

Complex Measurements of Fluorescence and Speckle Contrast in Laboratory Mice during Pancreas Ischemia Modeling

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Introduction

- Acute destructive pancreatitis is one of the urgent problems in abdominal surgery due to its high complications probability and mortality. Violations of microhemodynamics is leading mechanism triggering progression of pathological processes in pancreas.
- There is lack and low quality of diagnostic information about tissue metabolism and microcirculation during minimally invasive surgical operations.
- Possible solution for intraoperative online monitoring of tissues state is optical biopsy.

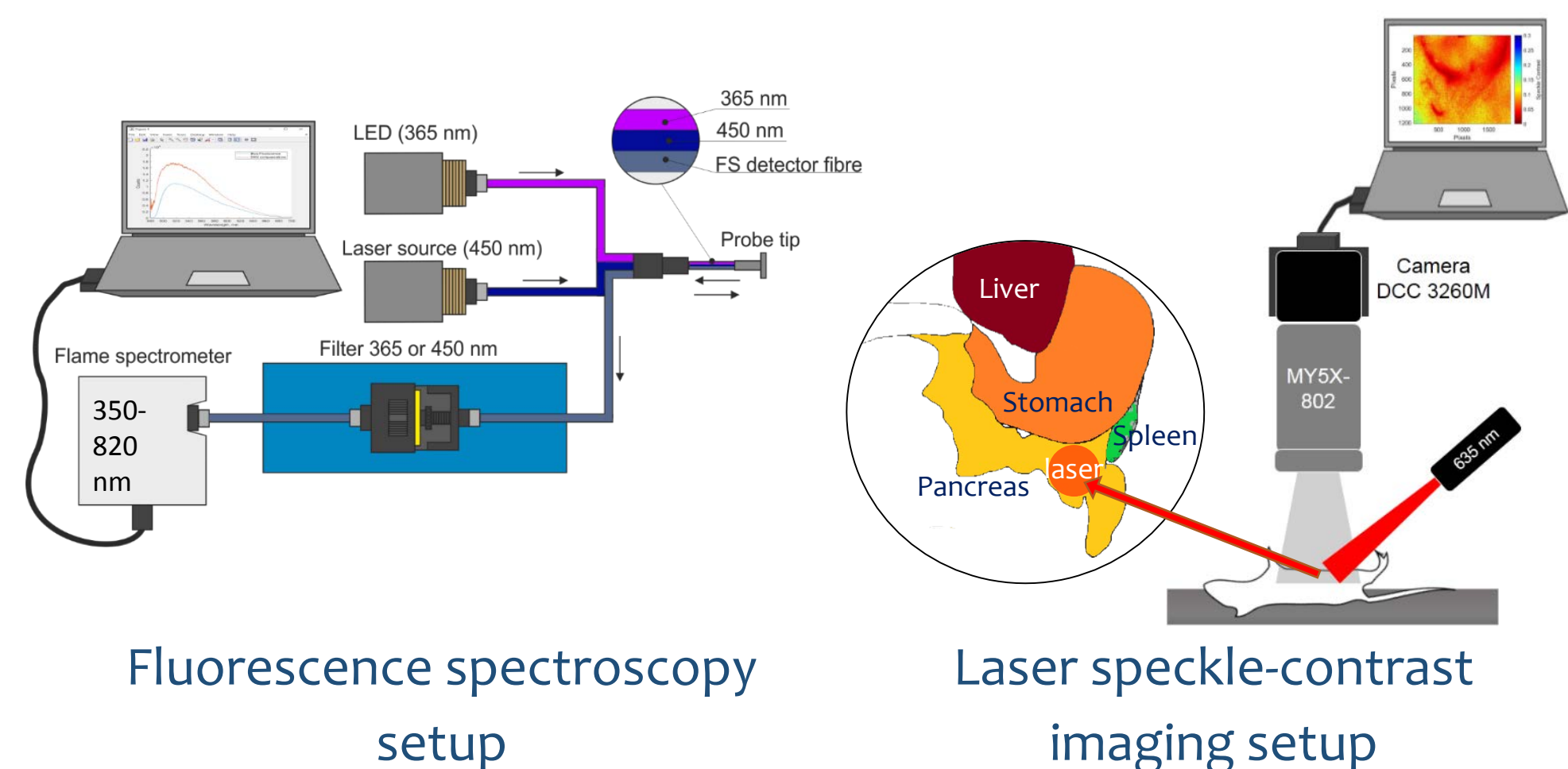
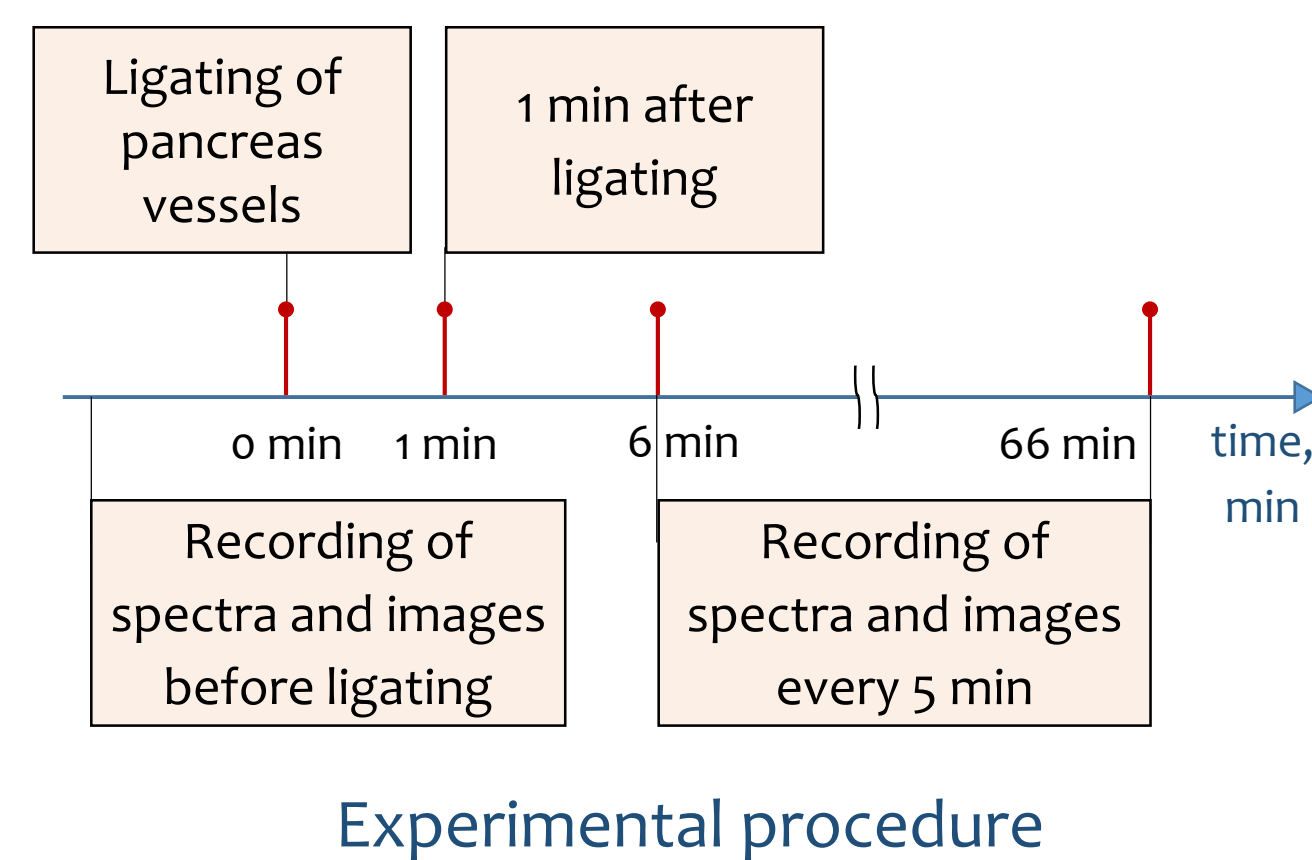
The aim – to study the possibility of combined application of optical spectroscopic and imaging techniques for multimodal diagnosing of the pancreas tissue state.

Materials and Methods

To model microcirculation violations occurring in pancreatitis ischemia was induced in laboratory mice.

The study was approved by Ethics committee of Orel State University (record of the meeting №10 of 16.10.2017).

Used methods included laser speckle-contrast imaging (for blood flow evaluation) and fluorescence spectroscopy (for metabolic state evaluation).

