

Double-AOTF-based aberration-free spectral imaging endoscopic system for biomedical applications

Alexander Machikhin* and Vitold Pozhar†

Scientific and Technological Center of Unique Instrumentation RAS Butlerova Street, 15, Moscow 117342, Russia *ilovemiracles@mail.ru † v _pozhar@rambler.ru

> Vladislav Batshev Bauman Moscow State Technical University 2nd Baumanskaya Street, 5, Moscow 105005, Russia batshev.vlad@gmail.com

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The problem of in vivo photoluminescence diagnostics of the tissues accessible by endoscopes is discussed. The spectral imaging module attachable to conventional rigid and flexible medical endoscopes is developed and described. It is based on a double acousto-optical tunable filter (AOTF) and a specialized optical coupling system. The module provides wide field of view (FOV), absence of image distortions, random spectral access, fast spectral image acquisition at any wavelength in the visible range and accurate measurement of reflectance spectrum in each pixel of the image. Images of typical biomedical samples are presented and discussed. Their spectra are compared to the reference data.

Keywords: Spectral imaging; spectroscopy; double monochromatization; acousto-optical tunable filter; photoluminescence diagnostics; endoscopy.

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1. Introduction

Noninvasive photoluminescence methods are very promising for diagnostics and therapy of tumor and inflammatory diseases.^{[1](#page-5-0)} The imaging systems which provide the hyperspectral (spectral and spatial) information are particularly interesting. They have several very important advantages: informativity, high sensitivity and clearness of data interpretation.²

Spectral imaging systems based on acousto-optic tunable filters (AOTFs) possess hyperspectral capabilities and also provide unique collection of

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features such as random spectral access, programmability and image transmission with high image quality — insignificant (less than $\frac{1}{0}$) distortion in the visible spectral range. Rather narrow bandwidth (down to 0.1 nm) and high spatial resolution (up to 1000×1000 dots), fast spectral tuning $(\sim 10 \,\mu s)$, compactness and absence of any moving elements make them a precise and ergonomic analytical tool capable of working in out-of-lab envi-ronment^{[3,4](#page-5-0)} for various applications, including biomedical analysis.

Spectral imagers based on other spectral elements do not provide possibility of recording images on arbitrary wavelengths (diffraction gratings), sufficient spectral range (Fabry-Perot filters) or speed of spectral tuning (liquid crystal filters).

Small-size AOTFs with large entrance pupil and wide field of view (FOV) can be readily integrated into optical schemes of existing equipment. For instance, there are AOTF-based imaging systems for microscopic fluorescence diagnostics of biomedical objects,^{[5](#page-5-0)} for detection of tumors by the injection of photoluminescence substances, 6 for noncontact oxygenation detection⁷ and other applications.

One of the most promising applications for AOTF-based spectral imaging in medicine is endoscopy, which is widely used for in vivo visualization and diagnostics. $4,8,9$ $4,8,9$ $4,8,9$ Being coupled with rigid or flexible medical endoscopes, AOTFs provide an extra capability which is reflectance spectrum quantitative analysis of inspected tissues and organs. It allows, for example, effective detection of mouse pancreas pathology and other malignant tissues.⁸

Most of AOTF-based spectral imagers for endoscopic photoluminescence analysis utilize single acousto-optic (AO) cell, which lacks spectral drift and chromatic aberrations of diffracted image.^{[10](#page-5-0)} That is why for spectral images matching, an additional correction procedure must be applied that complicates the analysis and makes it time-consuming and less reliable.¹¹ Another problem concerns the optical coupling between the endoscope ocular and the AOTF entrance pupil. The FOV and the light diameter of AOTFs differ significantly from endoscopes' ones. Therefore, directly attaching the AOTF to endoscope ocular causes losses in light power and decrease in the diffracted image quality.

Here, we present the endoscopic AOTF-based spectral imaging module free of these lacks. It comprises of double AOTF and special optical coupler. In this paper, we also describe the experimental approbation of this imager with real biomedical objects to demonstrate the efficiency and accuracy of spectrum measurement and applicability for in vivo endoscopic visualization of internal organs.

