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Laser Speckle Contrast Imaging of Abdominal Organs in Mouse Model

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ABSTRACT

Diagnosis and treatment of acute destructive pancreatitis is one of the most urgent problems of abdominal surgery. To obtain additional diagnostic information about the microcirculation state and metabolic processes, various imaging techniques are widely used during surgical interventions. One of the most perspective techniques for *in vivo* microcirculation assessment is laser speckle-contrast imaging (LSCI), allowing for evaluation of blood perfusion. The experimental results showed the possibility to evaluate the blood perfusion of the mouse pancreas in the simulation of ischemia using the LSCI method.

Keywords: speckle contrast, speckle pattern, blood flow, mice pancreas, ischemia

1. INTRODUCTION

Acute destructive pancreatitis became the one of the urgent problems of emergency surgery of the abdominal cavity in the last decade due to its high mortality and increased frequency of disease complications. Particularly, mortality of pancreatitis is 20-45% without differentiation of clinical forms. Moreover, mortality reaches up to 85% in infected pancreatic necrosis, and 100% in the fulminant course of the disease[1,2]. One of the main problems faced by surgeons is the need to obtain more diagnostic information during surgery operations. An important approach to obtain information about the clinical condition of the patient is the use of informative rapid diagnostic methods allowing the surgeon to timely assess the patient's condition during surgery and choose the right tactics.

The main factors that play a role in the progression of the disease combine microcirculation disorders of the pancreas, ischemic reperfusion injury, the transition from apical apoptosis to necrosis and the complex interaction of these factors. Ischemic-reperfusion injury of the pancreas is one of the trigger mechanisms of its tissue pathomorphosis. These mechanisms had identified subsequent events and manifestations of diseases[3,4]. Violations of microhemodynamics play a leading pathogenetic role in the progression of acute pancreatitis[4,5].

Currently, a large amount of optical imaging techniques has been implemented for detection of changes in microcirculation in tissues as well as for studying of various biochemical processes closely related to blood supply disorders. At the moment, the most effective methods for determining the basic parameters of microcirculation are the methods of diffuse reflectance imaging[6,7], laser Doppler imaging[8–10] and laser speckle-contrast imaging (LSCI)[11–14].

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LSCI is an effective method for full-scale monitoring of particle dynamics in heterogeneous media. This method is widely used in Biomedicine because it allows researchers and physicians to evaluate the course of physiological processes *in vivo* with high temporal and spatial resolution. In LSCI, the image of dynamic inhomogeneities is obtained by analyzing the local speckle contrast in the image plane[15]. If inhomogeneous object is illuminated by the coherent light, the randomly changing intensity pattern, produced by random interference in object, can appear. This pattern is widely known as speckle pattern. Movement of particles inside the illuminated medium causes the fluctuations in the intensity of speckle pattern and results in blurred image due to the finite exposure time of detector. Further, temporal and spatial statistics of the speckle pattern can be used for obtaining of information on the motion of scattering particles. The estimation of the contrast of time-averaged dynamic speckles depending on the averaging time of speckle-modulated images forms the data for analysis and processing. The spatial contrast of the speckle image is calculated from a single recorded image of the speckle field in the area.

Analysis of the effectiveness of new diagnostic methods for assessing the degree of acute pancreatitis in humans is impossible without preliminary experimental studies on phantoms and linear animals. There are many methods for modeling pancreatic diseases in the experiment, ranging from mechanical damage to its tissues to complex combined effects. This allows to reproduce acute, chronic and recurrent forms of the disease.

Currently, the most common models of acute pancreatitis[2] consider the occlusion of the loop of the duodenum, a choline-deficient diet, supplemented with ethionine, ligation of bile duct and pancreatic duct, injection cerulein, perfusion of the bile duct and pancreatic duct, the introduction of alcohol and the combined perfusion of the bile duct and pancreatic duct with simultaneous cerulein hyperstimulation[16–18]. In our experiment, a model of tissue ischemia was chosen by ligating the vessels of the body of the pancreas to create a blood flow failure in the vessels of the organ.

The aim of the work is to develop a method of application of LSCI for visualization and detection of pathological processes in various organs of the abdominal cavity by means of complex modeling of acute pancreatitis using pancreatic ischemia in mice.

