Visualizing Healthy and Malignant Tissues via Polarized Light Imaging and Chemical Staining

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Abstract. This study presents optical and chemical methods to visualize healthy and malignant sections of histological samples, by using polarized light imaging and staining with different contrast agents. This approach complements the diagnostic conclusions made by the physicians and improves qualitatively healthy versus tumor tissue differentiation, which may be in practical use for pathologists in their diagnostic conclusions.

Keywords: healthy and malignant tissues, polarized light imaging, histological staining.

1. INTRODUCTION

When tumors are growing, inevitable morphological and biochemical alterations in tissues occur, leading to changes in their optical properties (Novikova et al, 2012). Throughout histological analysis, pathologists rely on their empirically developed abilities to distinguish malignant from non-malignant tissues. Supplementary assistance by physical and/or chemical methods might be helpful for better diagnostic evaluation. After biopsy, tissue specimens of interest are usually placed and fixed on glass substrates to form biological samples, suitable for detailed examination under microscope. Depolarization of light by turbid media, such as biological tissues, is well known optical phenomena, which can be utilized for healthy versus tumor tissue differentiation (Ghosh et al, 2011). Example of such kind of optical technique is the commonly known polarized light imaging, used as assistance for the final diagnostic conclusion (Jacques et al, 2000). Furthermore, tissue staining by using contrast agents enhances the qualitatively healthy-tumor differentiation (Pierangelo et al, 2011). Hematoxylin and Eosin are most widely used for that purpose, which can be shortly denoted as H & E (Wittekind, 2003). H possesses the ability to stain the nuclei (usually in dark blue or violet), while E can stain the cytoplasm (usually in red or pink) (Day, 2014). The aforementioned contrast agents allow better recognition of cells under low magnification during examination under microscope. In this way, pathologists can have better insight about the presence of normal and abnormal cells and other tissue structures, fundamental for the final diagnostic conclusions. In this work, we present hybrid method for visualization and qualitative differentiation between healthy and tumor sections of the tissues by using polarized light imaging and chemical staining.

2. SAMPLES PREPARATION

In our work, *ex vivo* skin samples, provided from UH "Tzaritza Ioanna – ISUL" after approval from the ethical committee of the hospital, were used. Diagnosed and placed on glass substrates by the physicians, all samples contain regions with healthy and tumor sections, originating from *Basal Cell Carcinoma*. A 16 μm samples were obtained, with additional experiments carried, confirming no light depolarization of the glass substrates. Hematoxylin (Fig. 1a) and Eosin (Fig. 1b) have been chosen for contrast agents, in order to locate the histological sections of the samples.

A comparative image of stained and unstained samples is shown in Fig. 2.

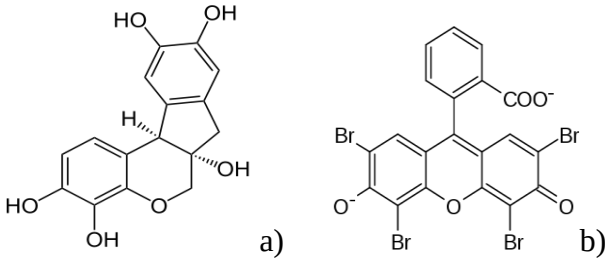


Fig. 1 Chemical structures of (a) Hematoxylin and (b) Eosin (Public domain, Wikipedia).

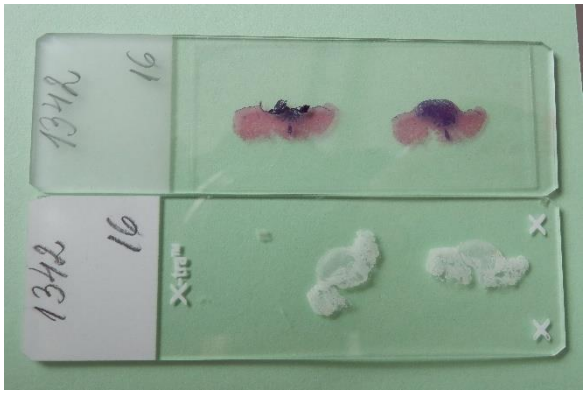


Fig. 2 Comparative image of the histological samples: stained (upper) and unstained (bottom).

In Fig. 2, violet sections represent the tumor sections, while the pale pinkish sections – the healthy zones. This is an example, that staining can facilitate differentiation between healthy and malignant tissues. In Fig. 3 enlarged image of unstained histological sample is shown with its corresponding regions of interests (ROIs) evaluated from the physicians.

