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Visualization of pathologic changes in liver tissue via polarized light

ANI STOILOVA[1, 2*], BLAGA BLAGOEVA[1], DIMANA NAZAROVA[1], ELENA STOYKOVA[1], NATALIYA BERBEROVA-BUHOVA[1], LIAN NEDELCHEV[1, 3], ALEXANDER MACHIKHIN[4]

[1]Institute of Optical Materials and Technologies – Bulgarian Academy of Sciences, Acad. Georgi Bonchev Bl.109, 1113 Sofia, Bulgaria

[2]University of Chemical Technology and Metallurgy, Department of Physics, 8 Kl. Ohridski Blvd., 1756 Sofia, Bulgaria

[3]University of Telecommunications and Post, 1 Acad. St. Mladenov Str., 1700 Sofia, Bulgaria

[4]Scientific and Technological Center of Unique Instrumentation – Russian Academy of Sciences, 15 Butlerova, 117342 Moscow, Russia

[*]Corresponding author: ani_st@web.de

In this work, we applied two polarized light based approaches to visualize histological patterns of liver pathologies. The first one involves acquisition of two images through a polarizing microscope, one image (P_{par}) acquired with the analyzer oriented parallel to the polarization of illumination and the other (P_{per}) acquired with the analyzer oriented perpendicular to the illumination. The final image is based on the polarization ratio, $P_{reconstructed} = (P_{par} - P_{per})/(P_{par} + P_{per})$. Using the second technique, the histological specimens were illuminated with a polarized laser beam with wavelength of 635 nm. Polarimetric parameters as azimuth, angle of ellipticity, degree of polarization and reflected power have been measured to quantify the change in the polarization state of the incident light after interaction with the sample of the healthy tissue and of the tissue with abnormal morphological changes.

Keywords: polarized light imaging, tissue polarimetry, liver tissue.

1. Introduction

Recognition of hepatic diseases under microscope, which is the classically used method, is often not an easy task, as the liver responds to a wide range of injuries in limited number of ways, namely through degeneration, necrosis, circulatory disorder, inflammation, fibrotic scarring, compensatory-adaptive changes or neoplasia [1]. However, certain features of morphological changes in the liver tissue could be of help in differentiating the origin of the injury if they become more clearly visible. One way of optimizing the medical image contrast and spatial resolution involves the use of polarized

light. The polarized light reduces the glare and increases the clarity of structures and shapes. There are different polarization-based techniques developed for improving the quality of medical images. Polarized light imaging relies on illuminating a histological sample with polarized white light and observing it through another polarizer, set orthogonally to the polarization state of the illuminating light [2]. The orthogonal state contrast imaging depends on two polarized images of the sample taken under white light illumination, the first one (P_{par}) taken by rotating the analyzer parallel to the polarization plane of the incident light and the second one (P_{per}) by rotating the analyzer perpendicular to the polarization plane of the incident light. The method was first proposed by JACQUES and LEE (1998) for visualization of skin lesions and was further developed by other research groups for imaging of malignant sections in breast and gastrointestinal tissue samples [3–5]. Polarimetric measurements provide quantitative evaluation of the degree to which the polarized light alters during propagation into the tissue. Measurable effects of polarized light interaction with biological samples as, for example, changes in the azimuth, the angle of ellipticity, the degree of polarization or the light power are used by this approach to map the borders between benign and malignant tissue regions [6–10]. This technique can be easily combined with optical imaging equipment such as endoscopes and microscopes [11]. Therefore, it has been actively developed in the last years for surgical and diagnostic applications [12, 13]. Most of the research works on the topic are dedicated to development of tools, which make the tumor formation detectable earlier and few of them are focused on characterizing pathology due to non-cancerous related diseases [14–17]. In the present study, we have used polarized light for visualization of morphological changes in human liver tissue caused by cancer and non-cancerous related diseases. We experimentally demonstrated the identification of amyloid degeneration, miliary tuberculosis, cirrhosis and hepatocellular carcinoma in liver tissue samples through polarimetric measurements and presented orthogonal state contrast images of histology slides, in which certain patterns of morphological changes, characteristic for the specific disease, are more clearly visible than in the total reflectance images of the samples.