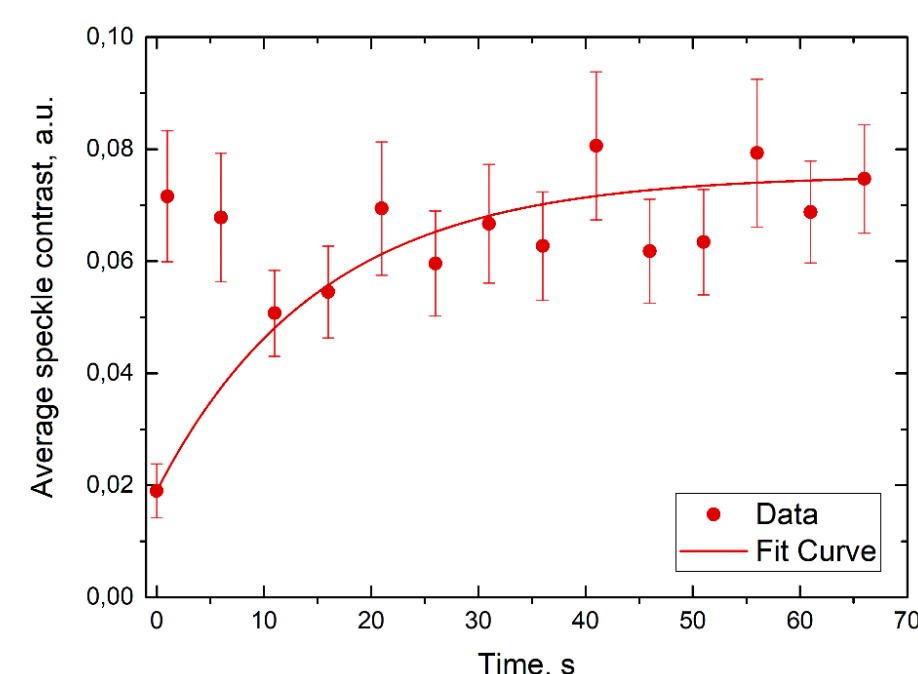


Results and Discussion

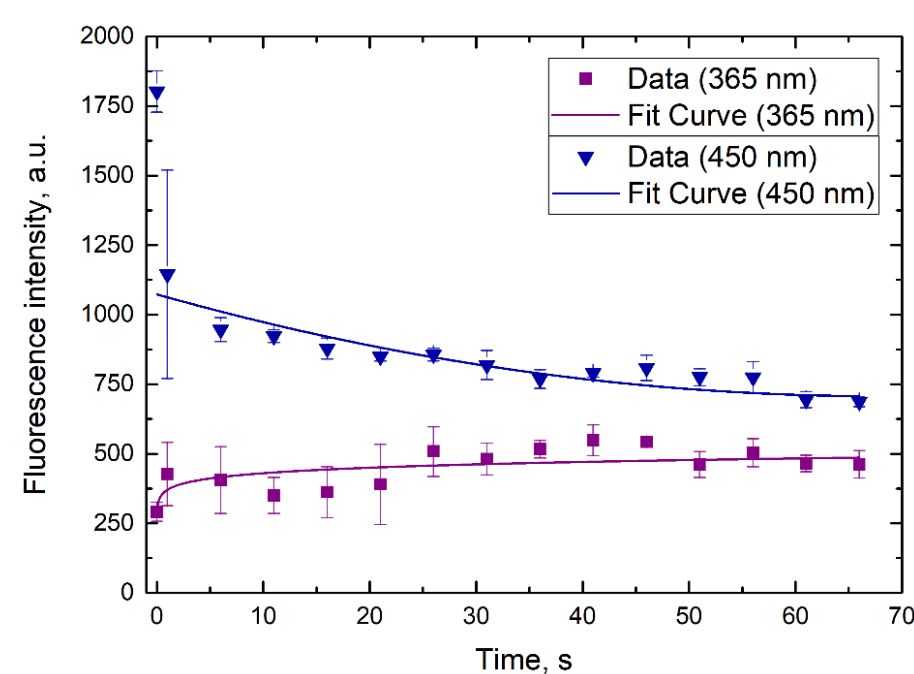
The standard spatio-temporal algorithm has been used for speckle contrast images obtaining. Average speckle contrast formula:

