PROCEEDINGS OF SCIENCE

Polarimetric characterization of collagenosis tissue samples

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Polarimetry is one of the emerging optical modalities for implementation in diagnostic. Polarimetric measurements are mostly informative about structural changes in the tissue. Collagenosis diseases affect connective tissue and arise from changes in collagen structure or metabolism. Their diagnosis is quite challenging, since the symptoms are not unambiguous. Considering that collagen is responsible for most of the skin structure, we investigated the feasibility of polarimetry for assessing tissue samples for different collagen degenerative diseases. We will present the result of histology tissue slides evaluated through Stokes polarimetry in transmission geometry.

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1. Introduction

In the recent years polarimetry is imposing as one of the diagnostically valuable optical tools for identification of skin pathologies. Most of the efforts for its clinical application are towards diagnostics of cancer. Two are the main approaches for application of polarimetry for tissue evaluation - Stoke's and Muller matrix polarimetry [1].

Detection of pathological changes in skin tissue sections was reported by Rovira et. al [2]. Also Ahmad et al. [3] demonstrated differentiation between cancerous and healthy tissue within a single tissue slide, based on their depolarization properties. The development of method for diagnosis through Stock's polarimetry is based on proof of principle for the in vivo application for the detection of skin cancer [4] followed by further development of the method for clinical applications[5, 6].

However in order to unravel additional aspects of the full diagnostic potential of this technique we propose a study on the possibility of differentiation between collagen related skin degenerative diseases, such as lupus erythematosus, psoriasis and syndrome of Reynaud. In the clinical practise the accurate diagnosis of those diseases is challenged by their various manifestations, which are often ambiguous. The accurate and timely diagnosis is essential for the treatment outcome and the patients quality of life. Currently this requires numerous hematological and immunological tests, and histological investigation of skin samples.

Hence a more robust method for diagnosis in real time would be beneficial for better health care. Polarimetry has proven useful for the diagnosis of cancer alterations in the skin, which often affect the collagen and extracellular matrix - similarly to the skin degenerative diseases. Based on this similarity we expect to be able to differentiate between skin tissue slides of different pathologies through different polarimetric parameters.

2. Materials and Methods

The measurement were performed through an experimental set up in transmission mode. The light source is diode laser emitting at 635 nm, to improve the intensity profile of the beam a pinhole is used before linear polarizer. In order to manipulate the polarization state of the beam a halve-wave plate and a quarter wave-plate were included before the sample holder. The sample holder is specifically designed to accommodate standard microscopy glass slides. Afterwards a microscopy objective with 10x magnification was placed in order to collect the scattered light and acuier better detection through commercially available a polarimeter (PAX1000VIS/M, ThorLabs Inc.). The output data of the polarimeter consists of Stokes vectors S_1, S_2 and S_3 measured in real time, and normalized to S_0, which represents the total power of the detected light. The vector S_1 describes the vertical and horizontal linear polarization state of the detected light. The linear polarization at 45° and –45° is accounted through the values of S_2 and S_3 is a measure of left and right circular polarization. Via the obtained values of Stokes vectors the degree of polarization (DOP) could be calculated with:

$$DOP = \sqrt{\frac{S_1 + S_2 + S_3}{S_0}}. \quad (1)$$

Additionally the degree of linear polarization (DOLP) is estimated with the equation:

$$DOLP = \sqrt{\frac{S_1 + S_2}{S_0}} \tag{2}$$

and analogously DOCP - degree of circular polarization:

$$DOCP = \sqrt{\frac{S_3}{S_0}}. \tag{3}$$

The samples included in this study are histology tissue slides. The standard procedure for their preparation consists of: tissue fixation with submerging in 10% neutral buffered formalin; dehydration; clearing with organic solvent; embedding in paraffin wax; sectioning to tissue slides with thickness around 4-5μm; mounting on microscopy glass slides; removing the paraffin; staining (typically with heamotoxylin and eosin); application of mounting media and coverslip. The whole process is followed up until the staining. Since our goal is to obtain contrast between healthy and pathological tissue based on their polarimetric properties we omit the staining. Additionally both heamatoxylin and eosin demonstrate high absorption of light in the range of 400 - 700 nm and 450 - 550 nm respectively [7]. Although the paraffin could protect the tissue slide from damage, it was removed since in our preliminary work it demonstrated high affect on the polarization state of the incident light.

