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Opponent's report on the dissertation thesis

Thesis title: Advanced Spectral and Image Processing of Hyperspectral Data

Thesis author: Mgr. Ondřej Vaculík

The dissertation thesis focuses on the field of hyperspectral imaging (HSI), bridging the gap between hardware instrumentation and advanced data analysis of the large HSI datacubes. The work includes developing a hyperspectral microscope, evaluating dimensionality reduction algorithms, and applying these tools to the analysis of algal samples. Given the increasing demand for HSI in biotechnology and material sciences, the chosen topic is highly topical and aligns with current trends in optics.

The main goals of the thesis were to build the entire HS analysis pipeline, from HS hardware through data compression and processing to advanced analysis, including machine learning (MCR-ALS, UMAP, t-SNE). The theoretical background provided in the thesis is sufficient, covering the necessary principles of spectroscopy and image processing to support the experimental work.

A significant contribution of this work is the development and calibration of the custom hyperspectral workstation. The author demonstrates a deep understanding of the instrumentation. In terms of data processing, the comparative study of dimensionality reduction techniques (PCA/MNF vs. UMAP/t-SNE) provides useful insights into the trade-offs between spectral fidelity and cluster separation. The practical utility of the work is highlighted by the case studies on algae that demonstrate unmixing spectral signals from different species. This represents a concrete scientific result with direct application potential in biotechnology.

The thesis is logically well structured. The progression from theory to hardware construction, followed by software algorithms and finally biological applications, makes the text easy to follow. The graphical presentation is generally of high quality, particularly the spectral maps and instrument schematics. However, in some cases, I would appreciate a more detailed discussion of the statements – for instance in the calibration steps (see questions below).

The student's publication activity, listed at the end of the document, includes an article in a respected journal in the field of optics, contributions to relevant conferences and prototype development, demonstrating an ability to disseminate research findings.

The dissertation demonstrates the author's ability to conduct independent scientific research, combining hardware engineering with complex data analysis. The work offers valuable contributions to the field of optical spectroscopy and biological imaging.

For the reasons stated above, I recommend this dissertation for defense.

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Questions and comments to be addressed during the defense:

1. In Section 3.2.1, you describe verification of the spectral resolution of the hyperspectral camera. Is it appropriate to measure only a single spectral profile in the camera? How does the resolution differ across different lines of the imaging spectrometer?
2. Figure 3.12 (page 40) shows the comparison between measured HS data (transmission) and NIST reference spectra, showing a significant offset between the measured and reference data. Is it possible to guess the origin of the offset? Since you compare relative values measured for each pixel, I assume that local sensor sensitivity or spectral sensitivity cannot be the reason behind.
3. Can you comment on the robustness of the HS data unmixing for the used neural networks with respect to studying a larger variety of samples? Would a small amount of unexpected “impurity” in the hyperspectral data (from an unwanted substance) distort the analysis?

Turnov, 19.2.2026

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