Comparative study of the influence of imaging resolution on linear retardance parameters derived from the Mueller matrix

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Abstract: Polarization imaging techniques are emerging tools to provide quantitative information of anisotropic structures, such as the density and orientation distribution of fibers in tissue samples. Recently, it is found that when using Mueller matrix polarimetry to obtain the structural features of tissue samples, some information can be revealed by relatively low-resolution polarization parameter images. Thus, to analyze what kinds of anisotropic optical and structural information contained in high-resolution polarization images are preserved in low-resolution ones, here we carry out a comparative study of the influence of imaging resolution on the Mueller matrix derived linear retardance parameters. We measure the microscopic Mueller matrix of human healthy breast duct tissues and ductal carcinoma in situ (DCIS) tissues, which have distinct typical fibrous structures, using objectives with different numerical aperture. Then we quantitatively compare a group of image texture feature parameters of the linear retardance parameters images under high and low imaging resolutions. The results demonstrate that the fibers density information contained in the texture features of linear retardance δ parameter image are preserved well with the decline of imaging resolution. While for the azimuthal orientation parameter θ which closely related to the spatial location, we still need high imaging resolution to obtain quantitative structural information. The study provides an important criterion to decide which information of fibrous structures can be extracted accurately using transmission Mueller matrix microscope with low numerical aperture objectives.

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1. Introduction

Polarization imaging techniques are widely used in biomedical studies and have been regarded as potential label-free tools for diagnosis, because of their sensitivity to microstructural changes in tissues [1–11]. As a method that can completely characterize the polarization properties of samples, Mueller matrix polarimetry is especially fit to detect the density and distribution behavior of anisotropic fibrous components of turbid samples [12–18], and has been applied to clinical practices to assist the diagnosis of several different types of diseases, such as Alzheimer’s disease, breast cancer and colon cancer [19–21]. Recently, to assist pathological diagnosis, we developed a modulus designed transmission Mueller matrix microscope by adding both the...
polarization state generator and analyzer to a commercial transmission microscope [21]. We applied the Mueller matrix microscope to the detection and staging of human breast carcinoma tissue samples, and found that the breast cancer progression has a close relation with Extracellular matrix (ECM) modification [22]. Although Mueller matrix is a comprehensive description of polarization characteristics of media, the individual Mueller matrix element can hardly reveal explicit information on the optical and structural properties. Therefore, several Mueller matrix analyzing methods, such as Mueller matrix polar decomposition (MMPD) [23,24], Mueller matrix differential decomposition (MMDD) [25] and Mueller matrix transformation (MMT) techniques [26], were proposed to derive sets of polarization parameters with specific physical meanings, which can be used to quantitatively obtain structural properties of media especially biomedical samples [24,27]. For example, the MMPD parameters were more and more prevalently used in biomedical studies including detection of cancerous tissues [28]. The studies indicated that the MMPD parameters can depict the polarization properties in various aspects to reflect different features of samples. Especially, linear retardation ($\delta$) is related with the birefringence generated by the fibrous structures, while orientation angle ($\theta$) is sensitive to the azimuthal orientation of the fibers [27–29].

On the other hand, objective with high numerical aperture (NA) is important to improve imaging resolution for traditional optical microscope. For optical microscopes including polarization microscopes, high resolution images can provide more microstructural information of the sample. However, for clinical applications the imaging speed is also important for quick diagnosis. The objective with low NA cannot offer enough imaging resolution to identify microstructures of biological samples effectively, imaging using a high NA objective means a smaller field of view (FOV) and slower imaging speed compared with that using a low NA objective. In our previous work, we found that when using Mueller matrix polarimetry to obtain the structural features of tissue samples, some information such as the density of small scatters can be revealed by relatively low resolution polarization parameters images [28]. Thus, to analyze what kinds of anisotropic optical and structural information contained in high resolution polarization images are preserved well in low resolution ones, a comparative study of the influence of imaging resolution on the Mueller matrix derived parameters is needed. It will be an important guidance and criterion to decide which information of fibrous structures can be extracted accurately using low NA transmission Mueller matrix microscope. In this study, we mainly focus on the analysis of influence of imaging resolution on linear retardance and azimuthal orientation parameters, which have shown great diagnostic potential of quantitatively detecting fibrous structures in various abnormal tissues [30]. Also, for thin pathology tissue slices, compared with other Mueller matrix derived parameters, the linear retardance parameters can be used to reveal the fibrous structural features more clearly [21]. It should be noted that there are more other polarization-sensitive parameters can be derived from Mueller matrix, which are related to different structural features of tissues and should be compared in our future studies. We prepare human healthy breast duct tissues and ductal carcinoma in situ (DCIS) tissues, which have different typical fibrous structures [30–32]. Then we use the Mueller matrix microscope to measure the unstained tissue slices’ MMPD parameters $\delta$ and $\theta$ images, which can reflect the linear retardance and azimuthal orientation induced by the fibrous structures in breast tissues, under 4×/NA 0.1 and 40×/NA 0.65 objectives. Here we select the 4×/NA 0.1 and 40×/NA 0.65 objectives for the comparative study because they are commonly used low and high NA objectives for pathological observations. Finally, we compare and quantitatively analyze the mean values and a group of image texture feature parameters of the MMPD parameters images under high and low imaging resolutions. The results suggest that under the premise of the enough imaging resolution, it is reasonable to exchange resolution for a large FOV and fast imaging speed for some parameters derived from Mueller matrix such as linear retardance parameter $\delta$, but for others like azimuthal orientation
parameter $\theta$ that closely related to the spatial location, high imaging resolution is necessary to accurately obtain the quantitative information for biomedical detections.