2. MATERIAL AND METHODS

The following LSCI-based experimental setup illustrated on the Figure 1 has been used in this study: 10 mW laser source working at 635 nm (Edmund Optics Inc, USA) illuminated the object. Further, CMOS- camera DCC 3260M (Thorlabs, USA) with 1936×1216 pixels and 5.86 μm pixel size was used in combination with 34 mm Mitutoyo Plan Apochromat Objective MY5X-802 (Thorlabs, USA) for raw speckle image acquisition. The obtained images were processed by a custom-developed algorithm in the offline regime using a MATLAB r2017b software environment. The spatial algorithm using 7x7 sliding window has been used for speckle contrast images obtaining . Totally, 20 speckle contrast images has been calculated and averaged in final speckle contrast image. Thus, the calculation of the final speckle contrast image was carried out according to the Eq. 1:

$$K = \left\langle \frac{\sigma_N}{\langle I \rangle_N} \right\rangle_k , \tag{1}$$

where $\langle\ \rangle$ – the symbol of averaging; N – the window of averaging NxN (N=7); k – the number of consecutive frames (k=20); $\langle I \rangle_N$ – average intensity in the window NxN; σ_N – standard deviation in the window NxN.

Prior to conducting animal studies, the flow phantom has been created for setup calibration and testing of its sensitivity. The phantom consisted of a capillary tube (diameter 1 mm). 20% Intralipid suspension was pumped through the capillary tube by a syringe. Changes in the speed of movement of the Intralipid in the tube were recorded by a camera, then, post-processing of data was performed according to Eq. 1

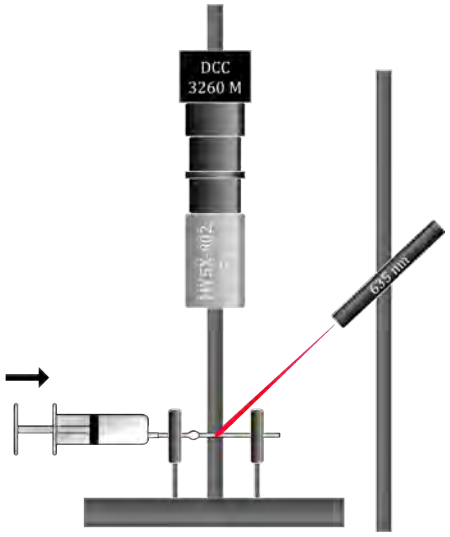

Figure 1 – The setup with capillary tube and syringe.

Experimental studies with animal model were performed on clinically healthy male Balb/c mice (n=6 in the group). Mice were obtained from the FSBI SCBT FMBA of. Russian Federation ("Andreevka" vivarium). Before the animals transfer to the clean area of the vivarium and the beginning of the experiment, the animals were kept in quarantine for 14 days. When placed in quarantine, the veterinarian conducted a primary assessment of the animals condition with the introduction of the examination results in the relevant statement and examined animals daily. The basic rules of maintenance and care corresponded to the standards of the sanitary rules for the arrangement, equipment and maintenance of experimental biological clinics and in the guide "Laboratory animals"[19]. All procedures for routine animal care were carried out in accordance with the Standart operational procedures of the J.-s.c. "Retinoids".

During the study, mice were anesthetized with Zolilet 100 (Vibrac, France), respectively, at standard dosages. The animal was fixed on a special platform in the position on the back. For each animal, a transverse laparotomy was performed. With the help of the tools, access was made to the upper part on the back wall of the abdominal cavity in the retroperitoneal space. After the opening of the operating field, the complex of organs containing the pancreas was carried out to the laboratory table. The body of the pancreas was isolated and ligature from polyester thread was applied. In addition, a cotton swab soaked in a 0.9% solution of sodium chloride (normal saline) was placed in the operating field. Then, the animal was placed under the optical system to visualize the study area and record the frame sequences (see Figure 2). The registration of the speckle-contrast image of the tissue was made on the setup before and after 1, 3, 6, 9 and 12 min the imposition of the ligature.

