

I. IDENTIFICATION DATA

Thesis title:	Automatic segmentation of cell structures from electron microscope images
Author's name:	Markéta Kvašová
Type of thesis :	master
Faculty/Institute:	Faculty of Electrical Engineering (FEE)
Department:	Department of Radioelectronics
Thesis reviewer:	Bartłomiej Zapotoczny
Reviewer's department:	Institute of Nuclear Physics of the Polish Academy of Sciences

II. EVALUATION OF INDIVIDUAL CRITERIA

Assignment	extraordinarily challenging
<i>How demanding was the assigned project?</i>	
<p>Fenestration in Liver Sinusoidal Endothelial cells has a size in the range of 50-350 nm, being mostly below the resolution of visible light. Even super-resolution techniques, as used by the Author Scanning Electron Microscopy (SEM) result in images with a pixel size of ~10 nm, which affects accurate fenestra recognition. What is more, SEM images result in multiple structures similar in size and contrast to fenestrae that must be excluded from the analysis. Considering the above, the creation of an automatic image analysis software for the recognition of fenestrae is extraordinarily challenging. In fact, up to today, there is no accurate automatic method for SEM image analysis allowing for accurate fenestrae detection and quantification of their parameters.</p>	

Fulfilment of assignment	fulfilled with minor objections
<i>How well does the thesis fulfil the assigned task? Have the primary goals been achieved? Which assigned tasks have been incompletely covered, and which parts of the thesis are overextended? Justify your answer.</i>	
<p>The resultant software allows for automatic image analysis with similar accuracy as a semi-automatic method which is based on contrast and which was introduced before. Still, the important factor is omitted by the proposed automatic method, which is the manual selection of the threshold in the semiautomatic method. This step results in discrepancies between the untrained users and may result in different data interpretations. What was lacking, in my opinion, was a discussion of the obtained results after the <i>results</i> section and an explanation of the advantages and drawbacks of the introduced method. The software has the potential to be published after the creation of detailed ground truth masks.</p>	

Methodology	outstanding
<i>Comment on the correctness of the approach and/or the solution methods.</i>	
<p>The Author used a novel methodology based on the quickly developing field of neural network-based image processing. The model was uploaded to Git Hub with a detailed readme file and can be further trained and used by the users.</p>	

Technical level	B - very good.
<i>Is the thesis technically sound? How well did the student employ expertise in the field of his/her field of study? Does the student explain clearly what he/she has done?</i>	
<p>All aspects of the thesis were properly introduced. A thorough introduction of cells and their role, fenestration, microscopy, and data analysis methods is difficult to fit into the limited space of a Master Thesis. Still, a more detailed introduction into the method would be beneficial for the reader. Is the model automatically restricted to the cell edges and excludes the cell nucleus or this part must still be manually done? Were there any other than the presented parameters of the training process tested?</p>	

Formal and language level, scope of thesis	B - very good.
<i>Are formalisms and notations used properly? Is the thesis organized in a logical way? Is the thesis sufficiently extensive? Is the thesis well-presented? Is the language clear and understandable? Is the English satisfactory?</i>	
<p>The language presented is very good. The thesis is well-organized and clearly presented. Short introductions were added before each paragraph (without the third) which is an advantage. The discussion chapter after the result presentation is lacking. However, some aspects were discussed in the <i>conclusions</i> including the future outlook.</p>	

Selection of sources, citation correctness**B - very good.**

Does the thesis make adequate reference to earlier work on the topic? Was the selection of sources adequate? Is the student's original work clearly distinguished from earlier work in the field? Do the bibliographic citations meet the standards?

There are 37 references in the thesis and most of them refer to scientific articles. Two articles in my opinion should be mentioned in the thesis. They do not fully cover the same aspects as investigated in the thesis, but were important steps in developing of certain standards in analysis and quantification of LSEC fenestration: one is dedicated to a standardized method of image analysis in electron microscopy for tissues (Cogger V. et al., 2015 doi: 10.3791/52698) and the second one is dedicated to analysis of fenestration by means of neural networks based on AFM data (Giergiel M. et al., 2022 doi: 10.1016/j.j.bspc.2021.103097).

Additional commentary and evaluation (optional)

Comment on the overall quality of the thesis, its novelty and its impact on the field, its strengths and weaknesses, the utility of the solution that is presented, the theoretical/formal level, the student's skillfulness, etc.

Considering the level of difficulty of a given task, despite the minor remarks I believe the Author deserves the highest grade for the thesis. The Author implements novel methods to address the unresolved (for ~50 years) issue of automatically detecting and quantifying fenestration in LSEC. The results of the thesis if developed further will be used by multiple laboratories working with LSEC fenestration around the World.

III. OVERALL EVALUATION, QUESTIONS FOR THE PRESENTATION AND DEFENSE OF THE THESIS, SUGGESTED GRADE

Summarize your opinion on the thesis and explain your final grading. Pose questions that should be answered during the presentation and defense of the student's work.

The grade that I award for the thesis is **A - excellent**.

1. The training sets, ground through masks and the results seem to have small structures scattered across the cell that are usually not clustered in sieve plates. Could they be removed based on the size criteria or could the Author suggest possible ways to overcome these artifacts?
2. The results from the automatic method seem to almost perfectly match with the data from the semiautomatic method when fenestrae frequency is compared. Nevertheless, when comparing fenestra size and porosity automatic method always shows larger values. Could the Author explain the origins of this result?
3. The Author presented several parameters quantifying fenestration. As porosity, fenestration diameter (presented as the diameter of a fenestration, when assumed it is circular), and fenestration frequency were well introduced before, the Author gives formulas to calculate the roundness and fenestration diameter of an ellipse. In the literature, the roundness and diameter of an ellipse are expressed differently. Does the Author mean circularity? Could the Author elaborate on this?
4. Could the method be implemented on other microscopy techniques, in particular optical nanoscopy techniques?
5. Does the Author think about the publication of the obtained result in a scientific journal?

Date: **10.6.2024**

Signature: