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Determination of blood flow velocity in vessels of the bulbar conjunctiva

Abstract. *The paper presents the methods and the system for registration of digital video recordings of the sclera with a spatial resolution of 2 μm. The pulsed illumination of the sclera was used to eliminate the image blurring caused by the continuous movement of the eyes. At evaluation of blood flow velocity the stabilization of the position of recorded images relatively to the first image frame is used and the trajectory of displacement of the bulbar conjunctiva of the eye is determined.*

Streszczenie. *W artykule omówiono metody oraz opisano system do rejestracji cyfrowych nagrań wideo twardówki z rozdzielczością przestrzenną 2 μm. Zastosowano impulsowe oświetlenie twardówki w celu eliminacji rozmycia obrazu spowodowanego przez ciągły ruch gałek ocznych. Przy ocenie szybkości przepływu krwi przeprowadza się stabilizację pozycji zarejestrowanych obrazów względem pierwszej ramki obrazu oraz wyznacza się trajektorię przemieszczenia spojówki gałki ocznej. (**Wyznaczenie szybkości przepływu krwi w naczyniach spojówki gałki ocznej**).*

Keywords: blood flow velocity, microcirculation, blood vessels of bulbar conjunctiva
Słowa kluczowe: prędkość przepływu krwi, mikrokrążenie, naczynia krwionośne spojówki gałki ocznej

Introduction

Early diagnostics of vessel diseases is based on evaluation of results of various research methods of vessels condition, hemodynamics and functions of different organs and tissues of the body [1]. Vessels of the eye fundus and the bulbar conjunctiva, being the branches of internal carotis artery, are the most accessible for direct observation and evaluation of their condition (Fig. 1). A number of corporations produce fundus cameras to obtain digital images of retinal vessels. It is possible to evaluate the different morphological parameters of retinal vessel network by processing these images [2]. Fundus cameras allow to register only unitary images, as the eye fundus is illuminated by single powerful white light pulse. After its influence, the pupil is narrowed during 200 ms interfering the obtaining of the next images. Only advanced unique laboratory equipment enables us to obtain video recordings of retinal vessels and to estimate the velocity of erythrocytes in retinal capillaries.

The vessels of the bulbar conjunctiva covering part of the sclera of the eye and its parts on corneal border are more accessible for research. The network of conjunctival vessels is a part of arterial arches of upper and lower eyelids and anterior ciliary arteries being the branches of ophthalmic artery extending from internal carotid artery. The branches of arterial and venous conjunctival vessels form the microcirculation vessel network which basic part lies in deeper layers, the smaller part lies in superficial layers of bulbar conjunctiva (Fig. 1).

The main parameters describing the blood microcirculation in small vessels, are the volume and linear velocity of blood flow. The existing methods of laser Doppler flowmetry give the integrated by scattering volume distribution of velocity projections. It does not allow to evaluate the blood flow velocity in certain vessels and capillaries. The known laser methods of blood flow velocity measurement in capillaries and small vessels [3, 4] are difficult in realization and do not find wide application in clinical practice.

The conjunctiva vessels are available for direct videorecording. The apparatus and the method for determining the blood flow velocity in these vessels are described in [5]. The apparatus include the expensive fast speed video camera (250 frames/s). The obtained frames are recorded in hard disk. As the eye is continuously moving, the stabilization of the position of the obtained images based on the SURF method is used.

Then the required area of the vasculature is chosen and the velocity of blood formed elements is determined using the shift of the blood formed elements during *n* frames. The drawback of this method is the large amount of data, which should be processed for determination of v_b.

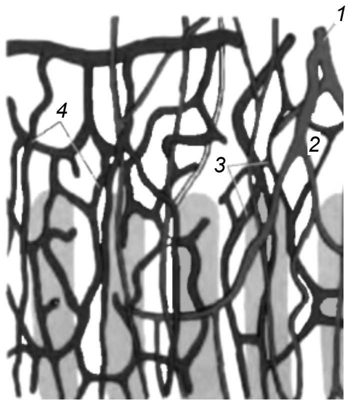

Fig.1. Scheme of conjunctival vasculature: *1* – anterior ciliary artery; *2* – episcleral arteries; *3* – episcleral venous network; *4* – lymphatic vessels of conjunctiva

The paper discusses the method and the technique for obtaining the digital images of conjunctiva vessels using the pulse sclera illumination, that allows to reduce the volume of the recorded and processed data in tens times.