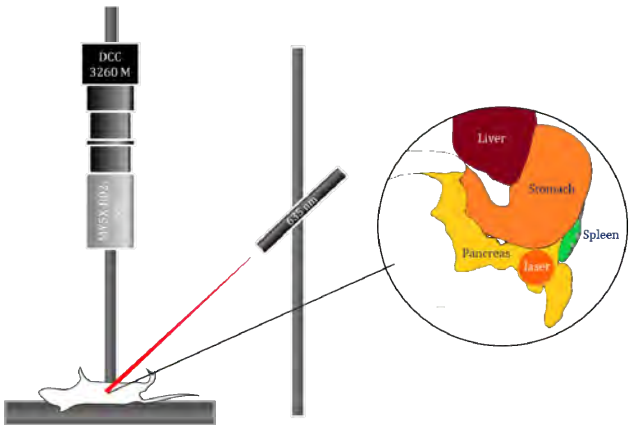

Figure 2 – The setup with the area of laser irradiation of the body of the pancreas.

3. EXPERIMENTAL RESULTS AND DISCUSSION

To check the sensitivity of the installation relatively the change in speed, a sequence of 20 frames was recorded for flow phantom. Three sequences of frames with capillary tube were registered: empty tube, with directed flow of fluid ; with Brownian motion of intralipid. After frames processing, speckle contrast images were obtained (see Figure 3).

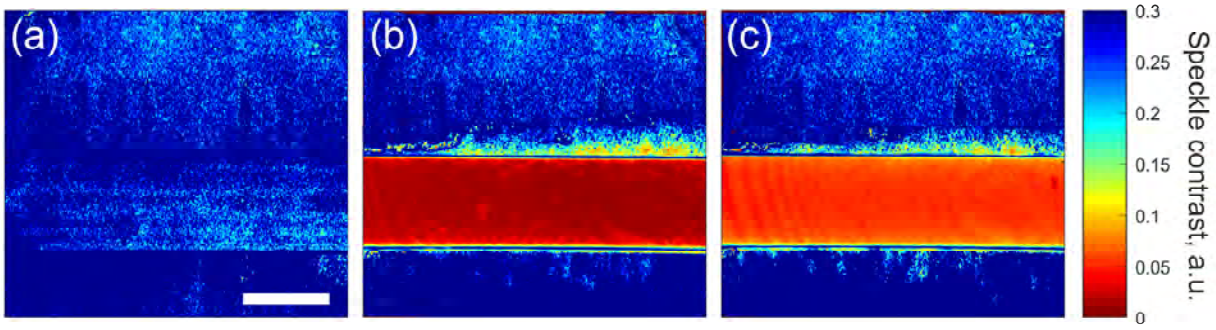

Figure 3 – Speckle contrast images from the phantom (a – without intralipid, b – fluid with direct flow, c – fluid with Brownian motion). Scale bar equals 1 mm.

Based on the analysis of the obtained frames, it is possible to detect the flow rate of the fluid with the help of the assembled setup. As the result, it was suggested that a similar sensitivity will also be noted for blood flow in a microvasculature.

Then, the developed setup is showed in Figure 3 was used to record the sequence of frames in mice. For each stage, a sequence of 20 frames was recorded. After image processing the space-time algorithm obtained spectral contrast images (see Figure 4).