2. Methods and materials

2.1. Mueller matrix microscopy

Here we use the transmission microscope based on the dual-rotating quarter-wave plate method [33,34], which is a robust and well-studied method to measure the Mueller matrix of samples. The theory of the dual-rotating quarter-wave plate method was proposed by Azzam [33], but then developed several different practical measurement schemes. In this study, we selected the scheme developed by Chenault et al. [34], which showed good measurement precision for biomedical samples. The schematic is shown in Fig. 1. During the measurement, the illuminating light of 633 nm from the LED (XLamp XP-E, 3.5W, Cree, Inc., USA) is collimated by a lens, passes through the polarization state generator (PSG) and interacts with the sample. Then the scattered light from the sample is collected by an objective lens, analyzed by the polarization state analyzer (PSA) and focused by an imaging lens to the CCD camera (QImaging Retiga ELECTRO, 1360 × 1024 pixels, 14-bit, 6.45 $\mu$m × 6.45 $\mu$m pixel size, Teledyne, Inc., Canada). The PSG and PSA have similar structure consisting of a linear polarizer and a quarter-wave plate as shown in Fig. 1(c). In the microscope both the linear polarizers are fixed at the same horizontal direction while the two quarter-wave plates rotate with the constant rates ($\omega$ and 5$\omega$ in the PSG and PSA, respectively). Then the Mueller matrix of the sample can be calculated inversely by using Discrete Fourier Transform (DFT) method shown as Eq. (1) [33],

$$I = a_0 + \sum_{n=1}^{12} (a_n \cos n\omega t + \beta_n \sin n\omega t)$$  \hspace{1cm} (1)

where $I$ is the light intensity captured by the CCD each time, $a$ and $\beta$ are the Fourier coefficients, $\omega$ is the rotating angle rate and set to be 6 degree per time constantly in the experiments. In other words, the rotating angle rate $\omega$ of the PSG is 6 degree per time, and the rotating angle rate 5$\omega$ of the PSA is 30 degree per time, $t$ in Eq. (1) (namely $q$ in the scheme developed by Chenault et al. [34]) is the number of measurement times (the $t_{th}$ measurement) whose range is 1 to 30. Obviously, the rotating angle rate $\omega$ should be different when another measurement scheme is used. However, the measurement result - the Mueller matrix - is invariant when ignoring the errors induced by different measurement schemes.

The Mueller matrix microscope was calibrated by using standard samples including air, polarizer and quarter-wave plate to ensure that the maximum errors of the measured Mueller matrix elements are less than 1%. More detailed information of the calibration process can be found in [35].

2.2. Mueller matrix polar decomposition

Mueller matrix polar decomposition (MMPD) developed by Lu and Chipman is a powerful tool for probing the microstructures of biomedical samples, especially pathological tissues [23]. MMPD method decomposes the interaction between light and sample into three main polarization properties: diattenuation ($D$), retardation ($\delta$), and depolarization ($\Delta$) shown as Eq. (2), representing the optical characteristics of tissue samples with some clear physical meanings [23]. Thus, in this study, we select the parameters $\delta$ and $\theta$ shown as Eqs. (3, 4) as the indicators to explore the changes of information contained in some MMPD parameter images of tissue
samples with different resolution [24].

\[ M = M_\Delta M_R M_D \]  
\[ \delta = \cos^{-1} \left\{ \left[ (M_R(2, 2) + M_R(3, 3))^2 + (M_R(3, 2) - M_R(2, 3))^2 \right]^{1/2} - 1 \right\} \]  
\[ \theta = 0.5 \tan^{-1} \left( \frac{r_2}{r_1} \right) \]

where \( M_\Delta, M_R \) and \( M_D \) are the sub-matrices of depolarization, retardation and diattenuation, respectively. \( r_1 \) and \( r_2 \) are the elements of the vector of retardance.