Methods for obtaining and recording the images

In order to reduce the number of video recordings of scleral vessels the pulsed illumination of the observed region of the sclera can be used. During recording the patient must fix the eyes on a bright enough point to eliminate the influence of saccadic eye movements. Then at pulse duration of 1 ms the shift of the sclera and the formed elements of blood will not exceed 1 μm. The spatial resolution of videorecording should be chosen no less than 2 μm.

The modulation of the intensity of eye illumination using LEDs is the most simple to realize. The brightness of the

LEDs is modulated by passing the current pulses with required duration and duty cycle through them. Modern LEDs are known to have a fast switching time. Therefore, the pulse generator may have the injection current rise and fall times of the order of dozen microseconds.

The fibre-optic equipment and focusing lens were used for the transmission of light that illuminates the eye. The focusing lens is placed laterally to the camera to avoid any shadow from eyelashes under illumination from above or below the camera. Modern high-power LEDs provide sufficiently high radiation fluxes, which can be introduced into the fibre of a diameter of 1 mm.

The diameter of the illumination spot focused on the certain area of the sclera should not exceed about 2 mm. This limitation is caused by two factors. Firstly, the eye fundus has a spherical shape. The depth of image field is small at high magnification of the lens. Therefore, the edge of the image will always be out of focus and there is no need to illuminate the blurred image areas. Secondly, the enlarged image formed by the lens on the photosensitive surface of video camera array is weakly illuminated. Good signal/noise ratio is ensured by the accumulation of the large number of photogenerated charges in photosensitive elements of the matrix. Therefore it is necessary to provide high illumination of the sclera, which is close to its daytime illumination in an open area. Such illumination is perceived by the patient as very bright when recording the images of the sclera under normal conditions of laboratory. It is necessary to reduce the diameter of the spot illumination in order not to fall the radiation into the pupil of the eye and not to feel discomfort by the patient. If diameter of light guide of the used fibre is small (less than 2 mm) the butt of the optical fibre should be shifted relatively to the point of the best focus. This allows to increase the diameter of the illumination area of the sclera.

The depth of image field of blood vessels of the sclera at high magnification of lens is small. In this case, the guidance of the camera’s field of view on the given area of the conjunctiva vessels and focusing of the image are strongly complicated. The image on the screen appears only in a narrow range of distances from the lens to the sclera. Therefore, an additional device for visual guidance and coarse focusing was included into the system. The device operation is based on the convergence of two laser radiation beams directed at the angle to each other in a single spot in the focal plane. Two laser diodes with a wavelength of 635 nm are used. They are placed above and below the camera lens. The laser diodes radiation is collimated using the small-size optics. The adjusting of the beam axes direction is carried out by special screws. These screws allow the convergence of laser radiation beams in a single spot in the centre of camera field of view in the focus plane. When using the device, the guidance on the selected area of the sclera and coarse focusing of the image are carried out in a few seconds.

A sequence of blood vessels images is recorded using a monochrome camera. This camera is connected (through GigE) to computer. The camera is controlled by means of the special program in Matlab. The image of scleral vessels is displayed on the computer screen when the camera and the device for pulse illumination are turned on. During the image observation the final focusing of the image is carried out. When you save images you have to choose the number of recorded frames N_{fr} and click on the button “Save” on the camera control panel. In this case, record will be saved to your hard drive.

Good contrast between images of white sclera and conjunctiva vessels can be achieved by proper selection of the spectral range of illumination radiation. The tissue of the sclera or conjunctiva is a diffusely scattering medium. This medium scatters back approximately 50% of the incident radiation. Formed elements of blood strongly absorb light (Fig. 2). Therefore, the blood vessels of surface layer of the conjunctiva are clearly visible on the white background of the sclera. The maximum absorption of the blood and hence the maximum contrast of vessels is observed when the sclera is illuminated by radiation in the range of 530-580 nm. Unfortunately, in this range of wavelengths the high-power LEDs are not available. Therefore, it is necessary to use commercially available blue, green, yellow and white LEDs with power consumption up to 3W.

