Depth selectivity for the assessment of microstructure by polarization studies

Xu Feng,* Liqun Sun, and Enyao Zhang

State Key Laboratory of Precision Measurement Technology and Instruments,
Tsinghua University, Beijing, 100084, China

*xfeng89@hotmail.com

Abstract: A polarimetric imaging system capable of continuously selecting imaging depth in a turbid media is demonstrated. The proposed system is based on the orthogonal polarization spectral (OPS) technique, and is able to detect microstructure and microvessel. First, we compare the performance of four polarization imaging channels on a biological phantom, and find that there is a linear relation between the degrees of ellipticity and image contrast in co-linear/co-elliptical channels. In addition, the cross-linear channel has the best image contrast. We then prove the performance of depth selectivity of microvessel in a mouse ear.

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1. Introduction

Microcirculation is the blood circulation between arterioles and venules. Adequate microcirculation is vital for the transport of oxygen and other nutrients, and for the removal of body waste. A traditional way of imaging microvessels is known as intravitral microscopy, a golden standard that allows for accurate detection of capillary and vascular morphology in vivo. However, the complicate surgery preparation and the need of fluorescence make it inconvenient for the assessment of human microcirculation [1,2]. Capillaroscopy was once a popular non-invasive in vivo assessment method [3,4], but its limitation is that it can only detect the peripheral regions of the tissue, especially the nail bed. Besides, sometimes fluorescence is needed to improve image contrast. During these years, Orthogonal polarization spectral (OPS) imaging [5], and its successor Sidestream dark-field (SDF) imaging [6] have become two important mainstream imaging methods based on similar principles. Results from several medical centers have shown that they have comparable performance [7]. OPS imaging is widely used to provide a semi-quantitative analysis of the microcirculation of several organs, including the sublingual region, liver, kidney, brain, et al [8–12]. It is relatively portable, inexpensive, and does not require the injection of fluorescence to obtain an excellent level of contrast. However, it only provides a two-dimensional visualization of the microvasculature, and is limited to detect photons coming from a deep channel [13–15]. So the cross-section layers between the deep channel and superficial layer can only be detected by mechanically scanning the sample.

In 2003, Morgan et al [16] used linearly and circularly polarized light to produce a polarization-gated image. They imaged an absorbing object at three different depths, and the results proved that the cross-linear polarization gate can penetrate as deep as 22 mean free paths (MFP), the co-circular polarization gate can reach 10 MFP, and the co-linear polarization gate is only 2 MFP. This proved the “polarization memory effect”, a conception introduced by MacKintosh et al [17]”, in which they said the depth detected by circular polarization is larger than that by a linear polarization. In another experiment they imaged a lentigo in four polarization channels. By adopting polarized-light subtraction, they are able to extract images that are free from surface reflections. The “polarization memory effect” was further developed by Monte Carlo simulations in a semi-infinite medium [18]. The degrees of polarization (DOP) for linear, elliptical and circular polarized light are measured under different MFPs. The simulation results show that linear polarized light becomes to depolarize only after 2 MFP, elliptical polarized light maintains its state of polarization under longer MFPs, and circular polarized light can keep a high DOP even after ten scattering events. They also found that elliptical polarization can be tuned between linear and circular polarization to reach different penetration depths. In 2011 Silva et al proved that the average depth into which elliptically polarized light can penetrate is between that of linearly and circularly polarized light [19,20]. They inserted a ruler in the liquid phantom with an angle, and tested the maximum visible depths under different degrees of co-elliptical polarization. As the angle
increases from 0° to 30°, the maximum visible depth increases from about 0.3MFP to 1MFP (MFP = 2.8mm).

Although the elliptically polarized light is proved to have the ability of depth selectivity in previous studies, the system fails to detect microstructure and microvessel. In [19] an in vivo experiment is performed on the cortex of a living mouse to test the feasibility of the method for the assessment of blood vessels, but it has the following problems: (1) The pixel size of the image is 42μm, so it is unable to detect microvessels with diameter much smaller than this value. (2) Background noises, coming from the light source and the specular reflection from the sample, are not well blocked out. (3) The central wavelength of the interference filter is not matched with the absorption spectra of the erythrocyte, which would greatly affect the imaging performance of microvessel. (4) The imaging performance of the four channels needs to be better explained and compared.

Here we adopted the approach proposed by [16,19], and extended it to the study of microstructure and microvessel. We also compared four polarization imaging channels and found the best channels for depth examination. Our proposed scheme has several advantages. (1) We take advantage of the OPS technology to obtain images with good contrast and micrometer resolution. The experimental setup efficiently blocks out background noise. (2) We can refocus the sample into different depths by simply tuning the state of polarization. (3) Subtraction of imaging channels is not necessary in our proposed scheme. (4) There is no need for mechanically scanning the sample.