### 2.3. Breast duct tissue samples

Fibrous structures play an important role in the breast duct tissues since they provide strength and cushioning to breast [36]. In our previous work, we found that the proportions and distribution of fibrous structures surrounding the breast ducts are different in healthy and DCIS tissues [22]. Such kinds of differences are easy to identify qualitatively with hematoxylin and eosin (H&E) staining. For example, in Fig. 1(b) we can observe that, under the 40×NA 0.65 objective, the light pink fibers in the healthy breast duct tissue represent an orderly arrangement while the fibers in the DCIS tissue have deeper H&E staining color and denser arrangement than the healthy tissues. The evident structural difference of the fibers makes breast duct tissue samples suitable to be explored using polarimetry to show the influence of imaging resolution on polarization properties.
The samples used in this study are 12-μm-thick unstained, dewaxed slices of human breast duct tissues at healthy and DCIS stages, together with correspondent adjacent 4-μm-thick H&E stained slices. All the breast duct tissues slices were prepared and evaluated by three certified pathologists from the Shenzhen Sixth People’s (Nanshan) Hospital. Here 13 cases of unstained, dewaxed slices were selected from the healthy and DCIS tissues, respectively, based on pathological observations of the correspondent adjacent 4-μm-thick H&E stained slices. To better show the influence of imaging resolution on the Mueller matrix derived linear retardance parameters, the sampling zones from 13 healthy and 13 DCIS tissues cases were carefully selected and confirmed by experienced pathologists and us to make sure they have typical fibrous structures. It should be noticed that the linear retardance parameter can be influenced by the physical thickness of tissue sample, and mitigating such kind of influence can increase the reliability of the linear retardance parameter as the tissue diagnostic index [37]. However, for the comparative study, we use the original data to avoid any processing which may affect the quantitative comparison. The reason is that, the aim of this study is to quantitatively compare the influence of imaging resolution on linear retardance parameters derived from Mueller matrix. For the comparisons between 4×/NA 0.1 and 40×/NA 0.65 objectives data, we choose the same zones from the identical tissue slices, meaning that thickness fluctuations have the same influence on the 4×/NA 0.1 and 40×/NA 0.65 objectives data, and the conclusion drawn from the comparisons would not be affected. For the regions of interest selection, we selected an original point according to the structural features of both unstained and H&E stained slices images, then established the coordinates in the whole images. The regions share identical coordinate values are the same zones in the adjacent H&E stained slice and unstained slice. During the Mueller matrix measurements, the tissue slice was not moved when changing the objective. The difference of field of view between 4×/NA 0.1 and 40×/NA 0.65 objectives can be corrected using a standard sample to select the zone on 4×/NA 0.1 image corresponding to the 40×/NA 0.65 image. This study was approved by the Ethics Committee of the Shenzhen Sixth People’s (Nanshan) Hospital.

2.4. Resolution reduction simulation algorithm

For the physics point of view, to better understand the difference of MMPD linear retardance parameters between the 4×/NA 0.1 and 40×/NA 0.65, we also reduced the Mueller matrix images digitally to simulate the impact of different NA objectives on the parameters, and to discuss the reason of differences in statistics between the linear retardance and orientation parameters. In the scheme of dual-rotating quarter-wave plate measurement used in this study, a Mueller matrix is constructed inversely using 30 raw intensity images [shown in Eq. (1)]. Therefore, the digital resolution reduction of the raw intensity images can influence the Mueller matrix elements images and certainly the derived linear retardance parameters. In the process, there are two factors can reduce the raw intensity image resolution. First, a low NA objective can be treated as a low-pass filter with a cutoff frequency determined by NA, which results in the loss of high-frequency information contained in the high resolution images. The light signal is sampled by the image sensor, the increase of FOV leads to the decrease of imaging size of CCD.