Samples of three degenerative skin diseases were evaluated: lupus, psoriasis and syndrome of Reynaud. Two polarization states of the incident light were used: linear polarization at 45° and circular polarization. For every sample tree areas were measured. Every measurement consists of continuous detection of 1000 values of the Stokes's vectors and derivative parameters. A total of 3000 values were averaged for every parameter.

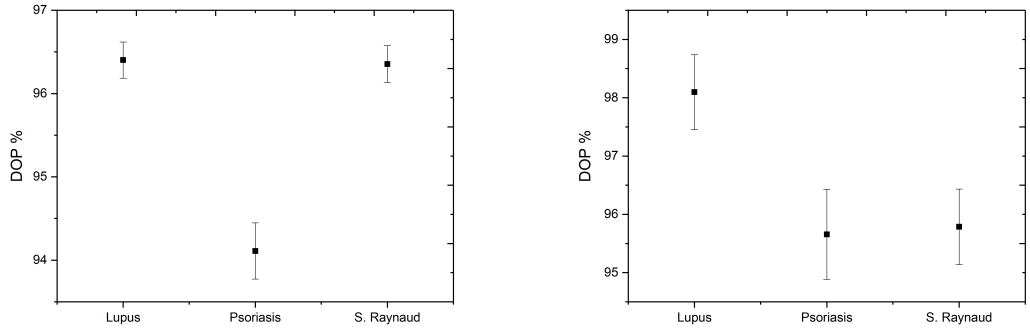
3. Results

The results for DOP for the two polarization states of the incident light are presented on figure 1. For incident light with linear polarization at 45° (figure 1a) the DOP for the sample of psoriasis demonstrates distinguishable value in comparison with the samples of lupus and syndrome of Raynaud.

The DOP of incident light with circular polarization (figure 1b after interaction with sample of lupus is noticeable less affected than after interaction with sample of psoriasis and syndrome of Raynaud.

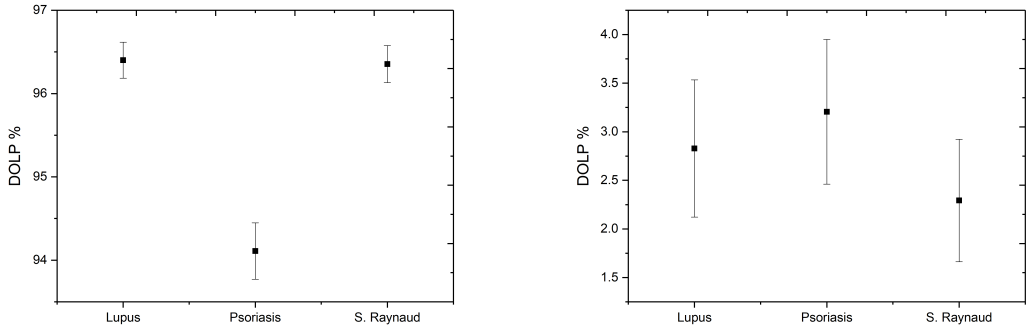
Figure 2 represents the values of DOLP. For the incident light with 45° linear polarization (figure 2a), although the values for lupus and syndrome of Raynaud are similar, the value for psoriasis is distinctive. Despite the fact that the value of DOLP for the sample of syndrome of Reynaud is diffrent than the values for psoriasis and lupus for incident light with circular polarization (figure 2b, their standard deviation are overlapping and we could not recognize this parameter as useful for differentiation.

The values of DOCP for incident light with linear polarization at 45° (figure 3a) are practically indistinguishable. But the value of DOCP of incident light with circular polarization after interaction with the sample of lupus is distinct.



(a) **(b)**

Figure 1: DOP of incident light with 45° linear polarization (a) and circular polarization (b) after interaction with tissue slides of lupus, psoriasis and syndrome of Raynaud.



(a) **(b)**

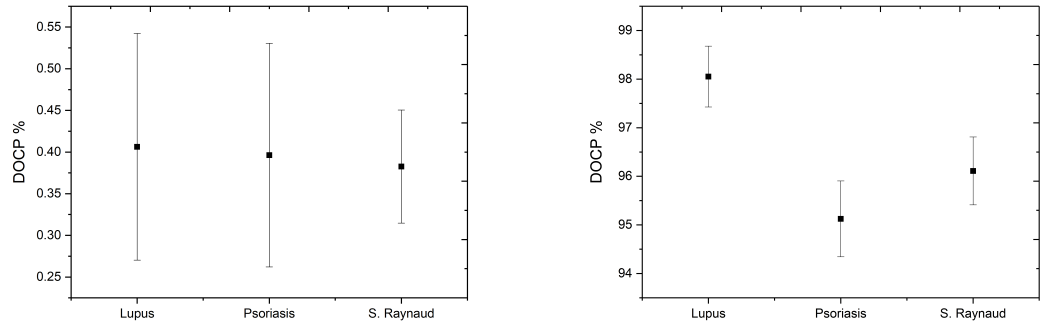
Figure 2: DOLP of incident light with 45° linear polarization (a) and circular polarization (b) after interaction with tissue slides of lupus, psoriasis and syndrome of Raynaud.