2. Experimental protocol

2.1. Polarized light imaging

In our experiments, a Zeta-20 Optical Microscope (Zeta Instruments) operating with polarized light in reflection mode was used. Objective lenses with 5× magnification and numerical aperture NA = 0.15 were chosen to obtain the images. We used colour images without extracting separately the data in the red, green and blue channels. The final image ($P_{reconstructed}$) is obtained as $(P_{par} - P_{per})/ (P_{par} + P_{per})$. P_{par} was acquired with the analyzer oriented parallel to the polarization of illumination and P_{per} with the analyzer oriented perpendicular to the illumination. Subtraction of P_{par} and P_{per} eliminates the part of the polarized light, which penetrates into the tissue, exhibits multiple scattering events, and exits the biological sample highly or fully depolarized. Normalization by

the sum of P_{par} and P_{per} eliminates sensitivity to variations in the incident light intensity and the surface properties of the sample. Comparison of the results obtained by color extraction methods will be reported separately.

2.2. Optical set-up for measuring the polarization parameters

For determining the azimuth θ, the angle of ellipticity χ, the degree of polarization (DOP) and the light power, we used a standard experimental set-up working in reflectance mode [6], shown in Fig. 1.

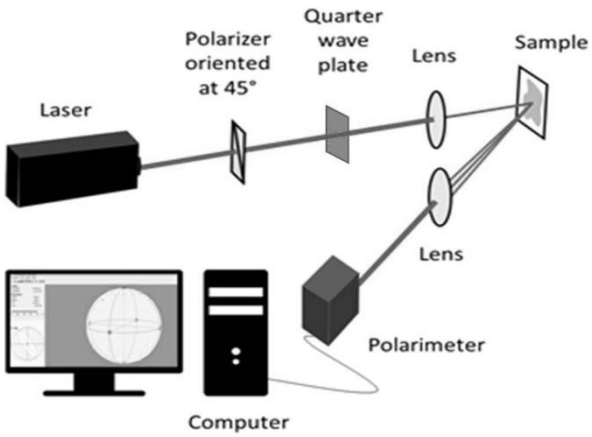

Fig. 1. Optical set-up for measuring the polarization parameters working in reflection mode.

The four Stokes parameters S_j ($j = 0, 1, 2, 3$), completely describing the power and the polarization state of an optical wave, were measured in real time by the polarimeter and visualized through the Poincaré sphere. In this case, the azimuth θ, the angle of ellipticity χ and the degree of polarization (DOP) can be respectively expressed in terms of the Stokes parameters following equations [18]:

$$\theta = \frac{1}{2}\tan^{-1}\left(\frac{S_2}{S_1}\right); \quad -\frac{\pi}{2} \le \theta \le \frac{\pi}{2} \tag{1}$$

$$\chi = \frac{1}{2}\sin^{-1}\left(\frac{S_3}{S_0}\right); \quad -\frac{\pi}{4} \le \theta \le \frac{\pi}{4} \tag{2}$$

$$\text{DOP} = \frac{\sqrt{S_1^2 + S_2^2 + S_3^2}}{S_0} \tag{3}$$

2.3. Tissues samples

Reference tissue samples containing hematoxylin and eosin stained human liver histological specimens were purchased from Konus Italia Group S.p.a. The tissue samples

are formalin-fixed and paraffin-embedded, thinly sectioned, stained and placed between microscope slides.

3. Results

Figure 2 presents the images of the liver tissue samples acquired and reconstructed in accordance with the orthogonal state contrast imaging approach and for comparison

