

Přírodovědecká Jihočeská univerzita fakulta v Českých Budějovicích Faculty University of South Bohemia of Science in České Budějovice

## STATEMENT OF THE BACHELOR/DIPLOMA\* THESIS REVIEWER

Name of the student: Johannes Grahammer

Thesis title: Preparation of Specimens for Transmission Electron Microscopy

using Freeze Substitution and Agitation

Supervisor: RNDr. Marie Vancová, Ph.D

Reviewer: Ing. Jana Nebesářová, CSc.

Reviewer's affiliation: Biology Centre of CAS

	Point scale <sup>1</sup>	Points
(1) FORMAL REQUIREMENTS	and annual to the term of the control to	and the control of th
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	1
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		12

Choose one

Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
ractical requirements – points in total		17
POINTS IN TOTAL (MAX/AWARDED)	29	(0-
		48)

## Comments of the reviewer on the student and the thesis:

The submitted bachelor thesis is methodical and is focused on the comparison of three different protocols of specimen preparation for the FS method. The prepared blocks with embedded samples were cut into ultrathin sections and examined in TEM to evaluate the preservation of the cell ultrastructure, mechanical damage of cells, resulting image contrast and extraction of the cell content. In addition, the use of HfCl<sub>4</sub> as a contrast agent replacing UA was evaluated. As can be seen from this overview, the objectives were too ambitious and inconsistent with the extent of the bachelor thesis. The result is that Johannes did a lot of work in the lab, he mastered many methods, but did not managed to describe all these methods in his thesis.

I have also comments to the structure of the thesis. The theoretical part is very brief, while the chapter devoted to the description of used methods is as extensive as the chapter devoted to the results, discussion and conclusions. The discussion represents the weakest point of this work. It is rather an enumeration of publications dealing with the same topic. Conclusions concerning e.g. comparison of the resulting image contrast are not sufficiently justified. There is no list of abbreviations.

<sup>&</sup>lt;sup>2</sup> Enter the number of points awarded.

## Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1/ Is it possible to change the speed of agitation? If so, what speed/level of agitation was used during specimen preparation?

2/ There are a lot of parameters which can influence the resulting image contrast in TEM: quantities of heavy metal atoms in the sample, thickness of ultrathin sections, accelerating voltage, beam current etc. How were these parameters included in the comparison of resulting image contrast?

3/ Do you have any explanation how the presence of HfCl<sub>4</sub> in the substitution solution could damage the plastid ultrastructure of *C. velia* cells?

## Conclusion:

In conclusion,  $\underline{I \quad r \in c \text{ o m m e n d}}$  the thesis for the defense and I suggest the grade  $\underline{very \text{ good } (2)}$ .

In Bechyně, date June 11, 2018

signature

Jana Mehora &

You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).