Hyper-spectral modulation fluorescent imaging using double acousto-optical tunable filter based on TeO$_2$-crystals

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Abstract. We have proposed a method for hyper-spectral fluorescent imaging based on acousto-optical filtering. The object of interest was pumped using ultraviolet radiation of mercury lamp equipped with monochromatic excitation filter with the window of transparency centered at 365 nm. Double TeO$_2$-based acousto-optical filter, tunable in range from 430 to 780 nm and having 2 nm bandwidth of spectral transparency, was used in order to detect quasi-monochromatic images of object fluorescence. Modulating of ultraviolet pump intensity was used in order to reduce an impact of non-fluorescent background on the sample fluorescent imaging. The technique for signal-to-noise ratio improvement, based on fluorescence intensity estimation via digital processing of modulated video sequence of fluorescent object, was introduced. We have implemented the proposed technique for the test sample studying and we have discussed its possible applications.

1. Introduction

Since first appearance of various fluorescent imaging techniques they have attracted considerable interest as possible methods of fundamental and applied research. They provide an ability for studying biological objects, single particles and cells, for visualizing structure and movements of object [1–8].

The methods of fluorescent imaging [8–16], fluorescent microscopy [17–22], and fluorescent tomography [23–25] have been developed during the last decades. Many approaches for fluorescent image processing, for tomographic reconstruction of the sample structure based on its fluorescent imaging were proposed, and many technical realizations of multi-spectral ultra-sensitive fluorescent image detection were developed. These techniques were successfully implemented for the purpose of medical diagnosis, including diagnosis of skin cancers [26,27]. However, the problem of development of hyper-spectral continuously-tunable fluorescent imaging systems, and the problem of fluorescent image processing remain very important for further progress in fluorescent imaging applications.
This paper presents the novel results of modulation fluorescent imaging system development. We are describing the fluorescent imaging system based on pumping the sample with modulated ultraviolet (UV) radiation of mercury lamp, equipped with 365 nm excitation filter. Double TeO$_2$-based acousto-optical filter, tunable in range from 430 to 780 nm and having 2 nm bandwidth of spectral transparency, is used for detecting the quasi-monochromatic images of fluorescent object. We utilize the pumping intensity modulation simultaneously with the technique for digital processing of video sequence of modulated fluorescent images, in order to achieve high signal-to-noise (SNR) ratio, as well as to reduce an impact of non-fluorescence background on the results of fluorescent measurements. We are implementing the proposed technique for the test sample studying; the results of experiments and possible applications of proposed method are discussed.

2. Materials and methods
At first we are describing the experimental set-up for fluorescence imaging. Figure 1 shows the schematic representation of experimental set-up. We are utilizing the UV radiation of mercury lamp with 100 W power of polychromatic beam to pump the sample, and we use excitation filter transparent around $\lambda_p = 365 \pm 5$ nm. The intensity of UV beam is modulated using mechanical modulator (beam chopper); thus, the time-domain intensity profile has the following character

$$I(t) \sim 1 + \sin(2\pi \nu_p t),$$  \hspace{1cm} (1)

where $\nu_p = 2.0$ Hz is a frequency of modulation, and the modulation depth is $m = 1.0$. Being radiated with UV, the sample fluoresces, and the intensity of fluorescence is also modulated with the same frequency, $\nu_p$. The video image of sample fluorescence is being detected by means of complementary metal-oxide-semiconductor (CMOS) camera equipped with double acousto-optical tunable filter (DAOTF) [28,29]. The frame rate of CMOS camera is much higher than the UV beam modulation frequency, $\nu_{frame} = 25.0$ Hz $\gg \nu_p$. Thus, the video sequence of sample fluorescence represents blinking of fluorescence intensity, allowing us to estimate the spatial distribution of fluorescence intensity by means of processing the video sequence.

