases the value of $A$ increases, whereas the value of $b$ decreases. The spectral characteristic features are different from what we observed in the SBM simulations (Fig. 8) and in the experiments (Figs. 5 and 7). The multispectral MC simulations based on SCSM and SBM indicate that birefringence due to the
interstitial medium is more likely responsible to the characteristic features in anisotropy for the cervical cancer and papillary thyroid carcinoma tissues.

We can also analyze further the relationship between the MMT parameters and the diameter or the scattering coefficient of small spherical scatterers, which may correspond to the size or density variations of intra-cellular organelles. In Fig. 9(c) and 9(d), we keep the other parameters of the SBM the same as Fig. 8 but change only the diameter of the small spheres from 0.2 μm to 0.5 μm and 0.8 μm. While in Fig. 9(e) and 9(f), we change the ratio between the scattering coefficients of the small and large spheres from 50:150 to 100:100 and 150:50. The simulations show that for the transmission imaging of thin slices of tissues, the size and scattering coefficient variations of the small scatterers have very limited influence on the MMT parameters. Based on the above simulations using different microstructural models, we conclude that the contrast mechanism of the transmission MMT imaging is the breaking down of birefringent normal tissues for cervical cancers, or the formation of birefringent surrounding structures accompanying the inflammatory reaction for papillary thyroid carcinoma tissues. The characteristic spectral features of polarization imaging offer additional information on the contrast mechanism. Meanwhile, although more studies of backscattered multispectral MMT parameters are still needed, the findings from this study will be useful for in vivo or endomicroscopic imaging of tissues.

4. Conclusion

In this paper, we take the transmission 3 × 3 linear polarization Mueller matrix images of the unstained thin slices of human cervical and thyroid cancerous tissues, and analyze their multispectral behavior using the MMT parameters $A$ and $b$. The experimental results show that for both cervical and thyroid cancerous tissues, when the incident wavelength increases, $A$ decreases and $b$ increases. The characteristic features of multispectral transmitted MMT parameters $A$ and $b$ can be used to distinguish the normal and abnormal cervical and thyroid tissues. Monte Carlo simulations based on the sphere-cylinder birefringence model (SCBM) proposed in our previous studies provide additional information of the relations between the characteristic spectral features of the MMT parameters and the microstructures of the tissues. By comparing the experimental and simulated data, we confirm that the contrast mechanism of the transmission MMT imaging for cancer detection is the breaking down of birefringent normal tissues for cervical cancer, or the formation of birefringent surrounding structures accompanying the inflammatory reaction for thyroid cancer. It is also testified that, the characteristic spectral features of polarization imaging techniques can provide detailed microstructural information of tissue samples, which can be useful for diagnosis applications.

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