Here to digitally reduce the resolution of the 40×/NA 0.65 images to a value that is similar to the 4×/NA 0.1 images, we simulate the imaging process of our incoherent imaging system. First, for each 0.65 NA raw intensity image, we can obtain a new 0.1 NA raw intensity image as Eq. (5),

\[ I_{\text{output}}(x, y) = |h(x, y)|^2 \otimes I_{\text{input}}(x, y) \]  

where \( I_{\text{input}} \) and \( I_{\text{output}} \) represent the input and output intensity images, and \( |h(x, y)|^2 \) is the incoherent point spread function in the spatial domain, \((x, y)\) is the coordinate of image. The incoherent transfer function can be generated with parameters of 40× magnification, 0.1 NA, 633 nm incident wavelength and 6.45 μm pixel size. After performing the low-pass filtering process in the Fourier domain, we obtained the new intensity output images of simulated 0.1 NA
objective. The low-pass filtering method used in this study was realized according to [38]. More details of the low-pass filtering process can be found in Section 1.2 of [38]. Second, we digitally averaged the new intensity images to a resolution that is similar to the 4x/NA 0.1 intensity images to simulate the imaging size difference. Then the 30 new digitally resolution-reduced intensity images were used to calculate the Mueller matrix and derived linear retardance parameters images.

2.5. Image analysis method

To explore the influence of imaging resolution changes, we first intuitively compare the H&E stained images, CCD images, MMPD $\delta$ and $\theta$ parameters images of a healthy breast duct tissue slice and a DCIS tissue slice as shown in Fig. 2(a) and (b). Then for a quantitative comparison, we analyze the image features of two typical Mueller matrix derived parameters MMPD $\delta$ and $\theta$, which can reflect the distribution behavior of the fibers. Here we use six image feature parameters for the evaluation including: Mean and Entropy shown as Eq. (6) are used to analyze the first-order statistic characteristics,

$$\text{Mean} = \sum_i z_i p(z_i),$$
$$\text{Entropy} = -\sum_i p(z_i) \log_2 p(z_i).$$

(6)

where $p(z_i)$ is the proportion of pixels with the value of $z_i$ to the total number of pixels [39]. The Gray level co-occurrence matrix (GLCM) parameters Contrast, Correlation, Energy, and Homogeneity are used to analyze texture features of the polarization parameters images. GLCM method is a powerful tool widely used to obtain image texture features. In GLCM, the co-occurrence matrix represents the joint probability occurrence of pixel pairs with a specified spatial relationship, which is defined by the gray levels ($N_g$) of the image, the interpixel displacement ($d$) and orientation values between them. Then several GLCM features can be derived from the co-occurrence matrix to reflect different texture features of an image as shown in Eq. (7) [40],

$$p_x(i) = \sum_{j=1}^{N_x} p(i, j), p_y(j) = \sum_{i=1}^{N_y} p(i, j),$$
$$\text{Contrast} = \sum_{n=0}^{N_g-1} n^2 \left\{ \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} p(i, j) |i - j| = n \right\},$$
$$\text{Correlation} = \frac{\sum_i \sum_j (ij)p(i, j) - \mu_x \mu_y}{\sigma_x \sigma_y},$$
$$\text{Energy} = \sum_i \sum_j p(i, j)^2,$$
$$\text{Homogeneity} = \sum_i \sum_j \frac{1}{1 + (i - j)^2} p(i, j).$$

(7)

where $N_g$ is the quantized gray levels. $P(i, j)$ is the relative frequencies with two neighboring pixels (whose gray levels are $i$ and $j$, respectively) separated by the interpixel displacement ($d$) in a specific direction occur on the image. $\mu_x$, $\mu_y$, $\sigma_x$, and $\sigma_y$ are the means and standard deviations of $p_x$ and $p_y$. Contrast measures the local variations in the gray-level co-occurrence matrix. A higher value of Contrast brings a stronger ability to distinguish various components of a static image. Correlation returns a measure of how correlated a pixel is to its neighbor over the whole image. For a perfectly positively or negatively correlated image, the Correlation value is 1 and
Fig. 2. Imaging and digitally resolution-reduced results from the non-duct area of (a) healthy breast duct tissue samples, (b) DCIS tissue samples. In each group, from left to right of the first row shows the images of adjacent H&E stained slice under 4×/NA 0.1 and 40×/NA 0.65 objectives observation, unpolarized light intensity images of the unstained tissue slice under 4×/NA 0.1 and 40×/NA 0.65 objectives observation. From left to right of the second and third rows show the MMPD δ and θ images of the same region under 4×/NA 0.1 and 40×/NA 0.65 objectives observation, the digitally low-pass filtered MMPD δ and θ images, and digitally simulate (averaged after low-pass filtering) 4×/NA 0.1 MMPD δ and θ images. The units for δ is radian angle, for θ is degree of angle. The white scale bar is 50 µm.
−1. Energy measures the joint probability occurrence of the specified pixel pairs, reflecting the orders in an image. A higher value of Energy means the texture of the image is more uniform. Homogeneity returns a value that measures the closeness of the distribution of elements in the co-occurrence matrix, reflecting orders of the local image.