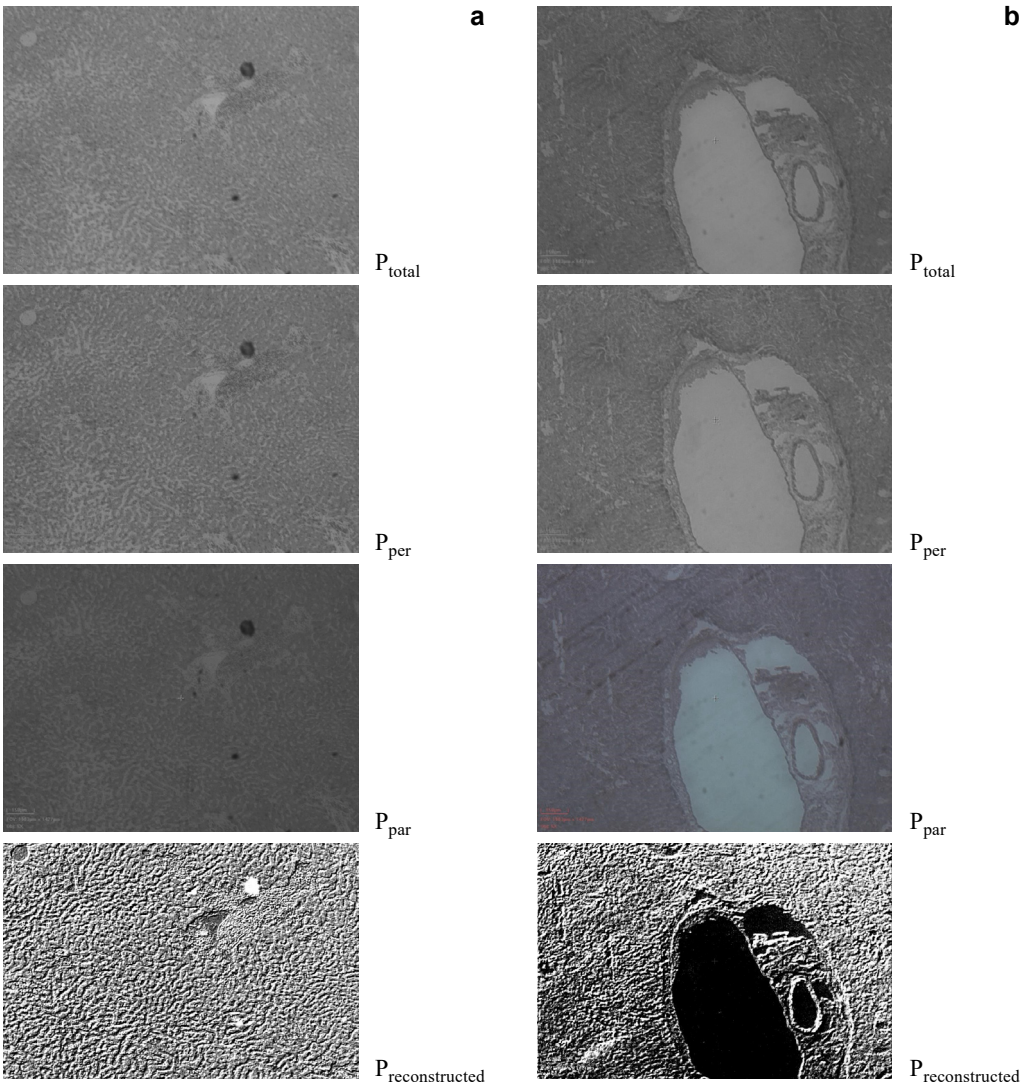

Fig. 2. Pathological changes in human liver tissue due to (**a**) hepatocellular carcinoma, (**b**) amyloid degeneration, (**c**) miliary tuberculosis and (**d**) cirrhosis. Ptotal - total reflectance image, P_{per} and P_{par} – images acquired by rotating the analyzer perpendicular and parallel to the polarization plane of the incident light and $P_{reconstructed} = (P_{par} - P_{per})/(P_{par} + P_{per})$.