2. Materials and methods

2.1 Polarization channels and imaging principle

Morgan’s theory indicates that there are four polarization imaging channels as follows:

Channel 1: Cross-linear
Channel 2: Co-linear
Channel 3: Co-circular
Channel 4: Cross-circular

Silva et al extend channel 3 and 4 to co-elliptical and cross-elliptical, respectively. The category of the emerging photons in the adapted four channels is summarized in Table 1.

| Channel | Illumination | Detection | Category of the emerging photons |
|---------|--------------|-----------|----------------------------------|
| 1       | Linear       | Cross-linear | Multiple scattered photons       |
| 2       | Linear       | Co-linear  | Surface-reflected photons, polarization-maintaining photons, multiple scattered photons |
| 3       | Elliptical   | Co-elliptical | Multiple scattered photons, polarization maintaining photons |
| 4       | Elliptical   | Cross-elliptical | Surface-reflected photons, multiple scattered photons |

Channel 1 is the experimental setup of OPS system (Fig. 1), in which the illumination is under linear polarization and the detection is under cross-linear polarization. In this circumstance, the polarization-maintaining light, including surface reflected light as well as light that undergoes small numbers of scattering events, are eliminated by the orthogonal analyzer. As a result, photons passing through the analyzer are mainly depolarized multiple scattering ones that come in large proportion from deep locations.

In channel 2 the detection is under co-linear polarization, so both the surface-reflected, polarization-maintaining and multiple scattering photons can be detected. Some researcher uses the subtraction of polarization images to obtain images of superficial tissues [16,21,22]. Since the multiple scattered light is randomized polarized, contributing equally to channel 1 and 2, the subtraction of channel 1 from channel 2 can eliminate the effect of backscattered photons and filter superficial backscattered photons. But image subtraction costs time, and the
resulting channel images suffer from a low signal-noise-ratio (SNR). In our proposed scheme, it is proved that without image subtraction the function of depth examination can also be realized.

Fig. 1. Principle of OPS imaging.

Channel 3 and 4 are under elliptically polarized illumination. In channel 3, the detection mode is under the same helicity as the illumination mode, while in channel 4 the helicity is reversed. When $\theta = 0^\circ$ channel 3 is the same as channel 2. As $\theta$ increases to $45^\circ$, channel 3 and 4 both become circularly polarized. It should be pointed out that co-circularly polarized light may have the same imaging performance as the cross-linearly one, mainly because when the propagation direction reverses, left-circular polarization would be changed into right-circular polarization, and vice versa [23]. So under co-circular channel a same polarizer can be used to block out specular reflection.

There are two types of emerging photons in channel 3: multiple scattered and polarization maintaining photons. But in channel 4 are multiple-scattered and surface reflected photons. It can be expected that in channel 4 the strong surface reflected light would overwhelm the weak backscattered light, so this channel would focus more on superficial information.

Unlike former studies in [19,20], here we use an appropriate spectrum for the imaging of microcirculation. In the visible and near-infrared regions of the erythrocyte absorption spectra, there are several isosbestic points: 522, 548, 587, 584, 805nm, where the molar extinction coefficients for HbO$_2$ and Hb are equal [24]. After taking into consideration the absorption efficiency, scattering depth and SNR, we finally choose 532nm as the central wavelength for illumination.

2.2 Experimental setup

The experimental setup is built by adapting an existing optical microscope (Fig. 2). A 100W tungsten-halogen light lamp was used as a light source. Two interference chromatic filters were used for illumination of different samples, $\lambda_1 = 633$nm and $\lambda_2 = 532$nm (FWHM = 10nm). The incident light passes through a linear polarizer and a quarter-wave plate, and is backscattered and analyzed with another quarter-wave plate and linear polarizer. A microscope objective with a magnification of 10 $\times$ and a numerical aperture of 0.25 is used. Two planar array CCD cameras (QHYCCD, China) were used for detection: CCD QHY6 (16 bit, 795 $\times$ 596 pixels, pixel size 6.5$\mu$m $\times$ 6.25$\mu$m) and IMG2S (14 bit, 1360 $\times$ 1024 pixels, pixel size 6.45$\mu$m $\times$ 6.45$\mu$m).

We use the polarizer/quarter-wave plate couple in the illumination path to generate different elliptically polarized illumination, by adjusting the angle $\theta$ between the fast axis of the quarter-wave plate and the linear polarizer axis. We then use another polarizer/quarter-wave plate couple for detection. The degree of ellipticity $\theta$ in the detection path is set the same as in the illumination path. So for each measurement, we need to tune the two polarizer/quarter-wave plate couples to obtain our desired state of polarization for illumination and detection.
Fig. 2. Sketch of the experimental setup.