In this study, the range of gray value of the MMPD $\delta$ and $\theta$ images was normalized to $[0, 255]$, the gray levels $N_g$ was set as 64, the interpixel displacement $d$ was set to 1 and 11 under $4\times/NA 0.1$ and $40\times/NA 0.65$ objectives, respectively, to compensate the differences induced by FOV. Each GLCM feature was the average of the features in four orientations ($0^\circ$, $45^\circ$, $90^\circ$ and $135^\circ$). Then, for the comparative study, we calculated the correlation coefficients of different feature parameters for MMPD $\delta$ and $\theta$ images under $4\times/NA 0.1$ and $40\times/NA 0.65$ objectives, and did the Student’s t-test between the healthy tissues and DCIS tissues cases to evaluate whether the image feature parameter is able to distinguish between the cases of two different stages. At last, we also show the results of fast screening of the fibrous structure which used a sliding window method in the pathological slices with a $4\times/NA 0.1$ objective. The sliding window was set to be $5\times5$ pixels and the step size was 1 pixel.

3. Results and discussion

3.1. Microscopic imaging results of different objectives

Figure 2 shows two groups of microscopic imaging and digitally resolution-reduced results from the non-duct area of a typical healthy breast duct tissue sample (Fig. 2(a)) and a DCIS tissue sample (Fig. 2(b)). In each group, from left to right of the first row shows the images of adjacent H&E stained slice under $4\times/NA 0.1$ and $40\times/NA 0.65$ objectives observation, unpolarized light intensity images of the unstained tissue slice under $4\times/NA 0.1$ and $40\times/NA 0.65$ objectives observation. From left to right of the second and third rows show the MMPD $\delta$ and $\theta$ images of the same region under $4\times/NA 0.1$ and $40\times/NA 0.65$ objectives observation, the digitally resolution-reduced MMPD $\delta$ and $\theta$ images whose raw $40\times/NA 0.65$ intensity images were only low-pass filtered and averaged after low-pass filtered (simulate $4\times/NA 0.1$).

Our previous work revealed that the MMPD parameters $\delta$ and $\theta$ are good indicators to reflect the density and distribution behavior of fibrous structures in tissues, thus can be used for breast ductal carcinoma tissues detection and staging [41]. First, it can be observed from Fig. 2 that, obviously all the images acquired by $40\times/NA 0.65$ objective are clearer and show more details to a greater degree than the images acquired by $4\times/NA 0.1$ objective. Albeit we can also roughly obtain the structural information, such as the density and location of fibers, from the $4\times/NA 0.1$ images the tiny fibrous structures can only be observed in the $40\times/NA 0.65$ images. For instance, in Fig. 2(b) the $4\times/NA 0.1 \delta$ image shows several isolated thick fibers. However, in the same locations of the $40\times/NA 0.65 \delta$ image we can see they are bundles of tiny fibers, which is closer to the morphologic feature of these tissues as represented by H&E stained image. Meanwhile the $40\times/NA 0.65 \theta$ images also provide more detailed azimuthal orientation information of the fibers than the $4\times/NA 0.1 \theta$ images. Second, comparing the results of healthy tissue (Fig. 2(a)) and DCIS tissue (Fig. 2(b)), their different fibers density and distribution can hardly be discriminated in the adjacent H&E stained images shown in the first row when the resolution decreased from $40\times/NA 0.65$ to $4\times/NA 0.1$. As for the unpolarized light intensity images of the unstained tissue slices shown in the first row, they do not represent the identification ability of tissues for both $40\times/NA 0.65$ and $4\times/NA 0.1$ observations. The MMPD $\delta$ and $\theta$ images of second and third rows demonstrate that, even for the $4\times/NA 0.1$ MMPD parameters $\delta$ and $\theta$ images with limited resolution, they still could be used to distinguish the different fibrous structures between the healthy and DCIS tissues. Specifically, the $\delta$ values of fibers for the healthy tissue are much smaller than those for the DCIS tissues, whose fibers are denser. The denser fibers also make the $\theta$ images of DCIS tissues clearer than the healthy ones under both $40\times/NA 0.65$ and $4\times/NA 0.1$ objectives.
The digitally resolution-reduced MMPD $\delta$ and $\theta$ images shown in Fig. 2 suggest that, first, we can see that the digitally resolution-reduced MMPD $\delta$ images of both low-pass filtered and averaged after low-pass filtered are very similar to the images measured with the 4x/NA 0.1 objective. Obviously, both in the experimental linear retardance $\delta$ images and the digitally resolution-reduced $\delta$ images, we can distinguish different fibrous structures and recognize two types of breast ductal tissue samples. The results indicate that most information of the linear retardance $\delta$ images are preserved. Second, for the digitally resolution-reduced MMPD $\theta$ images, it can be observed that the textural information contained in the images acquired with the 40x/NA 0.65 objective lost prominently as the low-pass filtering performed. It is noted that the information lost mainly happened in the process of low-pass filtering, and the image shows no obvious changes after the pixels averaging. The digitally resolution-reduced images demonstrate that, the main conclusion drawn from the comparisons here using experimental results would not be affected. That is the fibers azimuthal orientation information, which are closely related to the spatial position, derived from the features of high-resolution images cannot be completely obtained from relatively low-resolution images.