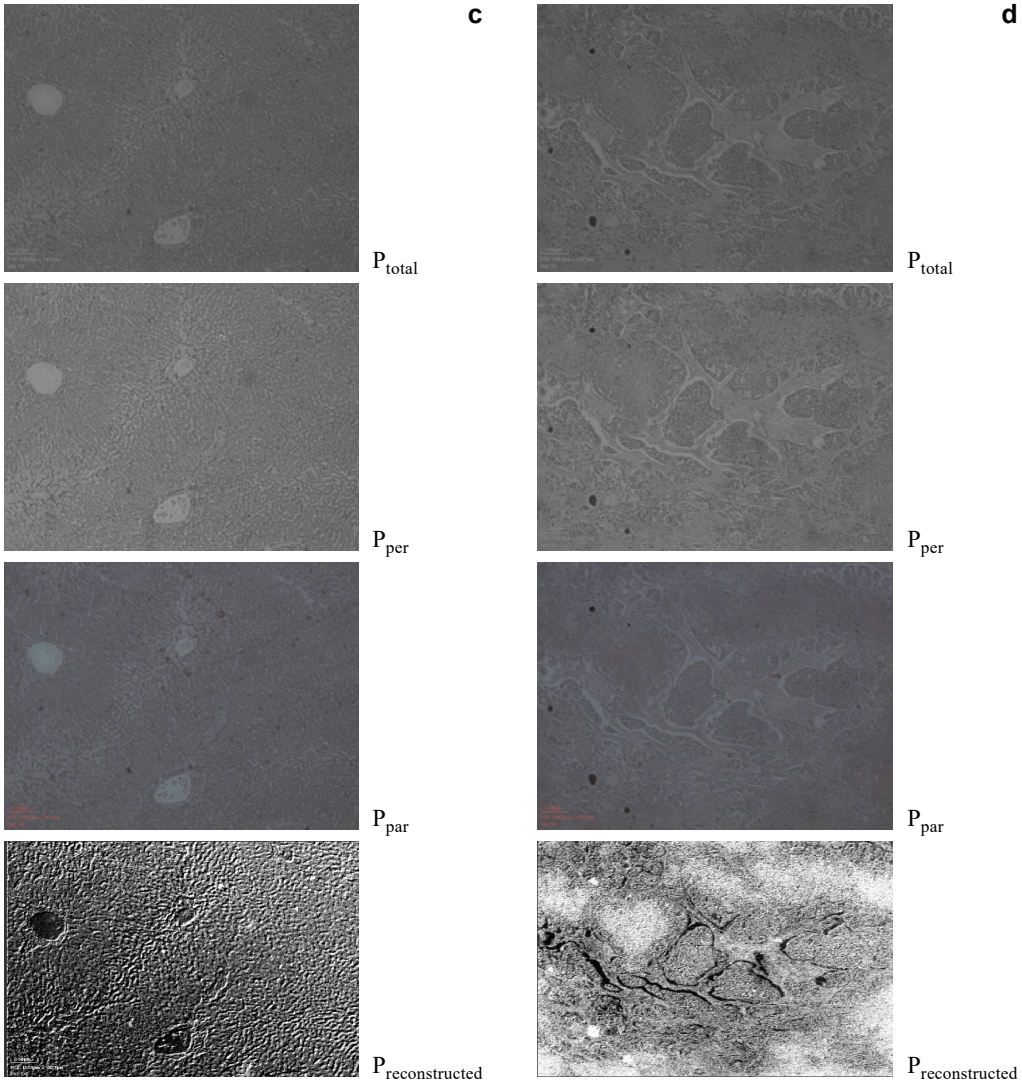

Fig. 2. Continued.

the total reflectance image of the sample. Architectural abnormalities such as vascular invasion, absence of portal tracts and hepatic lobules (**a**), amyloid deposition in portal vessels and hepatic sinusoids (**b**), granulomas with central amorphous granular material (**c**), regenerative nodules of hepatocytes surrounded by fibrous connective tissue (**d**), are visible at better contrast on the reconstructed images.

The results from the polarimetric measurements obtained for the four described above tissue samples are presented in Fig. 3 and compared to the healthy liver tissue.

Figure 3**a** shows a noticeable change in the χ values after interaction of the incident light with the tissue samples. This trend is more clearly expressed for the samples with