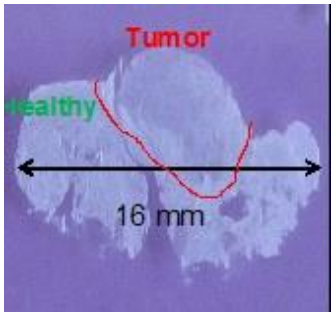


Fig. 3 Comparative image of the histological samples: stained (upper) and unstained (bottom).

Unstained histological sample with its correspondding ROIs (Background color is arbitrary chosen and is not related with the staining colors).

3. POLARIZED LIGHT IMAGING

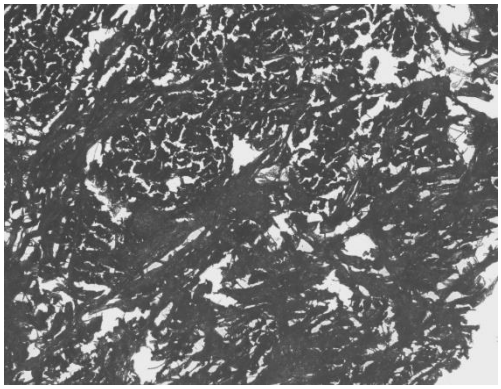
In turbid media with variations in the refractive index on a macroscopic scale, light is subjected to multiple scattering events, alongside changes in the magnitudes of the two orthogonal components of the electric field E_X and E_Y and also altering the phase difference φ between them. These events lead to partial or full loss of light polarization (Ghosh et al, 2011). For thin slabs of tissues transmission experimental geometry may be applicable, but for thick samples and further *in vivo* application, reflection geometry is required. An important contribution to polarized light imaging in medicine (to the best of our knowledge) has been introduced by Jacques et al (2000). Briefly described, such kind of experimental set-up operates in reflection mode and consists of: low coherent light source, in order to avoid laser speckle; a polarizer inserted after the light source and before the sample of interest; afterwards analyzer is placed after the sample oriented either parallel or perpendicular to the former optical element and a CCD camera, positioned after the analyzer. In the work of Jacques et al. (2000) two images are to be obtained: $I_{\parallel}$ (parallel polarizer and analyzer) and $I_{\perp}$ (perpendicular polarizer and analyzer). The photons which penetrate deeply in the tissue, exhibit backscattering processes and exit the biological sample, are subject to more scattering events and will be highly or fully depolarized, contributing more to the $I_{\perp}$ image. Their influence to the final image is undesired and, therefore, should be subtracted from the final polarized image I_{POL}. On the other hand, photons backscattered from the upper surface of the tissue will be less depolarized and will have higher contribution to the $I_{\parallel}$ image. I_{POL} image can be expressed as follows (Jacques et al, 2000):

$$I_{POL} = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \qquad (1)$$

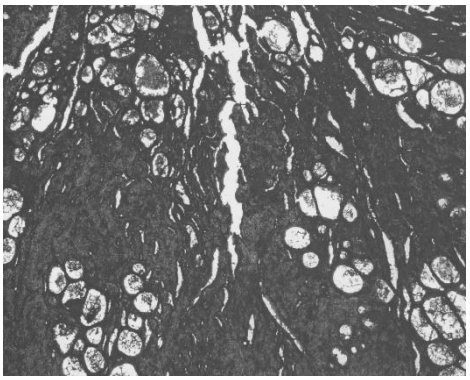
In our experiments, *Zeta-20 Optical Microscope (Zeta Instruments)*, operating in reflection mode, was used. The light source is high intensity LED with user controllable linear polarizer and analyzer. Furthermore, objective lenses with 5x magnification and numerical aperture NA = 0,15 were chosen to obtain the images presented in Fig. 4. All images have been converted to gray scale and processed in accordance to Eq. 1. In Fig. 4, images a), c) and e) correspond to the healthy section of the skin sample, while b, d) and f) correspond to the tumor section. Images obtained with parallel polarizer and analyzer refer to a) and b), while c) and d) refer to perpendicular polarizer and analyzer. The final images after processing are e) and f) and can be expressed as:

$$e) = \frac{a) - c)}{a) + c)} \tag{2}$$

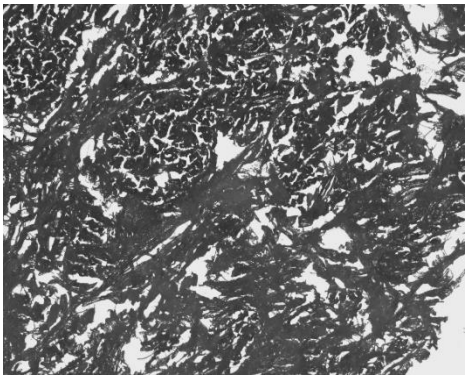
$$f) = \frac{b) - d)}{b) + d)} \tag{3}$$