At first we set $\theta = 0^\circ$ to obtain channel 1 and 2 images. In this circumstance, the two quarter-wave plates were not used. Next we tuned $\theta$ from $0^\circ$ to $45^\circ$ continuously to obtain a desired state of co-elliptical polarization (channel 3) for illumination and detection. Finally we inversed the direction of rotation to get images of channel 4. Images are cropped to proper dimension. For statistical analysis, the exposure time of the CCD was fixed, yet for display the exposure time of the camera was adapted to keep a high SNR.

2.3 Sample description

We conducted the experiments on two types of samples. Firstly, we used a piece of pork fat as a biological phantom. A hair (diameter, 92 $\mu$m) was inserted with a small tilt angle inside the fat. The hair in our field of view has a length of 548 $\mu$m, and a depth range of 0.16mm, so the estimated tilt angle is 16.3°. The reason we chose a hair as a target was that it was a strong absorbing object, and that its size was small enough in our system, so that we could use it for depth investigation in four imaging channels. The illumination light was filtered at 633nm. The detectors were CCD QHY6 and IMG2S with a fixed exposure time of 31ms and 100ms, respectively. Two areas of interests are detected in the following two experiments (Fig. 3 and Fig. 4). The former one was used to compare the performance of channel 3 and 4, while the latter one was to examine depth selectivity of channel 3.

Secondly, a four-week old male mouse was chosen for imaging the microvessel in the mouse ear in different polarization channels. Immersion oil was dabbed on the mouse ear to prevent it from drying, and a microscope coverslip was gently pressed on the ear to create a flat surface. Here, incident light with a central wavelength of 532nm was used for illumination, and CCD QHY6 with an exposure time of 47ms was used for detection.

3. Results

The above two samples are imaged under four channels, and the relation between image contrast and the state of polarization are examined and analyzed.

Some results obtained in biological phantom are shown in Fig. 3. It can be seen that channel 2 mainly collects photons in superficial layers, where the hair and the fat show strong specular reflection effect. There is a clear boundary at the point where the hair starts to enter the pork. Specular reflection is also displayed in channel 3 (from $\theta = 0^\circ$ to $20^\circ$), and in all the images in channel 4. Especially, as $\theta$ increases specular reflection is gradually eliminated in...
channel 3, and the boundary at the middle of the image becomes blurry, indicating photons in deeper layer become more and more evident. In other words, the multiple scattering photons account for a larger proportion from co-linearly to co-circularly polarized illumination. We also find that channel 1 has the richest tissue information coming from deeper layers, and it also has the best SNR among all the channels under the fixed exposure time of the CCD camera.

![Images of polarization images obtained at different degrees of ellipticity for biological phantom experiment.](image)

**Fig. 3.** First two row images: selected polarization images obtained at different degrees of ellipticity for biological phantom experiment. In this experiment, the hair is half exposed to the air and half inside the phantom. Imaging channels 2-4 are measured. The angle value measures the angle $\theta$ between the polarizer and the quarter-wave plate. Third row image: relation between image contrast and the corresponding $\theta$ in imaging channel 3 and 4. Pixel size 6.5$\mu$m x 6.25$\mu$m.

We use “contrast” to quantify the degree that a target could be discriminated as follows:

$$\text{contrast} = \left( I_{\text{background}} - I_{\text{signal}} \right) \bigg/ \left( I_{\text{background}} + I_{\text{signal}} \right)$$

where $I_{\text{signal}}$ and $I_{\text{background}}$ are the average grayscale on the hair and the pork, respectively. The numerator takes the difference between the two measured zones, and the ratio cancels out the common attenuation factor.

The third row image in Fig. 3 demonstrates that there is a positive relation between ellipticity $\theta$ and contrast in co-linear/co-elliptical polarization. Contrast increases along with $\theta$ except for $\theta = 40^\circ$. However, in channel 4 there is not such a relation, as contrast remains the same at $\theta = 10^\circ$, $15^\circ$, $25^\circ$ and $30^\circ$ but reaches the minimum value at $\theta = 20^\circ$ and $40^\circ$. Contrast in channel 1 is 0.0579, which is much larger than that in the rest three channels, so it is not shown in the figure.
Overall, channel 3 has relatively better image contrast than channel 4, and it shows a clear relation between contrast and the degree of elliptical polarization. The phenomenon is consistent with the analysis in Section 2.1 that channel 4 focuses on superficial information. So we will not discuss channel 4 in the following paper.