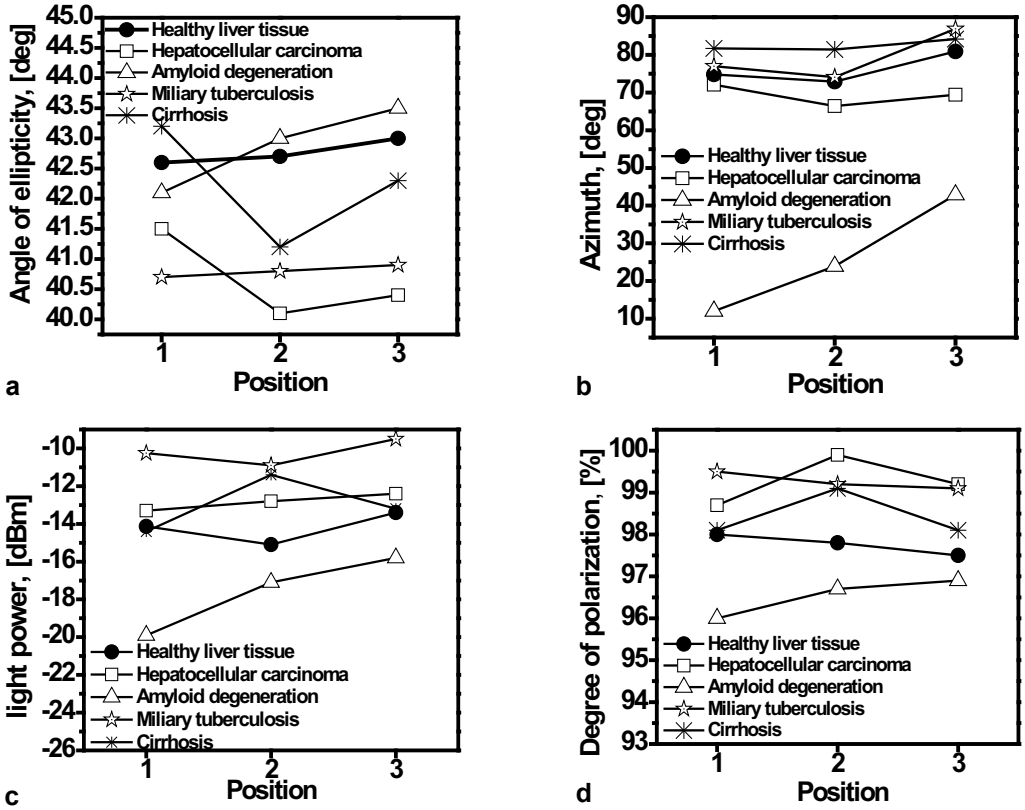

Fig. 3. Experimental results from tissue polarimetric study: (**a**) angle of ellipticity, (**b**) azimuth, (**c**) illuminating power, and (**d**) degree of polarization.

histological findings of hepatocellular carcinoma and miliary tuberculosis. Considering the azimuth in Fig. 3**b**, the values for all the samples are positive and assembled in the large range between 12° and 87°. The change in the value of θ is higher for the sample of hepatic amyloid degeneration. From Fig. 3**c**, it can be concluded that the samples with histological patterns of liver damages, except the sample with pathological changes due to amyloid degeneration, scatter less the incident light in comparison to the healthy tissue. The decrease in the beam power after interaction with the healthy liver tissue sample is significantly higher compared to the samples of hepatocellular carcinoma, miliary tuberculosis and cirrhosis, which means that they are optically more homogeneous than the healthy tissue. Figure 3**d** shows that the healthy liver tissue depolarizes the incident light more than the samples with pathologies, except the samples of hepatic amyloid degeneration. The values of all polarimetric parameters obtained for the histological slide of hepatic amyloid degeneration show larger fluctuations in comparison to the other samples studied. Amyloids are aggregates of peptides or proteins with fibrillar morphology and cross-β structure. They form stable, with high compactness and large