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

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

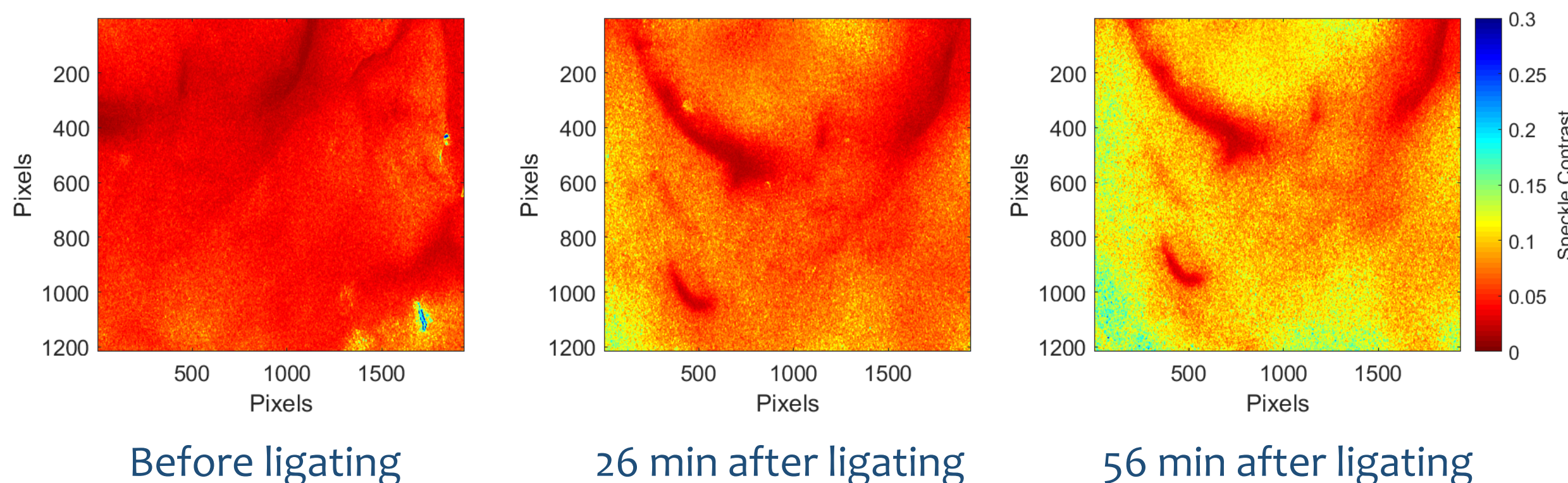


The average contrast ratio of the speckle image in the area of 100 x 100 pixels



Average fluorescence intensity maxima of fluorescence excited by 365 nm and 450 nm

Speckle images (scale bar equals 2 mm)



The intensity of fluorescence excited by 365 nm radiation increases as well as average speckle contrast. At the same time, the fluorescence excited by 450 nm radiation greatly decreased after ligation and continued to drop slowly. Such pattern indicate that progressing of tissue ischemia has led to accumulation of NADH, which is associated with a lack of oxygen. Increased consumption of FAD manifests in its fluorescence decrease. These effects are a sign of hypoxia and poor blood supply of pancreas tissue.

Pearson correlation between fluorescence intensity maxima and average speckle contrast

Data Sample 1	Data Sample 2	Pearson Correlation Coefficient ($p \leq 0.01$)
Average speckle contrast	365 nm fluorescence	0.6908
365 nm fluorescence	450 nm fluorescence	-0.67575
450 nm fluorescence	Average speckle contrast	-0.80386

Gradual increasing of speckle contrast value after ligating indicates the slowing of blood flow and development of tissue ischemia. Slow change of speckle contrast and its oscillations show that the venous blood flow still occurred as the blood left the vessels remained without arterial supply.

Conclusion

- Obtained results prove the possibility of using multimodal approach to evaluate metabolic activity and microcirculation rate by both spectroscopic and imaging methods.
- Combined measurements of fluorescence intensity and speckle contrast demonstrate close dependence of cell metabolism on oxygen supply. When blood supply in pancreas tissue is disrupted, associated metabolic disturbances occur, which we can see by the negative correlation between fluorescence intensities during the experiment.
- The results of this study will be used for adjusting the techniques of FS and LSCI for application in standard minimally invasive surgery tools.

Acknowledgements

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