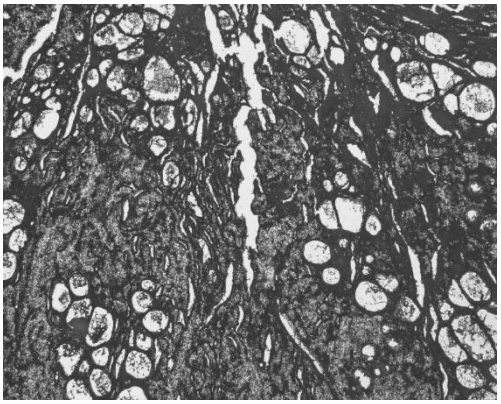
a) healthy - $I_{\parallel}$



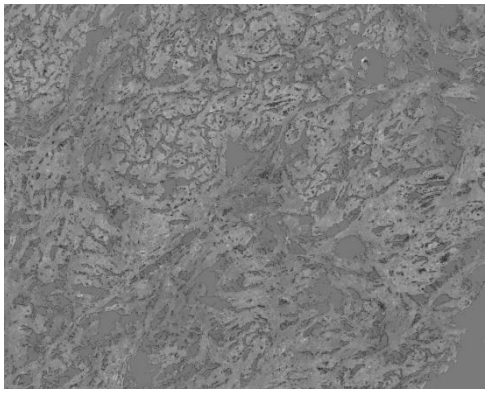
b) tumor - $I_{\parallel}$



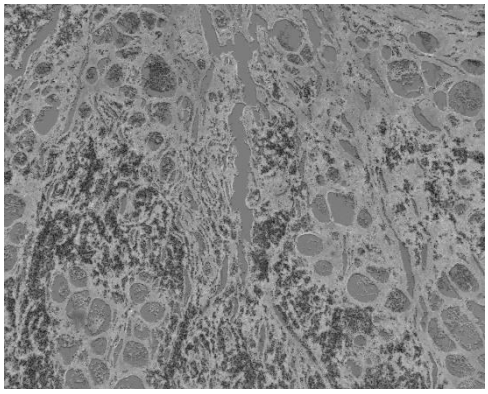
c) healthy - $I_{\perp}$



d) tumor – $I_{\perp}$



e) healthy – I_{POL}



f) tumor – I_{POL} .

Fig. 4 Images of the unstained skin sample.

It is very difficult to differentiate the tissue structures and their distribution on the glass substrates from $I_{\parallel}$ and $I_{\perp}$ images for both of the histological conditions respectively. All white spots from the images in Fig. 4 a) - d) represent gaps between the tissues and the glass substrates, while the dark regions correspond to the tissue itself. In the final I_{POL} images these spots appear with lower contrast, while tissue sections are brighter, but can be differentiated from the glass sections and, most importantly, more details in the tissue can be distinguished. For example, I_{POL} provide more detailed information especially for the tumor image, pointing out multiple, large clusters, darker than the rest part of the tissue. This is only noticeable after applying Eq. 1 in the image processing procedures. We assume and believe, that these areas have an important meaning and are to be consulted with the clinicians to evaluate their importance and meaning respectively.

4. CONCLUSION

In this work, two different approaches have been used: **i)** staining with contrast agents and **ii)** polarized light imaging. These approaches aim to facilitate pathologists for better diagnostic conclusions, especially when difficulties are arising to distinguish between malignant and non-malignant tissues. The chemical staining is well known and widespread method in a global scale, where in the inventory of pathologists' lab would be definitely included Hematoxylin and Eosin. On the other hand, polarized light imaging in Bulgaria is underdeveloped approach and holds promising perspectives, allowing to reach the following advantages: it is, painless, non-invasive, uses no ionizing radiation and provides qualitative diagnostic assistance on a low and affordable price. The presented polarized images are to be consulted with medical representatives for their better understanding and interpretation.

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CONFLICT OF INTERESTS

The Authors indicate that the article content has no conflict of interests of any party.

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