1.1. Double monochromatization and optical coupling

The main difference of the device from the existing AOTF-based spectral imagers is the double monochromatization. For this purpose, two AO cells are utilized, the second one being rotated at 180° in the polar plane. To provide identical conditions of AO interaction in both AO cells, the angles of incident light θ , diffracted light ψ and ultrasound γ should be related as follows $\theta_2 = \psi_1, \psi_2 = \theta_1, \gamma_2 = \gamma_1$ [Fig. [1\(a\)\]](#page-2-0). The cells implement the diffraction of orthogonally polarized light radiation: the first: $e \rightarrow o$, the second $o \rightarrow e$. Double monochromatiza-tion significantly increases the contrast^{[12](#page-5-0)} and the quality of the transmitted image¹³ in the whole spectral range. While each AO cell causes image $deformations, ¹⁰$ in the developed tandem double-AOTF, linear and nonlinear distortion aberrations are almost completely compensated[.13](#page-5-0)

Monochromator was designed and assembled as a separate PC-controlled device for image spectral filtration [see Fig. $1(b)$]. Its optical scheme includes two wide-angle noncollinear $TeO₂$ AO cells placed in series as shown in Fig. $1(a)$. Monochromator provides spectral resolution $\sim 1.5 \,\mathrm{nm}$ (at $630 \,\mathrm{nm}$) and spatial resolution ~ 600 resolved elements in each direction in the wavelength range 450–750 nm.

The second feature of the developed imaging system is the specialized optical coupler between the eyepiece of the endoscope and the AOTF. It significantly increases the luminosity and the FOV of the system.

FOV behind the standard endoscope eyepiece is $2\omega_{\rm oc} = 20-50^{\circ}$, and the diameter of eyepiece exit pupil $D_{\text{oc}} = 2-5$ mm. AOTF parameters differ significantly from these values: $2\omega_{\text{AOTF}} = 3^{\circ}$ and $D_{\text{AOTF}} \leq 8 \,\text{mm}$. So when installed just behind the eyepiece without an optical coupling system, AOTF uses only a part of the entire eyepiece FOV and the AOTF input pupil is filled only partially. To solve this problem, the optical coupler should be an afocal system with magnification $\Gamma < 1^{14}$ $\Gamma < 1^{14}$ $\Gamma < 1^{14}$ The coupler increases the input FOV of the AOTF to the value

Note: q, k_i, k_d — wave vectors of ultrasound, incident and diffracted light; n_o , n_e — refractive indexes of crystal for ordinary and extraordinary polarized light.

Fig. 1. Wave diagrams of the diffraction (a) and double-AOTF monochromator, (b) 1 — mechanical adapter for optical coupler, 2 — two AOTFs inside the cover, 3 — mechanical adapter for objective lens; 4 — driving radiofrequency unit; 5 — USB control port, 6 — power supply port.

Fig. 2. Optical system of the coupler. 1 — mechanical adapter for endoscope ocular, 2, 4, 5 — lenses, 3 — focusing tool.

 $2arctan(\tan(\omega_{\text{AOFF}})/\Gamma)$ and decreases the AOTF light beam diameter to the value ΓD_{AOTF} . Matching of the diameters leads to the luminosity increase by $(1/\Gamma)^2$ times. For the majority of medical endoscopes, the optimal magnification of the coupler is $\Gamma = 0.4$. In this case, the increase in the FOV of the spectrometer is 2.5 times and luminosity rises 6 times. It was proved experimentally. $9,15$ The main novelty of the device described in this paper in comparison with Refs. [13](#page-5-0) and [14](#page-5-0) is that the spectral drift of the image is eliminated and there is the no noticeable image distortions at the full FOV on any wavelength. This allows us to compare the images obtained at different wavelengths without postcorrection. In particular, it allows us to quickly and reliably obtain a spectrum of different image points.

For the present research, we measured the image aberrations, including the chromatic drift, caused by AOTFs with various angular configurations.[16](#page-5-0) The measurement setup was based on the wave-front analysis by means of Shack–Hartman sensor. Using the experimental results we developed the new optical coupler where the single lens was replaced with a doublet. The new coupler has the same features as in Refs. [9](#page-5-0) and [15](#page-5-0) but has a new very useful feature. It demonstrates a very small chromatic shift (less than $50 \,\mu\text{m}$) and thus need not refocusing while spectral tuning. Figure 2 shows the coupler structure. The focusing tool 3 is required to change and fix the position of the focus when changing endoscopic probe. The mechanical adapter 1 is standard endoscopic mount so that the coupler is compatible with medical rigid and flexible endoscopes.

2. Experimental Setup

The optical scheme of the experimental setup is shown in Fig. [3](#page-3-0). Inspected object 1 is illuminated by wide-band visible or ultraviolet light from source 3.

Fig. 3. Optical scheme (a) and appearance (b) of experimental setup. 1 — inspected object; 2 — rigid endoscope probe; 3 — wideband light source; 4 — optical coupler; 5, 7, 9 — polarizers; 6, 8 — identical wide-angle AO cells; 10 — objective lens; 11 — digital camera; $12 - PC$; 13 — control unit. Home-made AOTF-based module with optical coupler is marked by dotted line rectangle.

Reflected and scattered light as well as reemitted fluorescence radiation is collected by endoscope front lens and transmitted through the rigid endoscopic probe 2, optical coupler 4 and double-AOTF monochromator 5–9 consisting of two AO cells 6, 8 and three polarizers 5, 7, 9. Selected narrow-band light is focused by the objective lenses 10 at the sensor of the digital monochrome camera 11.

Parameters of the sensor (exposure time, gain, binning, etc.) and the wavelength of the monochromator can be tuned interactively by the user from PC 12. The developed software provides both hardware control and spectral image processing.

The rigid endoscope (diameter 5.5 mm, length 45 cm) 2, the 150W halogen light source 3, objective lens $(f = 90 \text{ mm})$ 10 and videocamera The Imaging

Fig. 4. Spectral images (500–740 nm) of human skin (thumb) and measured reflectance spectra at three different points.

Fig. 5. Spectral images (500–740 nm) of mouse testicle and measured reflectance spectra at three points: 1 — testicle membrane, 1 — testicle body, 3 — fixative compound.

Source DMK 31BU03 (sensor Sony ICX204AL, 1024×768 pixels) 11 are standard devices widely used in medical endoscopy. The coupler 4 and double-AOTF-based monochromator 5–9 are developed to be totally (optically and mechanically) compatible with them so that the user can either carry out conventional endoscopic analysis or insert the coupler and monochromator for photoluminescence research.

To eliminate the influence of the light source, inconstancy of the CCD quantum efficiency and transmission of optical components, the whole optical system was preliminary calibrated by the measurement of the test-object spectra.

To demonstrate the applicability of developed AOTF-based endoscopic system, typical biomedical tissues were analyzed: human skin and mouse testicle. The spectral images shown in Figs. [4](#page-3-0) and 5 are uncorrected in order to show the raw data obtained by the spectrometer. All images in Figs. [4](#page-3-0) and 5 was obtained under the same illumination conditions using the halogen light source with well-known spectrum.

A series of spectral images of a human thumb was detected in the range 450–750 nm with a step 2 nm. Reflectance spectra at three points are shown in Fig. [4.](#page-3-0) All the spectra are quite similar and have a minimum in the range 500–740 nm. The shape corresponds well to the spectral reflectance of main skin layers: stratum corneum, epidermis and dermis. [17](#page-5-0)

A series of spectral images of a mouse testicle in the range 450–750 nm with a step 2 nm is shown in Fig. 5. The measured reflectance spectra of testicle membrane, testicle body and the fixative compound (glue) differ significantly. Despite the wide usage of testicles for the analysis of medicine influence and diseases progress such a spectral

Table 1. Comparison of the described and conventional approaches.

Parameter	Type of the spectrometer	
	Single-AOTF-based without optical coupling	Double-AOTF-based with optical coupling
FOV .	$3 - 10$	$30 - 60$
Luminosity inhomogeneity over the image, $%$	up to 70	< 10
Spectral contrast, %	12	1
Monochromatic distortion, %	up to 5	${}_{0.5}$
Chromatic distortion, %	up to 3	< 0.1
Spectral image drift, μ m	up to 300	< 50

imaging of mouse testicles is carried out for the first time.

3. Discussion

The endoscope-attachable double-AOTF-based system combines reliable spectral imaging with the collection of important features: absence of distortions and chromatic drift, high spectral contrast, no-moving-part design, fast electronic tuning, compatibility to commercially available endoscopes. To summarize the merits of the described spectrometer, in Table [1](#page-4-0) its main technical parameters are compared with those of the single-AOTF spectral imagers without optical coupling.

4. Conclusion

A novel endoscopic spectral imager for biomedical applications based on double monochromatization of light and precise optical coupling provides high spectral contrast and aberration-free imaging. The imager has several new important features: compensated spectral and spatial image distortion, minimization of optical losses and compatibility with standard medical endoscopes both rigid and flexible. Experimental research of real biomedical tissues shows the effectiveness of the developed AOTF-based endoscopic spectral imager. Comparison of measured reflectance spectrums with reference ones demonstrates the correctness of obtained data. The spectral imager has the potential for noninvasive optical diagnostics in colonoscopy, bronchoscopy, gastroscopy, etc.

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