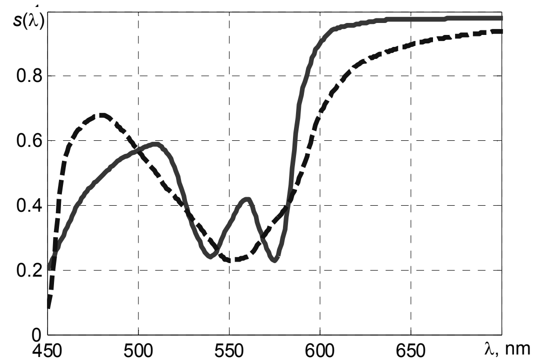

Fig. 2. The transmission spectra of 100 μm thickness of oxygenated (solid line) and deoxygenated (dashed line) blood columns [6]

Design features of the system

The computerized system for recording the digital images of the scleral vessels is based on the platform of slit lamp. This platform allows to move the camera with both the pulse illumination unit and the device for impulse illumination and focusing in the three coordinates *x*, *y*, *z* with the fixation of the chosen position. There is also the mechanical system for fixing the patient's face. It is possible to move the camera to the right and to the left for comfortable recording of the scleral vessels of both right and left eyes. The axis of rotation is located close to the plane of the eyes.

Monochrome video camera Imperx Bobcat IGV-B1410M is used in the system. It is advanced, intelligent, high-resolution, progressive scan, fully programmable and field upgradeable CCD camera. The camera is designed for applications with high requirements for sensitivity, image quality and simplicity of use. It is based on the highly sensitive sensor ICX-285 (Sony). The ICX285AL is a diagonal 11 mm (Type 2/3’) interline CCD solid-state image sensor with a square pixel array 6.45×6.45 μm^2. It forms the 10-bit digital image consisting of 1040 lines and 1392 columns. The maximum relative spectral sensitivity ICX285 is in the region of 530 nm, and its nonuniformity is not worse than 20% in the range 450-680 nm. The camera is controlled using the programs Vision Sclera, created in the GUI Matlab. Maximum frame rate is 23.2 Hz when using 16-bit format (uint16) of image.

The graphic window Vision Sclera shown in Fig. 3 includes the output region of the obtained digital images and two panels: "Obtaining and recording the sequence of frames" and "Viewing recordings and saving the individual frames". Top panel "Obtaining and recording the sequence of frames" is designed for initial adjustment of the recording process of digital images. It contains three buttons Start, Stop, and Save, as well as several editable input-output fields for setting the parameters of the camera and the video flow. Fields located on the left half of the panel are designed for photometric measurements when evaluating a bidirectional scattering coefficient of the sclera. Recording of the required number of frames N_{fr} is carried out when clicking on the button “Save”. Data are saved in the .m file on hard disk in the form of packaged three layer ($1392{\times}1040{\times}N_{fr}$) array of 16-bit numbers.

