Development of Dual-Mode Hyperspectral/Fluorescence Lifetime Imaging System

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This paper presents a microscopic diagnostic system that combines hyperspectral and frequency domain fluorescence lifetime imaging to record the content of chromophores and high-speed changes in cell and tissue metabolism. The efficiency of the system was tested on liver tumor slices of a laboratory mouse.

Keywords: FLIM, hyperspectral imaging, microscopic system

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I. Introduction

The implementation of a multimodal imaging system based on the use of a hyperspectral approach and fluorescence lifetime imaging (FLIM) is a rather difficult task but potentially has a wide diagnostic capability. Research papers presenting such systems are relatively few, and most of them are based on the use of time-resolved fluorescence lifetime measurements and commercial microscopic systems [1, 2]. Such an implementation of fluorescence lifetime visualization is associated with spatial scanning of the sample and does not allow measuring fast processes in dynamics. However, recent advances in measuring the fluorescence lifetime in the frequency domain make it possible to use special cameras for such tasks [3]. At the same time, modern acousto-optic or liquid crystal filters allow the development of compact custom-built hyperspectral systems.

Thus, the aim of this work was to develop a microscopic system that combines the hyperspectral method based on the study of diffuse reflected light and the frequency domain FLIM method.

II. Materials and Methods

The developed microscopic system is schematically shown in Figure 1 and conventionally consists of 3 parts: the main optical channel including MY5X-802 objective, MD416 dichroic mirror, FELH0 400 nm optical emission filter and 50:50 UVFS plate beamsplitter; the hyperspectral channel including Kurios liquid crystal tunable filter, AC254-050-A-ML achromatic duplet, IDS UI-3060CP camera and OSL2 broadband light source together with FRI61F50 ring illuminator; the fluorescence channel including PCO FLIM camera, AC254-050-A-ML achromatic duplet and BDL-SMN laser light source (375 nm, 80 MHz).

The fluorescence lifetimes were estimated by the phase shift and the change in the modulation index of the recorded fluorescence radiation relative to the amplitude-modulated exciting radiation. Hyperspectral cubes were processed using a specially developed algorithm.

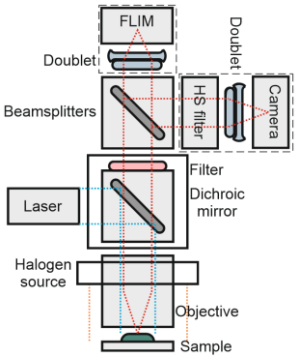

Fig. 1. Structural scheme of the developed system

III. Experimental Testing of System

To approbation of the developed system, tumor slices (hepatocellular carcinoma) of the liver of a laboratory mouse were studied. Using the hyperspectral channel, hyperspectral arrays containing information on diffuse reflected light in the wavelength range 400-1000 nm were obtained. Using FLIM, maps of the fluorescence lifetime distribution were obtained as well as phase-specific fluorescence lifetimes clustered in the regions of 2 and 5.5 ns.

The results obtained in this work may assist the transition of the technique to biomedical practice to identify characteristic patterns of physiological parameters in healthy and pathological cells.

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