A study of hemodynamics and the structure of capillary blood flow using the method of laser speckle contrast analysis LASCA

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Abstract. The LASCA method can be applied to the study of microcirculation of superficial microvessels. A chicken embryo of the 5th - 7th day of incubation was used as a model of the vascular system. The effect of laser occlusion of a vessel on blood microcirculation in the vascular system was studied. Using the LASCA method, we can conclude about the presence or absence of the blood flow in the vascular system. The contrast value C, calculated by the method, shows the blood redistribution in the vascular system before and after occlusion.

1. Introduction

Observation of blood microcirculation is an important issue of modern medical diagnostics. There are numerous cardiovascular diseases: chronic venous insufficiency, diabetes and other diseases which cause changes in the capillary blood flow. Identification of hemodynamic pathologies and structure of the capillary blood flow is a prerequisite of making a medical decision at both stages: diagnosis and during treatment [1,2].

Now the most effective methods for determining hemodynamics and structure of the capillary blood flow are the methods of dynamic light scattering. One of them is laser speckle visualization [3].

This method makes it possible to estimate the average value of the speed of the capillary blood flow. Using speckle microscopy, an assessment of changes in speckle pattern contrast for individual capillaries is carried out.

2. Laser speckle contrast

J. David Briers and Sian Webster developed a laser speckle contrast analysis method LASCA [3, 4]. The method of LASCA is based on spatial and time statistics of the speckle.

For information about the movement of diffusers, one can analyze the intensity fluctuations of individual speckles. The image is integrated over the exposure time. Thus, to detect the components of spatial distributions with high speeds, e.g. when studying the capillary blood flow, the minimal exposure time should be sufficiently small, i.e. some milliseconds, for the method to work effectively in real time [5].

By the speckle microscopy method, the changes in the contrast of speckle images for the selected capillaries can be assessed.

The contrast of speckles is determined as the ratio of the standard deviation of the fluctuation of intensity to the average intensity [5].
The contrast determines the blurring level of the speckle image and the time of the exposition. If the object shows no or a little movement, there will be no blurring, or it will be insignificant. The speed is maximal when the exposition is minimal.

3. Results

3.1 Experimental setup

In order to study the hemodynamics of the blood flow, an experimental setup (Figure 1) has been used. This setup is an optical system of capturing and processing images (Figure 2), which consists of a digital microscope; a laser ($\lambda=650\text{nm}, W=30\text{mW}$) which provides the scattered radiation to create a speckle-structure on the surface of the researched object; a laser ($\lambda=405\text{nm}, W=60\text{mW}$) which is used to create occlusion of the microcirculatory vessel.
The “microscope - 405nm laser” system was placed with micrometer screws on a slide, where the laser was fixed. The laser beam was focused on the microvessel. The field of the microscope in the plane of the object was 2400 x 2000 μm. The image of microvessels was recorded using a microscope consisting of a lens (magnification x3, numerical aperture NA = 0.11, tube length 160), a cylindrical tube and a digital monochrome CMOS camera DCC1545M (Thorlabs, Germany), with a resolution of 1280 x 1024 pixels using USB2.0.

The camera was connected to a personal computer (PC). The PC was used to display and process images of microvessels. The microscope focusing on the object was moving the tube along the optical axis using a screw adjustment device mounted on a fixed base.

Microvascular images were recorded using software (Figure 3) developed in the LabVIEW 8.5 Professional Development System (National Instruments, USA) programming environment.

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C_y = \sqrt{\frac{1}{(n+1)^2} \sum_{x=1}^{n} \sum_{y=1}^{n} I_{x,y} - \left( \frac{1}{(n+1)^2} \sum_{x=1}^{n} \sum_{y=1}^{n} I_{x,y} \right)^2}
\]

where \(I_{i,j}\) is the intensity of pixel \(i, j\) and \(n + 1\) is the size of the area over which the contrast is calculated.

3.2 Chicken embryo microcirculation study by LASCA method

The practical implementation of the methods of registering and processing the images has been performed on a superficial microcirculatory vessel of a chicken embryo on the 5\textsuperscript{th} – 7\textsuperscript{th} day of incubation before and after occlusion of these vessels (Figure 2).

Figure 4 shows blood redistribution after occlusion of the vessel. Images A, F are original and images B, G demonstrate the results of the process received using the method of LASCA.
Figure 4. Blood redistribution in the vessel after occlusion. A and B – vessels before occlusion; F and G – after occlusion.

It can be observed that the vessel which has undergone laser occlusion is seen in the original image (Figure 4F). However, the image which has been processed with the method of LASCA (Figure 4G) demonstrates the lack of the blood flow in this vessel.

Figure 5a shows the vessels of the embryo and specifies the values of the contrast in the control points. The value of the contrast has been determined according to the average value of the contrast received from 100 framed images. The values are averaged in order to take into consideration the influence of the changes in blood during the process of pulsation. The vessel FG has been cauterized with the laser. After laser occlusion (Figure 5b), the cauterized vessel disappeared because the blood flow had stopped. By the value of the contrast, we can make conclusions about blood redistribution. At points A B the contrast has increased in 10%, at points C D the contrast has decreased. Therefore, we can say that at point A the speed of blood flow has decreased and at point D it has increased because of the occlusion conducted on the vessel between points F and G.

Figure 5. The vessels with the values of the contrast before (a) and after the occlusion (b).
Findings of research show that LASCA is a real-time, full-field, non-invasive and non-contact method of capturing images of different kinds of flows such as the capillary blood flow. For this method, accessible equipment and user-friendly mathematical tools are used. The main disadvantage of LASCA is the loss in the image resolution which is due to the necessity to receive the average value of the contrast in a block of pixels in order to have spatial statistics used in the analysis. However, the advantage of this method working real-time, without scanning, outweighs the problems in resolution, especially for biomedical application.

4. Conclusion
An optical in-vivo method was used to measure blood microcirculation. We presented the results of the study of superficial microvessels of a chick embryo on the 5th - 7th day of incubation, the study being carried out using the method of LASCA. The effect of laser occlusion of a vessel on microcirculation of blood in the vascular system by the LASCA method has been studied.

The vessel that was subjected to laser occlusion is visible on the original image. However, an image processed by the LASCA method indicates the lack of the blood flow in it. According to the contrast values, we arrive at the conclusion concerning blood redistribution in the vascular system after occlusion.

References
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