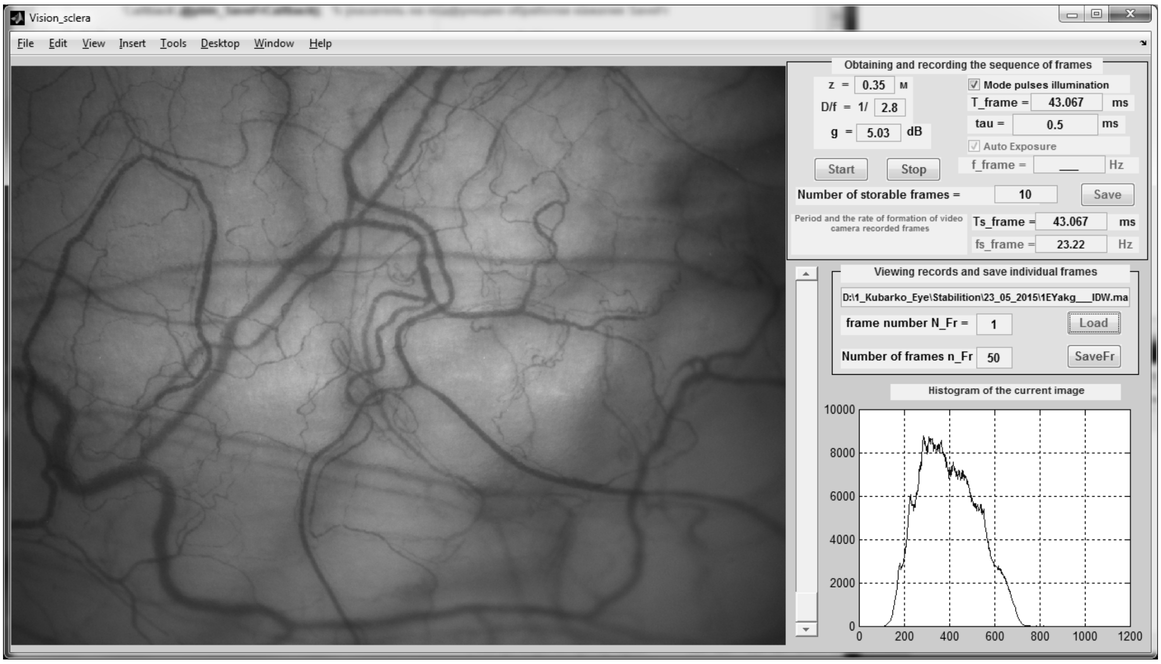

Fig. 3. The main window of the program of system for obtaining and recording the digital images of the sclera

Input/output fields on the right half of the panel are used for:

- setting the exposure time in ms when the checkbox “Auto Exposure” (tau) is set;
- setting the number of frames "Number of saved frames";
- setting the frame period (T_frame) when the checkbox "Mode pulses illumination " is reset;
- setting the frame period (Ts_frame) during recording.

The bottom panel is used for viewing the recorded frames of images. You can read the recorded .m files and view any of the chosen recording frames by using the vertical slider at the left of the panel. The directory, file name and the total number of frames in the recording are displayed in the corresponding windows of the panel. You can save a chosen frame in .mat and .bmp file formats.

In order to control visually the efficiency of the use of registered signals range the histogram of the current image is displayed on the screen.

Different colour LEDs can be applied in this system. Colour choice of high-power LEDs is limited. Therefore, five types of LEDs are used in this system. Their spectra are shown in Fig. 4. Warm White LED allows to obtain the low-contrast images of blood vessels located in the deeper layers of the conjunctiva, and high-contrast images of subsurface blood vessels (see Fig. 3) due to a great contribution of blue part of the spectrum to the integral spectrum of radiation of the LED (curve 5 in Fig. 4). Amber colour LED allows to obtain low-contrast images of vessels.

It is better to apply Green LEDs when determining the velocity of blood formed elements in subsurface veins and arteries of the conjunctiva. In this case, the correlation of images of moving blood formed elements which are in transparent plasma is observed for several consecutive frames (Fig. 5). Transparent gaps filled with plasma are clearly visiblein the images of capillaries. The gaps in images of venules also contain the plasma but they have a worse contrast and are visible during a few frames.

The system allows to conduct photometric measurements. Therefore, modulation of the current flowing through the LEDs is disabled when the checkbox "Mode of pulses illumination" is reset. There is a possibility to change the integration time «tau» of photogenerated charges and the frame period. The exposure time could be set automatically.

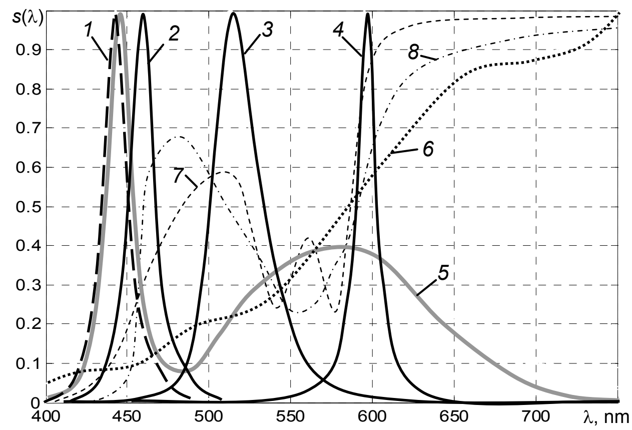

Fig. 4. The emission spectra of the royal blue (1), blue (2), green (3), amber (4) and white (5) LEDs, and a halogen lamp (6). The absorption spectra for oxygenated (7) and deoxygenated (8) blood

The strobe pulse required for the formation of pulsed radiation is generated by generator built into the IGV-B1410M camera. This pulse is applied to the gate of the FET that controls the current through the LEDs. The repetition period of the pulse coincides with the frame period (T_frame in acquisition mode or Ts_frame in recording mode). The duration of the pulse may be set by the operator in the window «tau =» and may vary from 0.2 to 2.5 ms.