The main feature of presented set-up is associated with the presence of DAOTF [28,29]. It consists of two single acousto-optical filters (AOF) based on TeO$_2$ crystals, three CaCO$_3$ polarizers and additional parts for acoustic wave generation and control. If linearly polarized electromagnetic wave is propagating througth the TeO$_2$-crystal simultaneously with the acoustic wave, and if the condition of non-collinear light diffraction is satisfied

$$k_s = k_i + Q,$$ \hspace{1cm} (2)

where $k_i$ corresponds to wave vector of incident electromagnetic wave, $k_s$ corresponds to wave vector of scattered electromagnetic wave, and $Q$ corresponds to the acoustic wave vector, we are observing a change of electromagnetic wave polarization. Thus, the initial linear polarization changes to the opposite linear one. For a certain direction of electromagnetic wave propagation through the TeO$_2$-crystal we could change the polarization of electromagnetic wave at a certain wavelength, $\lambda$, and this wavelength is directly related to the acoustic wave frequency, $f$. By varying $f$ we could continuously tune the wavelength, $\lambda$, which changes the polarization, and this wavelength could be easily isolated from the polychromatic spectrum by using the polarizer.

The principle of DAOTF operation could be described as follows [29]. Initial polychromatic radiation emitted by the object of interest is passing through the linear polarizer ($P_1$) and reach the TeO$_2$-crystal of first AOF. This AOF changes the polarization of particular wavelength, $\lambda$, within the polychromatic spectrum, and this wavelength is defined by the acoustic wave frequency, $f$. By passing the beam through the second polarizer ($P_2$), which is transparent for $\lambda$ polarization and almost non-transparent for polarization of the polychromatic spectrum, we are
Figure 1. Schematic representation of the set-up for modulation fluorescent imaging, where M is a mirror, P₁, P₂, and P₃ are polarizers transparent for the indicated polarizations, L₁, L₂, and L₃ are lenses, collimating the pumping beam and build the object image at the plane of CMOS detector (f₁, f₂, and f₃ define focal lengths of the lenses). Wide arrows in front of polarizers and after them indicates the light polarization states.

filtering the particular wavelength, \( \lambda \). Almost monochromatic beam reaches the second TeO₂-crystal, where it undergoes the polarization change for the second time. After this rotation of electromagnetic wave polarization, the optical beam passes through the third polarized (P₃), which suppresses the remaining low-power polychromatic component, leaving the \( \lambda \)-component untouched. Thereby, we could observe quasi-monochromatic radiation after the DAOTF.

For the particular DAOTF we are observing 2 nm narrow bandwidth of spectral transparency with the ability to continuously tune the wavelength of electromagnetic wave within the range from 430 to 780 nm [29]. The aperture of the beam passing through the DAOTF reaches \( D = 8 \) mm, the angular field of view reaches \( 2\omega = 3^\circ \) with the angular resolution of \( \delta = 0.7 \) radians, providing the ability for imaging the sample. The mirror (M) in the fig. 1 is used for the compactness of pumping optical system, the lenses (L₁, L₂, and L₃) are used to collimate the pumping UV beam and to built the monochromatic images at the plane of CMOS sensor (image plane). Note, the pumping system, the DAOTF and the CMOS camera is controlled via the PC. Thus, the video images of the object fluorescence in narrow bandwidth of electromagnetic spectrum could be detected utilizing the described system.

Let us define the monochromatic video sequence with \( s_\lambda(x, y, t) \) \( (x \) and \( y \) represent spatial coordinates at the image plane, \( t \) represents the time of image registration, and index \( \lambda \) defines the central wavelength of DAOTF transparency). The object fluorescence could be describe as a spatial distribution of the fluorescence intensity, \( p_\lambda(x, y) \), and we are estimating the latter one utilizing the following procedure

\[
p_\lambda(x, y) = \tilde{s}_\lambda(x, y, \nu = \nu_0),
\]
where

$$
\tilde{s}_\lambda(x, y, \nu) = \int_{-\infty}^{+\infty} s_\lambda(x, y, t) e^{-i2\pi\nu t} dt
$$

stands for Fourier transform applied to time-domain data at each point of the image plane. Equation (3) helps us to both prevent an impact of non-fluorescent background on $p_\lambda(x, y)$ reconstruction, and to suppress the digital noises inherent to single image of the video sequence, $s_\lambda(x, y, t = t')$, where $t'$ corresponds to the certain moment of time.

