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Assessment of Tissue Ischemia of Nail Fold Precapillary Zones Using a Fluorescence Capillaroscopy

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ABSTRACT

An optical instrument for nailfold fluorescence capillaroscopy and image registration has been developed. With this instrument, an effect of increasing fluorescence intensity in the spectral range of NADH fluorescence during ischemia was detected.

Keywords: fluorescence, capillaroscopy, imaging, metabolism.

1. INTRODUCTION

Currently, pre-clinical and clinical studies show that the use of diagnostic methods based on detection of fluorescence radiation, can improve the early diagnosis of a cancer and other destructive processes in various organs¹. New designs of instruments and analytical tools for obtaining new diagnostic information are currently intensively developing. However, instruments still require a significant improvement for their large-scale introduction into clinical practice.

Many factors which have a strong impact on the results of fluorescence measurements should be considered. In case of in vivo and in situ measurements, new approaches of analysis and processing of obtained data are required to get reliable diagnostical information. So, fluorescence spectroscopy measurements of the two places of tissue with the same distribution and concentration of fluorophores, but with a different blood volume, will provide significantly different spectra due to the strong impact of blood on parameters of light absorption in spectral range of visible light. Without appropriate data correction of the measurements, the accurate determination of fluorophore concentration and even their ratio cannot be achieved²⁻⁴. For the successful applications of the methods of fluorescence spectroscopy in clinical practice it is important to find out reliable, but easy to use method of the correction.

To solve the task in this study, we have simultaneously applied the measurements of several parameters of biological tissue. Fluorescence and diffuse reflectance imaging techniques^{5,6}, as well as the method of videocapillaroscopy^{7,8} during local occlusion tests were chosen for the approach. Areas of fingers' proximal nail fold was chosen for the investigations. This area is easily available for visual control by the videocapillaroscopy method to quantitatively assess the parameters of blood flow as well as fluorescence intensity of adjoining precapillary zones. The areas are of particular interest to in vivo tissue gaseous exchange investigation. Such measurements provide comprehensive diagnostic information with the possibility of correction of the recorded fluorescence data. It makes it possible to study the effects of delivery and consumption of oxygen in vivo and in situ, as well as the impact of blood flow on metabolism of living biological tissues.

Thus, the aim of the study was investigation of respiratory chain parameters by recording NADH fluorescence intensity of nail fold epithelial tissues supplemented by simultaneous tissue reflectance measurements during local tissue ischemia.

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2. MATERIAL AND METHODS

In this study, the area of the proximal nail fold of the middle finger was illuminated by LED light source with a 365 nm central wavelength (power ~2 mW) and a broadband halogen source HL-2000 (Ocean Optics, USA, 360-1000 nm, ~7 mW). A high-aperture microscope objective with a numerical aperture of 0.12 and a projection long-focus objective were used to form the scaled-up image on a monochrome CCD sensor. Filtering of fluorescent images was carried out by using bandpass optical filters (Fig.1).

To induce tissue ischemia and dynamically monitor the changes of fluorescence intensity, the occlusion tests was conducted (by imposition of the cuff on the brachial artery). Thus, the artificial state of ischemia was created. NADH molecules formed in the sixth glycolysis reaction, depending on the presence of oxygen, have two paths of further transformation. They can either stay in the cytosol and enter the eleventh reaction of glycolysis (anaerobic conditions), or to penetrate the mitochondria and become oxidised in the respiratory chain of the Krebs cycle (aerobic conditions). In the conditions of hypoxia, mitochondrial NADH oxidation is slowed down. Also in the case of the glycolysis (anaerobic) path, NADH formation is activated. In this regard, it can be assumed that the detected level of fluorescence during this experiment should increase⁹.

In the protocol of the study, occlusion tests with pressure of 220 mmHg during 1.5 min were conducted. A pair of images (fluorescence and reflectance) were recorded before and at the end of the occlusion test. At the next stage, image drift stabilization of the study area and frame alignment were conducted.