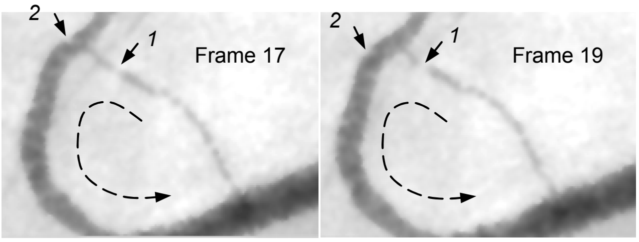

Fig. 5. Fragments of the image of venules and capillaries received in the 17th and 19th frames (Δt = 86.134 ms) of the recording 1PLekg___IDG during illumination of the sclera using green LED

Normalization of images and stabilization of their position

Continuous eye movements define the unstable position of images of scleral vessels [5]. Therefore, the program "Image Stabilization" can be used to view the records. This program allows you to perform some additional processing operations for received video recordings.

The illumination spot formed with the help of fibre-optic technique and the lens has a non-uniform illumination. Therefore, the image of the white diffuser surface should be registered before recording each series of images of blood vessels of patients. Then this image is processed by the program “Image stabilization”, smoothed by median filter, normalized by dividing it by its maximum value and saved to the hard disk of your computer in the form of normalizing matrix D_{norm} with a maximum value of 1. When you load the vessels image records, each image is divided by the normalizing matrix that allows you to adjust the brightness of the frame area.

The image stabilization of the sclera is carried out by determining the displacement vector of *k*-th image relatively to the first image. For various shifts of *k*-th image Δx and Δy the certain surface formed by standard deviation of the difference in brightness is calculated. The displacement vector of the image will match the coordinates of the global minimum of surface. To reduce the amount of calculation the step of grid is selected to be 5 pixels. The position of the minimum is refined by means of two-dimensional subpixel cubic interpolation of the surface in vicinity of the global minimum. This method allows to determine the displacement vector of k-th image with a resolution of 0.1 pixels, i. e. 0.2 μm.

The patient should keep the direction of gaze on a bright point. Herewith the shift of the image of the sclera should be small. Stabilized image is cropped at the edges. The width of the removed area corresponds to the value of maximum shift. For large shifts the eye stabilization of image is not full (Fig. 6).

Features of blood flow in vessels of the bulbar conjunctiva and methods for blood flow determination

The evaluation of the volume velocity of blood flow in the retina or bulbar conjunctiva is a complex and difficult task requiring the measurement of linear speed of blood flow and internal gleam of vessels. Usually the volumetric speed of blood flow is evaluated on the basis of measurement of average values of linear speed of blood flow in retinal artery (for example, central retinal artery) and its diameter.

The methods described in the paper allow to determine only linear speeds of blood formed elements in superficial blood vessels of the conjunctiva. Using appropriate software it is possible to receive a map of distribution of linear speeds in parts of blood vessels marked by the researcher. The speed was determined visually at viewing several consecutive frames of the recording of the digital images of blood vessels. For example, the linear speed of blood formed elements in Fig. 5 equals 0.23 mm/s for the first fragment and 0.2 mm/s for the second fragment. The error of visual determination is large enough; therefore the algorithms of its automatic determination in parts of blood vessels marked by the researcher are required.

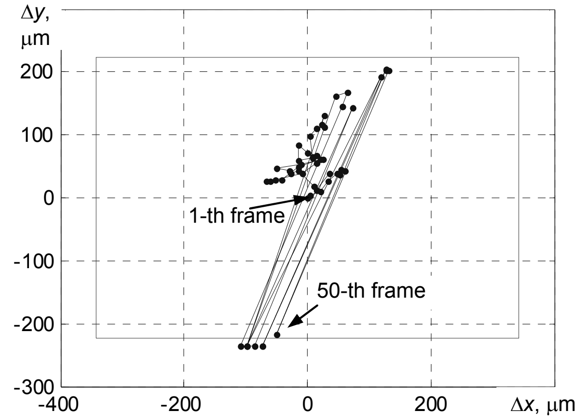

Fig. 6. The trajectory of eye movement obtained by the stabilization of the image recording 1EYakg___IDW

Summary

The described computerized system and method of obtaining the digital images of vascular bulbar conjunctiva are the initial stage of search of effective diagnostic features of vascular pathologies at the preclinical stage of their manifestations. The development of this direction will allow to obtain the quantitative data about microvascular blood flow. The possibility of direct recording of the images of the bulbar conjunctiva in digital form with good spatial resolution is shown. This allows to evaluate the morphological parameters and the distribution of blood flow in the capillaries and small vessels at different effects on the cardiovascular system of the patient.

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