The results shown in Fig. 2 demonstrate that the high resolution Mueller matrix microscopic images have the power to provide some detailed quantitative structural information for pathological detections of human breast ductal carcinoma tissues. However, it can be noticed that some structural characteristics of fibers useful for diagnostic purposes could also be identified and quantitatively evaluated with low resolution images, which have the advantages of fast imaging speed and wide FOV.

### 3.2. Quantitative analysis of the polarimetric imaging results with different resolutions

In this section, to further analyze what kind of structural information contained in high resolution Mueller matrix polarimetric images are preserved in the corresponding low resolution polarimetric images, we did more quantitative comparisons. Figure 3 and 4 show the comparative analyzing results of the image feature parameters Mean, Entropy, Contrast, Correlation, Energy, and Homogeneity of MMPD $\delta$ and $\theta$ images for 13 healthy breast duct tissues samples (blue dots) and 13 DCIS tissues samples (orange dots). The sample dot over, on and under the black diagonal dashed line, namely reference line, represents its value under 40x/NA 0.65 objective is larger than, equal to and smaller than the value under 4x/NA 0.1 objective, respectively. The correlation coefficient (R) of all 26 samples between 4x/NA 0.1 and 40x/NA 0.65 groups was calculated and labeled in each panel when the image feature parameter shows a strong correlation ($R > 0.8$). The higher value of R represents two data groups are more relevant. The quartiles of healthy samples (blue quartile) and DCIS tissues samples (orange quartile) were given in the X-axis and Y-axis for 4x/NA 0.1 and 40x/NA 0.65 groups, respectively. The P-value in each resolution group was also labeled separately to show the significance between healthy and DCIS tissues.

From Fig. 3 we can see that all the six image feature parameters of $\delta$ show strong correlation between the 40x/NA 0.65 and 4x/NA 0.1 objectives data sets, which means some distinguishing features are similar in both the high and low resolution images. For example, the R values of Mean ($R=0.991$) and Energy ($R=0.958$) suggest that for these two features, most information are still preserved when using low resolution objective. We can see from Fig. 3(a) that, the Mean values of 40x/NA 0.65 data group (0.0307-0.0672) are slightly larger than those of 4x/NA 0.1 data group (0.0255-0.0580), probably resulted from the wide-angle photons received by the higher NA objective propagated longer distances in the sample and accumulated more retardance. It can be observed from Fig. 3(b) that the Entropy values of 4x/NA 0.1 data group (4.1903-5.4140) do not decrease significantly than those of 40x/NA 0.65 data group (3.9029-5.1484), indicating that most entropy information are preserved with imaging resolution declined [42]. Figure 3(c) shows that, compared to the 4x/NA 0.1 data group (1.0233-4.8148), the Contrast values of the 40x/NA 0.65 data group (6.9569-26.7912) are much larger, which shows that the $\delta$ images would
Fig. 3. Comparative analyzing results of MMPD δ parameter images of 13 healthy human breast duct tissue samples (blue dots) and 13 DCIS tissue samples (orange dots) between the 4×/NA 0.1 (X-axis) and 40×/NA 0.65 (Y-axis) data sets. (a)-(f) Mean, Entropy, Contrast, Correlation, Energy and Homogeneity. In each panel, the correlation coefficient R between the 40×/NA 0.65 and 4×/NA 0.1 objectives data sets are provided, and the significance P values between healthy and DCIS tissue samples of the 4×/NA 0.1 and 40×/NA 0.65 data sets are also listed in X-axis and Y-axis.