3. Results

We are implementing the described experimental set-up for studying the test sample in order to verify the proposed method of modulation fluorescent imaging. We are studying the samples of polymer fluorescent thread, containing fluorescent dye and injected into a peace of white low-fluorescent paper. During the study we are setting the spectral band of DAOTF transparency to 612 nm, and we are utilizing maximal power of the UV pump. Figure 2 shows schematic representation of video sequence of fluorescent images of test object: (a) shows the theoretical time-domain profile of the UV pump intensity, $I(t)$ (equation (1)), and (b) shows the separate images of the video sequence corresponding to different pumping conditions (1$^{st}$, 2$^{nd}$, and 3$^{rd}$ fluorescent images in the figure 2 (b) correspond to (1) maximal, (2) medium, and (3) minimal power of UV pump, respectively). One could observe strong difference between the object fluorescence intensity at this array of images.

By applying the procedure (3) we are reconstructing the parametric image of object fluorescence, $p_\lambda(x, y)$, and figure 3 shows the results of these calculations: (a) corresponds to simple visual image of object corresponding to the case of no UV pump is applied, and (b) presents the result of modulation fluorescent imaging implementation (equation (3)). We observe strong suppression of digital noises, as well as suppression of non-fluorescent background in the experimental parametric image in comparison to the ordinary images of the video data.

The described technique (equation (3)) helps to significantly suppress the digital noises in the fluorescent parametric image, $p_\lambda(x, y)$, and an approximate increase of fluorescent data SNR.
could be described as

\[ K = \frac{P_{\Delta \nu}}{P_{\nu_0}} = \nu_{\text{frame}} T, \]

where \( P_{\Delta \nu} \propto \nu_{\text{frame}} \) stands for the power of the digital noise localized in the entire Fourier-domain, \( P_{\nu_0} \propto 1/T \) stands for the power of digital noise localized near the modulation frequency, \( \nu_0 \), in the Fourier-domain, and \( T \) corresponds to the duration of entire video capture. According to equation (4), the higher the duration of video sequence we are detecting, the higher resolution in the discrete Fourier-domain we are achieving, and the lower the power of digital noises localized within the elementary discrete Fourier-domain window, centered near \( \nu_0 \), we are observing. For example, for a given experiment conditions, \( \nu_{\text{frame}} = 25.0 \, \text{Hz} \) and \( T = 4.0 \, \text{sec.} \), we could achieve \( K = 10^2 \) times enhancement of SNR by using the modulation fluorescent imaging in comparison to the ordinary one. This estimation is in complete accordance with the results of experimental implementation of the algorithm.

The main advantage of the describe set-up for modulation fluorescent imaging is associated with the ability to continuously tune the central wavelength of the DAOTF transparency, \( \lambda \), within the wide operating range from 430 to 780 nm. Thus, a set of \( N \) fluorescent parametric images of the sample

\[ p_{\lambda_1}(x,y), p_{\lambda_2}(x,y), \ldots, p_{\lambda_N}(x,y), \]

with small spectral step \( \Delta \lambda = \lambda_{i+1} - \lambda_i \) form an effective basis for further inverse problem solutions, object detection, recognition, and identification. A list of potential applications for the described technique, which we are going to consider, includes imaging and microscopy of biological tissue and cells, medical diagnosis, for instance, diagnosis of pigment skin neoplasms and skin cancers [30].

4. Conclusion
In this paper we have proposed method for multi-spectral fluorescent imaging based on acousto-optical filtering. We have used double TeO\(_2\)-based acousto-optical filter monochromator in order to construct the acousto-optical filter, tunable in range from 430 to 780 nm and having 2 nm bandwidth of spectral transparency. We have utilize modulation of UV pump intensity to reduce an impact of non-fluorescent background, as well as to suppress the digital noises in fluorescent
images via the digital processing of modulated video sequence. We have studied the test sample in order to implement the proposed technique and to study the increase of signal-to-noise ratio in the fluorescent parametric image.

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