The second experiment illustrates depth selectivity of channel 1-3 (Fig. 4). The change from specular reflection to multiple scattering is similar to the above experiment, so we will not discuss again. Here, we extract the grayscale along two dashed lines: grayscale on the hair (white line) and on the background (black line). The relation between ellipticity θ and contrast is displayed in Fig. 4, where the three curves represent three imaged positions: the first, middle and last 20 pixels along the Y axis. To reduce noise, grayscale at each position is the average grayscale of the 20 pixels.

It is understandable that the hair inserted at a shallower layer (larger pixel value) is more visible. A more important fact is that if we only focus on each curve, we can find that from linearly to circularly polarized illumination, image contrast gradually becomes larger, which means it becomes easier to discriminate a target at that position. Suppose that contrast = 0.04 is a threshold for a good display, then for the hair located at the first 20 pixels, images with θ≥12° could reach the goal. However, for the hair located at the middle and the last 20 pixels, θ needs to be at least 26° and 45°, respectively. In other words, only light with larger degree of elliptical polarization could reach a deeper layer. This phantom experiment proves that a continuous depth examination can be realized by tuning the state of polarization. Besides, it can be seen from the last two points on the three curves that co-circularly polarized illumination can rival cross-linearly polarized illumination from the aspect of imaging depth.

Fig. 4. First two row images: polarization images in channel 2 and 3. The hair is fully inserted with a tilt angle of 16.3°. Left bottom: channel 1 image. X and Y axis indicates the pixel numbers. The hair is inserted in a shallower layer as the pixel number along the Y axis increases. Right bottom: comparison of contrast in three regions under the corresponding θ. Pixel size 6.45μm × 6.45μm.

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In this experiment, we also found that image subtraction is unnecessary. According to previous researches, there are two ways of image subtraction [16]: (a) direct subtraction, (b) normalizing the image by the total intensity before subtraction. We used method (a) for contrast analysis, but because the grayscale of the background in channel 1 is much larger than that in the other channels, a subtraction of channel 1 from channel 2 can lead to grayscale inversion. Under this circumstance, contrast of the subtraction image is a negative value. This contradicts our definition that the target is invisible if contrast<0. We then used method (b) for a similar analysis, but it was turned out that normalization was not very effective. Finally, we used images without subtraction, and the results show that even without subtraction there is a linear behavior between imaging depths and the degrees of ellipticity in channel 3.

Next, we test the feasibility of this method by imaging the auricle microvessel of a nude mouse. Images are acquired at 532nm with a detection area of 770μm, shown in Fig. 5. There are a network of blood vessels, including one large vessel, and several small ones. Imaging channel 1 has the best SNR, and contains the most various vessel details, while channel 2 mainly focuses on the superficial layer. In channel 3, from $\theta = 0^\circ$ to $20^\circ$ the vessels becomes more visible, and the incident light penetrates deeper into the tissue, but as $\theta$ grows larger, the discrepancy is not very evident.

Two microvasculature zones are chosen for analysis: zone A is a large microvessel on a main branch, and B is a smaller one. We also analyzed the relation between contrast and ellipticity $\theta$ in these two zones by using Eq. (1). A linear behavior is found in zone A when $\theta$ is between $0^\circ$ and $20^\circ$, while in zone B $\theta$ is between $5^\circ$ and $25^\circ$. The growth rate is also different in two zones. Unlike the above biological phantom, which has a quite uniform absorption pattern in different layers, the auricle vessels are much more complicated due to the random distribution of erythrocytes in the tissue.

It should also be noticed that co-circular polarization does not perform as well as that in the biological phantom experiment. A reasonable reason is related to the category of photons in the co-circular channel. In co-circular channel the polarization-maintaining photons are the dominate ones. These photons come from the middle layers of the tissue, and the DOP can be maintained for 10 MFPs. The value of MFP in biological tissues is much smaller than that in biological phantoms, mainly because the anisotropy factor of erythrocytes could be as large as...
0.995 [25]. A more specific study about the optical properties of the sample need to be further investigated.

Finally, for a continuous depth examination ellipticity $\theta$ should be within a proper range, such as $0^\circ$ to $25^\circ$ here. The subdivision of angle can further improve axial resolution.

4. Conclusion

In this paper, we testify the feasibility of depth examination by tuning the state of polarization in four imaging channels, based on an OPS imaging system. Experiments were conducted on a biological phantom and the auricle microvessels of a mouse. The results show that in the co-linear/co-elliptical channels the imaging depth, characterized by image contrast, has a linear relation with the degrees of ellipticity. Besides, the cross-linear channel has the highest SNR. The proposed method can be used for microscopy sectioning of microstructure and microvessel without mechanically scanning the sample. Further work includes modeling of erythrocyte in turbid media, and to find the exact depth and the range of ellipticity suitable for the assessment of microcirculation.

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