# Posudek práce

**předložené na Přírodovědecké fakultě JU**

❑ posudek vedoucího ☑ posudek oponenta
❑ bakalářské práce ☑ diplomové práce

Autorka: Ayya Tashlieva
Název práce: Visualization of surface antigens by correlative microscopy
Studijní program a obor: Biofyzika
Rok odevzdání: 2018

Jméno a tituly oponenta: Mgr Tomáš Fessl, Ph.D.
Pracoviště: Ústav chemie a biochemie
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**Odborná úroveň práce:**
❑ vynikající ☑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

**Věcné chyby:**
❑ téměř žádné ☑ vzhledem k rozsahu přiměřený počet ❑ méně podstatné četné ❑ závažné

**Výsledky:**
☑ originální ❑ původní i převzaté ❑ netriviální kompilace ❑ citované z literatury ❑ opsané

**Rozsah práce:**
❑ veliký ☑ standardní ❑ dostatečný ❑ nedostatečný

**Grafická, jazyková a formální úroveň:**
❑ vynikající ❑ velmi dobrá ☑ průměrná ❑ podprůměrná ❑ nevyhovující

**Tiskové chyby:**
❑ téměř žádné ☑ vzhledem k rozsahu a tématu přiměřený počet ❑ četné

**Celková úroveň práce:**
❑ vynikající ☑ velmi dobrá ❑ průměrná ❑ podprůměrná ❑ nevyhovující

**Slovní vyjádření, komentáře a připomínky oponenta:**

In this thesis, Ayya Tashlieva showed the ability of systematic and hard work. She investigated 13 different acrylic resins and protocols for sample preparation for correlative light and transmission electron microscopy as an alternative to high-pressure freezing and freeze substitution. Acrylic resins with varying water content were chosen to preserve fluorescence.

Unfortunately none of these procedures preserved fluorescence signal on detectable level. In some cases, polymerization using high temperature and UV exposure probably caused bleaching of all GFP fluorescence.

As is generally accepted, thoroughly described negative results in science are extremely important. Ignoring the vast information source that is negative results is troublesome in several ways. Firstly, it skews the scientific literature by only including chosen pieces of information. Secondly, it causes a huge waste of time and resources, as other scientists considering the same questions may perform the same experiments.

This thesis could be used as a great source of useful negative results for resin embedding for cross correlative microscopy, since all the protocols and experimental procedures are described in a great detail.

Errors student should avoid in the future:

a) I would appreciate if all the references were in English, especially since the younger generation of scientists doesn't speak Russian any more.
b) Next time, I would suggest asking native speaker for grammar correction, at least in introduction. On the other hand site vocabulary is rich and scientific terms are used properly.
c) In discussion, I would recommend Ayya Tashlieva to try to explain in bigger detail, why used procedures failed. For future work, it might be also useful to indicate next steps leading to possible improvements.

**Případné otázky při obhajobě a náměty do diskuze:**

a) Why did you choose Köhler illumination to detect fluorescence?

b) Since there was no fluorescence signal detected after embedding of GFP tagged *B.burgdorferi* in resin, could you show positive control, i.e. GFP images prior to this step?

c) In heat polymerization methods, you expose GFP tagged samples to temperature over 60°C for approximately 24 hours. Based on known thermal stability of GFP, could you estimate what percentage of original fluorescence signal would be lost after this exposure?

d) UV light polymerization methods (@360 nm). GFP has non-negligible absorption at 360 nm. Do you think that choosing different fluorescence protein and/or different UV wavelength could help to preserve fluorescence? If yes, which GFP variant would you choose?

**Práci**
☑ doporučuji
❑ nedoporučuji
uznat jako diplomovou.

**Navrhuji hodnocení stupněm:**
❑ výborně ☑ velmi dobře ❑ dobře ❑ neprospěl/a

Místo, datum a podpis oponenta:

České Budějovice, 11th January 2018

Tomáš Fessl

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Autor/ka: **Ayya Tashlieva**
Název práce: *Visualization of surface antigens by correlative microscopy*
Studijní program a obor: Biofyzika
Rok odevzdání: 2017

Jméno a tituly oponenta: Mgr. Radek Kaňa PhD.
Pracoviště: Mikrobiologický ustav AVČR, Třeboň
Kontaktní e-mail: kana@alga.cz

**Odborná úroveň práce:**
❑ vynikající ❑ velmi dobrá ☒ průměrná ❑ podprůměrná ❑ nevyhovující

**Věcné chyby:**
❑ téměř žádné ❑ vzhledem k rozsahu přiměřený počet ☒ méně podstatné četné ❑ závažné

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## Slovní vyjádření, komentáře a připomínky vedoucího/oponenta:

In the presented thesis *Visualization of surface antigens by correlative microscopy* by Ayya Tashlieva, author summarizes process of optimizing sample preparation for correlative light-electron microscopy (CLEM). As biological model system, a virulent *B.burgdorferi* Bb914 expressing GFP has been selected. Unfortunately, there is no detailed description of the strain (e.g. where GFP is expressed), and the main aim of the project is not directly mentioned in the thesis. Based on the title of the thesis, it is not clear if author aimed to characterize ultrastructures of *B.burgdorferi* Bb914 by CLEM method, or just tried to optimize method of sample preparation. I guess, student finally focused only to the optimizing method of sample preparation for CLEM, even though title would indicate rather biological questions (*Visualization of surface antigens by correlative microscopy).*
The thesis contains all necessary parts, Introduction, Results and Discussion. From formal point of view, I have notice several English misspelling, there are also no links to figure in the text therefore it was very hard to understand results and discussion. There is also no biological part in Introduction that could at least briefly describe *B.burgdorferi* Bb914 for non-expert. I have not found any details about GFP construct (see enclosed my questions). In the main part of the thesis, result/discussion, several methods of sample preparation has been tested (data are presented in descriptive tables), and on the very end few TEM pictures are enclosed. It seems that Ayya Tashlieva has not finally managed to get both – TEM picture and fluorescence signal of GFP by fluorescence microscope. She has concluded that it is because of sample bleaching. However, there could be also some other explanations (see my questions below). As a conclusion, she has managed to tested several methods of sample preparation for CLEM, but it is not clear which method she would advise for TEM of *B.burgdorferi* Bb914 (see my question below).

**Conclusions:** Based on the number of method for sample preparation she has tested, she has to spend many hours in laboratory. Therefore it is pity that the final version of the thesis does not reflect the fact and the amount of work/time that has been invested. Even though these formal/practical objections towards presented thesis, the text fulfils the necessary pre-requirements for Master thesis.

## Případné otázky při obhajobě a náměty do diskuze:

1. How can UV polymerization affect presence of GFP inside of sample? What was the wavelength of UV used for polymerization? Would not be possible that the UV irradiation during polymerization reduced GFP stability resulting in absence of GFP signal in sample? Do you know absorption spectra of GFP expressed in *B.burgdorferi* Bb914, do they overlapped with UV spectra of the lamp used for polymerisation?
2. Have you checked GFP in native cells before sample preparation by some independent method? (e.g. by fluorescence spectroscopy?).
3. I would expect rather a low level of GFP fluorescence from the thin section sample *B.burgdorferi* (below 100nm) if wide field fluorescence microscope was used. Have you tried/think to use another method, like confocal microscopy for imaging?
4. Based on author expertize, which method of sample preparation seems to the most promising for future CLEM imaging of *B.burgdorferi* Bb914?

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❑ výborně ☒velmi dobře ❑ dobře ❑ neprospěl/a

V Třeboni 11.01. 2018

Karla Radek