be clearer with high imaging resolution. However, the value of Correlation (Fig. 3(d)), Energy (Fig. 3(e)) and Homogeneity (Fig. 3(f)) in the 4×/NA 0.1 group are smaller than those in the 40×/NA 0.65 group, showing that the image textures with lower resolution are more uniform, similar and in good order. The reason is that the lost of many image details can cause the image become smoother and the textures look fuzzy. Moreover, from the t-test results listed in Fig. 3 we can see that, for the δ images, the feature parameters Mean, Entropy, Contrast, Energy and Homogeneity have the similar distinguishing abilities between healthy and DCIS tissue samples using both 4×/NA 0.1 and 40×/NA 0.65 objectives (P < 0.01), while the distinguishing ability of Correlation decreased using 4×/NA 0.1 objective (P > 0.01) compared with using 40×/NA 0.65 objective (P < 0.01). The quantitative analyzing results shown in Fig. 3 suggest that, the low resolution MMPD δ images can preserve some important texture feature information contained in the high resolution δ images. These information can be used to reveal the characteristics of fibrous structures in tissues.

Figure 4 shows the analyzing results of MMPD θ images. It can be seen that compared with the results of δ images, none of the six image feature parameters represent strong correlation between the 4×/NA 0.1 and 40×/NA 0.65 objectives data sets (R is 0.650 for Mean, 0.168 for Entropy, 0.698 for Contrast, 0.149 for Correlation, 0.260 for Energy and 0.036 for Homogeneity). The Mean (Fig. 4(a)) and Entropy values (Fig. 4(b)) of the DCIS samples (orange dots) using 4×/NA 0.1 and 40×/NA 0.65 objectives are almost equal. However, the Entropy values of healthy samples (blue dots) using 40×/NA 0.65 objective are larger than those using 4×/NA 0.1 objective, indicating that the azimuthal orientation of fibrous structures in healthy breast duct samples distributed more randomly than that in DCIS samples. As shown in Fig. 4(c) the Contrast values of 40×/NA 0.65 data group are larger than those of 4×/NA 0.1 data group, indicating that compared with 4×/NA 0.1 images, the θ images with high resolution offer more details to make them look clearer. What is more, the Correlation (Fig. 4(d)), Energy (Fig. 4(e)) and Homogeneity (Fig. 4(f)) data are all under the reference line, showing that the textures are more well-distributed. Last, the P values listed in Fig. 4 show that when using 40×/NA 0.65 objective,
the parameters Entropy, Correlation, Homogeneity are able to distinguish different orientation distribution behavior of fibers between healthy and DCIS tissues. However, when using 4×/NA 0.1 objective, all the six feature parameters show no significant difference between these two types of fibrous structures (P>0.01). The comparative analyzing results in Fig. 4 suggest that enough imaging resolution is necessary if θ images were used to identify characteristic orientation distributions of fibrous structures. Especially, for tissue samples with a small amount of fibrous structures such as healthy breast duct tissues, the information of fibers’ azimuthal orientation derived from Mueller matrix may lose prominently when the imaging resolution is not enough.

Fig. 4. Comparative analyzing results of MMPD θ parameter images of 13 healthy human breast duct tissue samples (blue dots) and 13 DCIS tissue samples (orange dots) between the 4×/NA 0.1 (X-axis) and 40×/NA 0.65 (Y-axis) data sets. (a)-(f) Mean, Entropy, Contrast, Correlation, Energy and Homogeneity. In each panel, the significance P values between healthy and DCIS tissue samples of the 4×/NA 0.1 and 40×/NA 0.65 data sets are provided around X-axis and Y-axis.

In summary, we can see that the fibers density information contained in the texture features of MMPD δ parameter image are preserved well with the decline of imaging resolution. It suggests that when using features of MMPD δ images to distinguish between different fibrous structures according to their density distribution, the relatively low resolution images may serve as a better choice, which provide similar information as high resolution images together with fast imaging speed and wide FOV. However, it is also demonstrated here that the fibers’ azimuthal orientation information, which are closely related to the spatial position, derived from the features of high resolution MMPD θ images cannot be completely obtained from relatively low resolution images. Thus, imaging resolution is crucial to improve the accuracy for the polarimetric detection and staging method based on the azimuthal orientation to recognize fibrous structure in tissues.