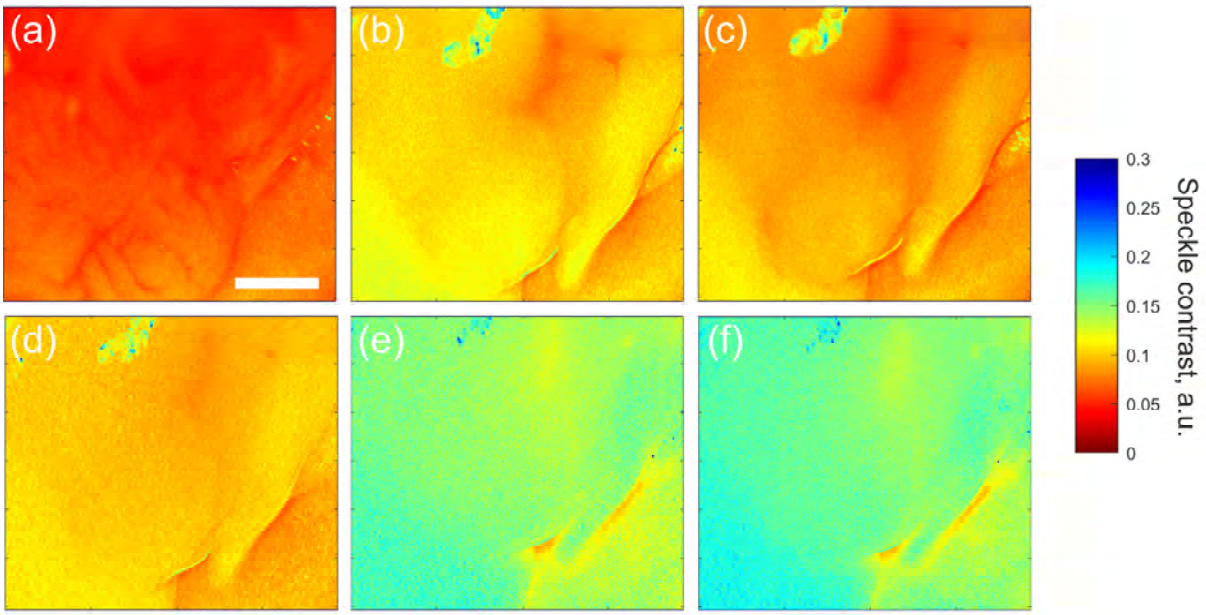

Figure 4 – Speckle images from mice before (a) and 1 (b), 3 (c), 6 (d), 9 (e) and 12 (f) min after ligating pancreas. Scale bar equals 1 mm.

The average contrast ratio of the speckle image in the area of 100x100 pixels was calculated using the Eq. 1 (see Figure 5).

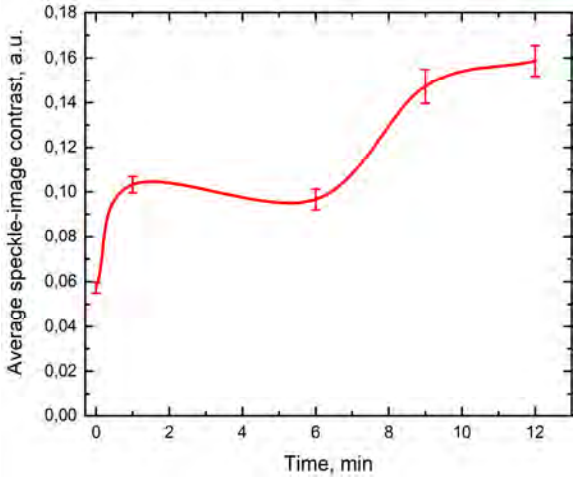

Figure 5 – Evaluation of averaged speckle contrast value taken within part of camera field of view (100 x 100 pixels) during ligation process.

Figure 5 showed ischemia development after ligature registered by speckle-contrast imaging. Increment in averaged speckle contrast value indicates a slowing of blood microcirculation in the pancreas. A decrease in the point corresponding to 6 minutes of the experiment may indicate the occurrence of venous blood flow in the body in the absence of arterial blood flow[20]. This effect is observed with the usual occlusion of the limbs. After 6 minutes, the increasing trend was stayed to the end of recording.

4. CONCLUSION

Performed experiments showed differences in the characteristics of the speckle fields of non-ischemic and ischemic tissue. Changes in the microcirculation registered by LSCI allow physicians to assess the degree of development of pathological disorders.

Preliminary data suggest that the registration of changes by laser speckle contrast imaging is capable for representing different degrees of blood perfusion failure of pancreatic tissue in complex modeling of ischemia. The experiment demonstrated a difference in the contrast of spotty fields in healthy and pathologically altered tissue.

The presented method of imaging potentially allows physicians to find out the degree of organ blood supply during the operation. Also, the development can provide additional diagnostic information and allows the doctor to adjust the course of the operation. Despite the fact that there are respiratory and cardiac oscillations of the tissue, it is planned to reduce the influence of these factors with the help of image processing and stabilization algorithms. The main results of this work will be used to develop a laparoscope with an integrated LSCI channel for intraoperative tissue analysis.

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