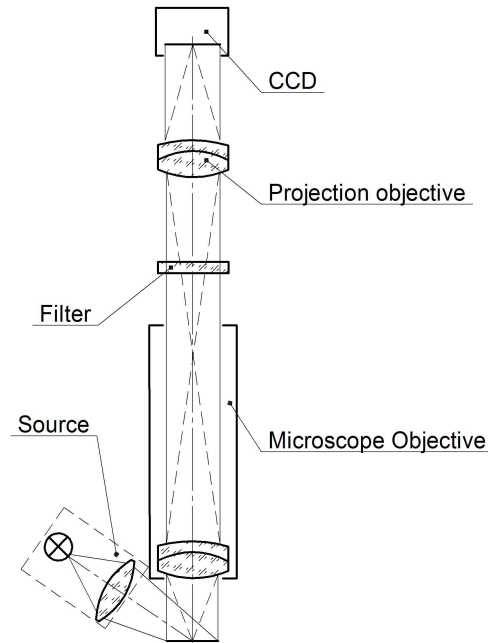


Figure 1. The experimental setup.

Reflectance images were calculated as follows below:

$$I_d(\lambda) = \frac{I_t(\lambda) - I_b(\lambda)}{I_{PTFE}(\lambda) - I_b(\lambda)} \quad (1)$$

where $I_t(\lambda)$ – the measured reflectance image of biological tissue; $I_{PTFE}(\lambda)$ – the measured reflectance image of the etalon optical diffuser (PTFE); $I_b(\lambda)$ – the background image obtained without any illumination.

3. EXPERIMENTAL RESULTS AND DISCUSSION

Obtained fluorescence images of precapillary zones normalised to the image of the diffuse reflection from experimental studies are presented in Fig. 2.

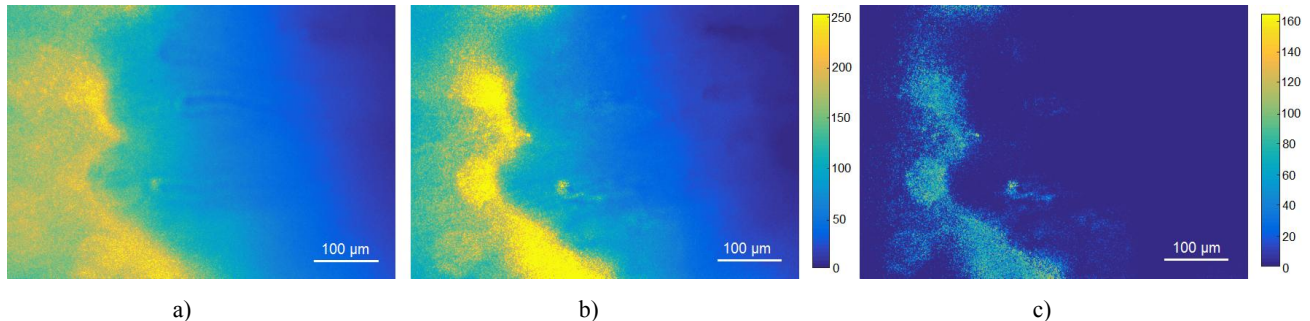


Figure 2. Fluorescence images before (a), in the end (b) of occlusion test and difference between stages (c)

It can be seen that by the end of the occlusion test, the fluorescence intensity significantly increases. The effect is especially bright in the precapillary zones, which may indicate a significant accumulation of NADH due to tissue hypoxia.

Thus, the proposed approach can be used to study the dynamics of changes and NADH in vivo and in situ. In vivo registration of NADH fluorescence as a noninvasive marker for the detection of cell death is promising for the development of highly efficient ways to detect and treat cancer^{10–12}.

4. CONCLUSION

These promising and substantial results demonstrate the ability of spectroscopic techniques to provide useful information for disease classification in a noninvasive manner. Specifically, fluorescence changes of NADH will provide details about tissue biochemistry.

The conducted study can be of particular interest in the research area of cell metabolism as well as find applications in clinical practice. However, further research is needed to understand the reliable diagnostic criteria based on imaging of NADH fluorescence in epithelial tissues.

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