3.3. Fast pathology screening of fibrous structures using the sliding window method

Now we can see that the 4×/NA 0.1 MMPD parameter δ preserves enough linear retardance information induced by the fibrous structures. Compared with a high NA objective such as 40×/NA 0.65 objective, the FOV of a 4×/NA 0.1 objective is more than one hundred times (125 times in our study) wider (the blue dashed box in Fig. 5(b) shows the FOV of the 40×/NA 0.65 objective), which indicate that we have to take hours to measure the sample using a 40×/NA 0.65 objective, but several minutes using a 4×/NA 0.1 one. Thus, here we demonstrate the application that uses the Mueller matrix microscope of 4×/NA 0.1 objective for fast screening of the tissue slices and quickly finding the region of interest to evaluate the pathological features.
Fig. 5. Images of (a) 4-µm-thick adjacent H&E stained slice, (b) unpolarized light intensity of unstained slice, (c) MMPD parameters $\delta$, and (d) GLCM parameters of DCIS tissue sample under 4×/NA 0.1 objective. The breast duct, non-duct and the fat tissue area are marked with identifiers of blue triangle, red star and black arrow in (a), respectively. The dashed box in (b) shows the FOV of 40×/NA 0.65 objective. The white dashed lines in (c) show the boundary of the duct. The white scale bar is 500 µm.
Figure 5 shows the results of fast pathological screening of fibrous structure of a DCIS tissue sample using 4×/NA 0.1 objective. Here in this section, we use the sliding window method in the MMPD δ image with the 4×/NA 0.1 objective. The whole images shown in Fig. 5 were imaged with the 4×/NA 0.1 objective, and the blue dashed box in Fig. 5(b) shows the FOV of the 40×/NA 0.65 objective. First, we measured the MMPD δ image as shown in Fig. 5(c). Figure 5(a) and (b) show the correspondent H&E stained image and unpolarized intensity image of the unstained tissue slice. In Fig. 5(a), the area marked with the blue triangle, red star and black arrow are breast duct, non-duct and the fat tissue area, respectively. With the increase of FOV, we can recognize various different areas of breast tissue rather than just one area shown as the blue box in Fig. 5(b). We can see that there are less fibrous structures in the duct area, while its intact boundary indicates that the cancer cells did not penetrate the basement membrane to invade the duct of breast. Second, Fig. 5(d) shows the results of using sliding window method mentioned in Section 2.4 to present different local image texture information of the MMPD δ image to visually reflect more features of fibrous structures in the DCIS tissues. As we can observe from Fig. 5 that, compared with other uniform and isotropic areas, the non-uniform fibrous structures areas marked with red stars in Fig. 5(a) have larger values in the Contrast and Correlation images shown as Fig. 5(d). Meanwhile, the uniform fat areas which are isotropic [27] marked with black arrow in Fig. 5(a) have larger values in the Energy and Homogeneity images shown as Fig. 5(d) compared with the non-uniform fibrous structure areas. It can be found that the Contrast and Correlation images mainly show the distributions of non-uniform textures, while the Energy and Homogeneity images mainly show the uniform and regular textures. Compared with the first-order statistic characteristics Mean and Entropy, the GLCM parameters are related to the location of the pixels, which can be used to reveal detailed local structural features in sliding window method. Obviously, this wide FOV polarimetric method may help doctor screen pathological structures quickly and provide numerous texture information for diagnosis.

4. Conclusion

In this study, we applied the Mueller matrix microscope developed in our previous studies on two tissues of distinct typical fibrous structures: healthy breast duct tissues and DCIS tissues. The MMPD δ and θ parameters images under 4×/NA 0.1 and 40×/NA 0.65 objectives of the unstained tissue slices were calculated and analyzed. Then, the mean values and a group of image texture feature parameters of the δ and θ images under high and low imaging resolutions were compared quantitatively. For the MMPD δ images, we can see that the fibers density information contained in the texture features Mean, Entropy, Contrast, Energy and Homogeneity are preserved well with the decline of imaging resolution. It suggested that when using features of MMPD δ images to distinguish between different fibrous structures according to their density distribution, the relatively low resolution images may serve as a better choice, which can provide similar information as high resolution images together with fast imaging speed and wide FOV. However, for the MMPD θ images, it was demonstrated that the fibers’ azimuthal orientation information, which are closely related to the spatial position, derived from the features of high resolution images cannot be completely obtained from relatively low resolution images. Thus, imaging resolution is crucial to improve the accuracy for the polarimetric detection and staging method based on the azimuthal orientation to recognize fibrous structure in tissues. Although statistical analysis on more tissue samples are still needed, the results here indicate that the information of scalar linear retardance is preserved under different image resolutions, contrary to the azimuth of optical axis. Since the imaging speed is also important for clinical pathological detections, the results provide an important criterion to decide which information of fibrous structures can be extracted accurately using transmission Mueller matrix microscope with low numerical aperture objectives to assist quick scanning diagnose clinically such as breast ductal carcinoma.
Funding
National Natural Science Foundation of China (61527826); Shenzhen Fundamental Research and Discipline Layout Project (JCYJ20170412170814624); Overseas Research Cooperation project of Tsinghua Shenzhen International Graduate School (HW2018005).

Disclosures
The authors declare that there are no conflicts of interest related to this article.

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