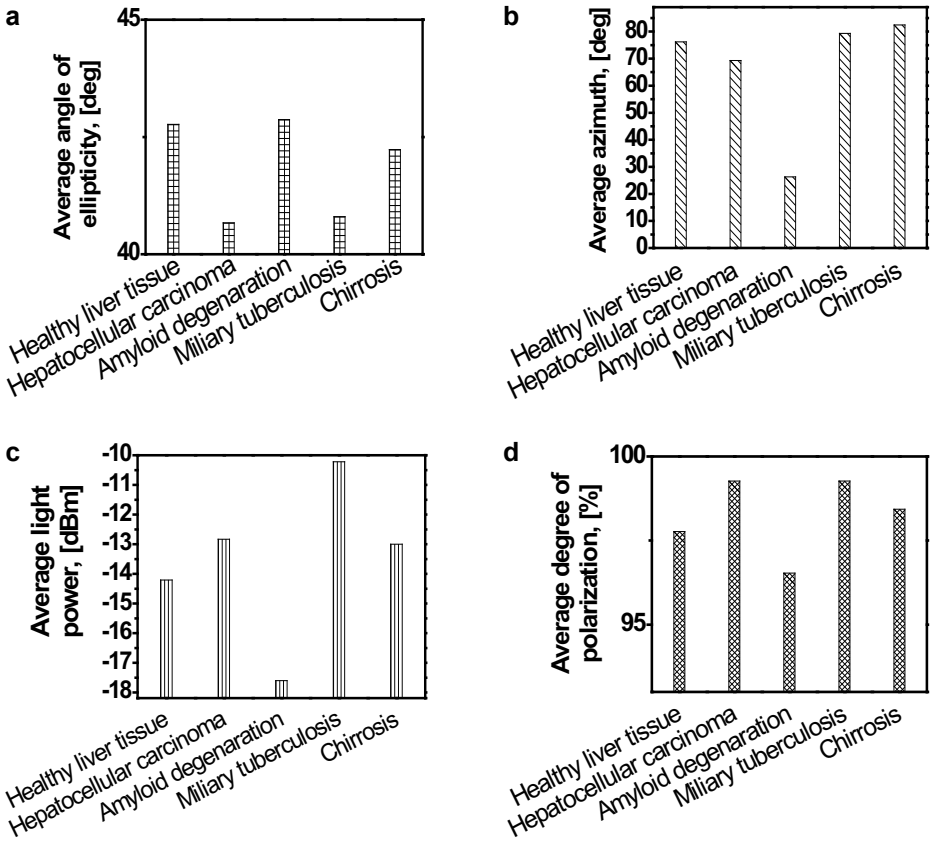

Fig. 4. Average values of the angle of ellipticity (**a**), the azimuth (**b**), the illuminating power (**c**) and the degree of polarization (**d**).

size deposits in the central nervous system or in other tissues including the heart, spleen, liver, and kidney, giving rise to a diverse range of diseases including, for example, type II diabetes, atrial amyloidosis, Alzheimer's disease and Parkinson's disease [19].

Figure 4 presents the average value for each of the polarization parameters obtained from measuring the corresponding sample in the three positions on its surface. Analysis of the angle of ellipticity and the degree of polarization provide little or no information for histological discrimination. The light power and the azimuth indicate applicability for differentiation between healthy and abnormal tissue, as well as between samples with pathologies due to different diseases.

4. Conclusion

In this work, polarized light has been used for enhanced visualization of histopathological changes in human liver specimens associated with amyloid degeneration, miliary tuber-

culosis, cirrhosis and hepatocellular carcinoma. The images acquired and reconstructed in accordance to the orthogonal state contrast imaging approach show higher contrast in comparison to the non-reconstructed images and to the total reflectance image. Tissue polarimetry has been applied to measure the degree to which the polarized light alters during interaction with the healthy and damaged liver tissue. The results have shown that the azimuth and the light power could be used to discriminate among liver tissue pathologies of different origin, although this study is still in its early stage. If the two polarized light based approaches used above are to be compared, it should be noted that the development of some diseases, as cancer for example, starts with abnormal changes being not yet visible microscopically but may alter the light polarization state, which makes the tissue polarimetry an effective tool for early diagnostics.

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Authors' contribution statement
Ani Stoilova – carried out the polarization microscopy, the computer image processing and wrote the manuscript in consultation with Dimana Nazarova, Elena Stoykova, Lian Nedelchev and Alexander Machikhin;
Blaga Blagoeva – performed the polarization parameters measuring;
Dimana Nazarova – conceived and planned the experiments, helped by the computer image processing, contributed to the analysis of the results;
Elena Stoykova – contributed to the design and implementation of the research, supervised the project;
Nataliya Berberova-Buhova – worked out almost all of the technical details;
Lian Nedelchev, designed the study, contributed to the analysis of the results and the writing of the article;
Alexander Machikhin – contributed to the final version of the manuscript, supervised the project.

Conflict of interest
The authors declare that they have no conflict of interest. The authors certify non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
This article does not contain any studies with human participants or animals performed by the authors. The studied in this work human liver histological specimen were purchased from Konus Italia Group S.p.a.

Informed consent
The authors have agreed for authorship, read and approved the manuscript, and have given consent for submission and subsequent publication of the manuscript.

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