Additionally two more parameters were evaluated - ellipticity and azimuth [8]. We have chosen to present the azimuth obtained for incident light with linear polarization at 45° (figure 4a) since it demonstrates a possibility for an interesting trend for increasing value respectively for the tissue slides of lupus, psoriasis and syndrome of Reynaud. Furthermore similar is the observation for the values for the ellipticity for incident light with circular polarization (figure 4b) interaction with samples of psoriasis, lupus and syndrome of Raynaud. However this observations are not explicit.

4. Discussion

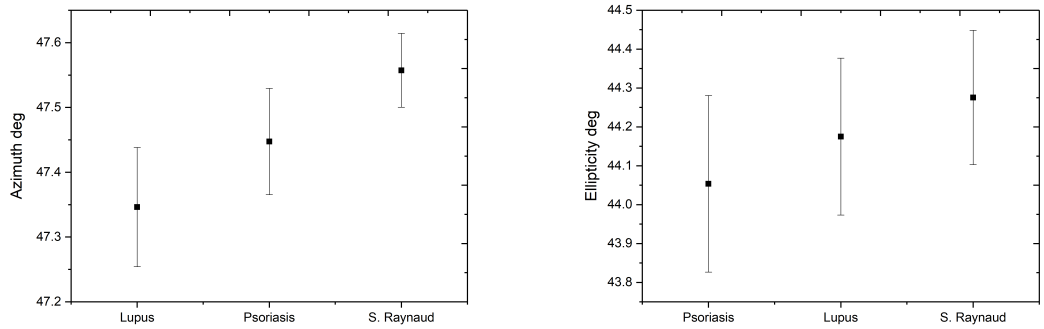
Considering the results presented in the previous section for incident light with linear polarization at 45° highest depolarization is observed by the sample of psoriasis (figure 1a and figure 2a). Typical for this pathology is the abnormal accumulation of collagen in the tissue [9], which is the most likely reason for this peculiarity in its depolarization properties.

For histology skin samples of lupus there are more than 15 specific alterations, which are considered diagnostically valuable. One of the most consistent among the different stages of



(a) **(b)**

Figure 3: DOCP of incident light with 45° linear polarization (a) and circular polarization (b) after interaction with tissue slides of lupus, psoriasis and syndrome of Raynaud.



(a) **(b)**

Figure 4: Azimuth of incident light with 45° linear polarization (a) and ellipticity of circularly polarized light (b) after interaction with tissue slides of lupus, psoriasis and syndrome of Raynaud.

the diseases is follicular hyperceratosis [10]. This is characterised with keratin build-up, which inevitably disrupts the skin architecture and the typical structure of the extracellular matrix, which is the main source of depolarization in tissues. This could be the reason for the lower depolarization observed by the tissue slide of lupus (figure 1b and figure 3b).

Those differences in the depolarization properties of the samples from psoriasis and lupus are manifested with application of incident light with different polarization state and this could be due to the variable orientation of the structures in the samples.

At general the syndrome of Raynaud affects mostly the skin arterioles and causes exaggerated cold-induced vasoconstriction. This however is not explicitly manifested in the skin structures mainly responsible for its interactions with lights polarization state.

5. Conclusion

In this study of the possible application of polarimetric parameters for differentiation between different skin collagen degenerative diseases - namely lupus, psoriasis and syndrome of Raynaud

we could surmise: 1. the psoriasis sample could be differentiated from the other two based on its depolarization properties (DOP and DOLP) in the case of incident light with linear polarization at 45°; the sample of lupus is distinguishable nased on its lower depolarization demonstrated in the case of incident light with circular polarization (DOCP, figure 3b); the sample of syndrome of Raynaud did not manifest any dissimilar values for the observed parameters.

In order to obtain a more robust and diagnostically valuable differentiation we forsee the application of different wavelengths and/or different polarization states of the incident light.

Acknowledgments

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