

**Posudek práce předložené k obhajobě
na Matematicko-fyzikální fakultě Univerzity Karlovy**

- posudek vedoucí/vedoucího posudek oponentky/oponenta
 bakalářské práce diplomové práce

Název práce: Analysis and Reconstruction of Dynamical Speckle Patterns in Interferometric Scattering Microscopy

Jméno, příjmení a tituly autorky/autora práce: Vítězslav Lužný

Studijní program: Matematické modelování

Rok odevzdání: 2023

Jméno a tituly vedoucí/ho nebo oponentky/-ta: mgr. Łukasz Bujak, PhD.

Pracoviště: Ústav fotoniky a elektroniky AV ČR, v. v. i.

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Hodnocená oblast	Hodnocení
Oborová úroveň (zejména fyzikální)	Výborná
Didaktická úroveň (zejména metody sběru a analýzy dat v didaktickém výzkumu, přiměřenost vzniklých materiálů)	Výborná
Práce s literaturou	Výborná
Jazyková úroveň práce (srozumitelnost textu, členění textu, stylistika, pravopis)	Výborná
Grafická úroveň práce (formátování textu, typografie, přehlednost tabulek, kvalita obrázků, videí)	Velmi dobrá
Zásady pro vypracování práce	Splněny

Slovní hodnocení (včetně upřesnění případných výhrad; mj. lze posoudit originalitu a kreativitu zvolených řešení, náročnost tématu apod.):

[Nahradte vlastním textem]

The bachelor thesis titled "Analysis and Reconstruction of Dynamical Speckle Patterns in Interferometric Scattering Microscopy" by Vítězslav Lužný showcases a commendable level of originality and creativity in its approach. The thesis explores the application of deep neural networks (DNNs) in the analysis of speckle patterns within the context of interferometric scattering microscopy (iSCAT), which is a relatively novel and emerging field of research. By combining advanced microscopy techniques with machine learning and deep learning methodologies, the author presents a unique and innovative approach to addressing the challenges associated with the analysis and interpretation of dynamic speckle patterns. The thesis is well-structured and easy to follow, and the author provides a clear and concise overview of the topic. The study conducted by Vítězslav Lužný successfully demonstrates the potential of DNNs in addressing the challenges associated with the analysis and interpretation of dynamic speckle patterns.

The thesis begins with an introduction that establishes the importance of studying dynamic speckle patterns. It highlights how these patterns contain valuable information about the motion and behaviour of microscopic particles, enabling researchers to investigate various phenomena such as Brownian motion, particle interactions, and dynamic processes within biological samples.

The review part of the thesis provides an in-depth examination of relevant topics. It starts by discussing different microscopy techniques, including optical microscopy and its diffraction limit. The limitations of optical microscopy lead to the exploration of fluorescence microscopy, which allows for higher-resolution imaging. The thesis then introduces iSCAT, a cutting-edge microscopy technique that utilizes interferometric measurements to capture and analyse speckle patterns with exceptional sensitivity and resolution. The thesis cites over 30 sources, predominantly journal publications.

The thesis incorporates machine learning techniques to facilitate the analysis and reconstruction of dynamic speckle patterns. It explores various aspects of machine learning, such as supervised learning, which involves training models with labelled data to make predictions. The thesis also discusses specific machine learning algorithms like minibatch stochastic gradient descent (SGD), which optimizes model parameters, and Adaptive Momentum Estimation (Adam), a widely used optimization algorithm. Furthermore, the thesis explores different neural network architectures, including multi-class logistic regression, multi-layer perceptron, convolutional neural networks (CNNs), residual networks, and the U-Net architecture. These deep learning models provide powerful tools for extracting meaningful information from speckle patterns and enabling automated analysis.

In the practical part of the thesis, data simulations are conducted to gain practical insights. The thesis describes the experimental setup necessary for modelling the signal obtained with iSCAT, specifically capturing speckle patterns. Detailed information is provided about the computer setups and software employed for the simulations, ensuring transparency and reproducibility in the experimental process. The thesis also explores the concept of the point spread function (PSF), which characterizes the blurring effect caused by the imaging system. This understanding of the PSF is crucial for comprehending the fidelity and resolution of the captured speckle patterns. Furthermore, the thesis introduces the technique of sparse subpixel convolution, which plays a vital role in enhancing the accuracy of the reconstruction process. By utilizing this technique, the thesis addresses the challenge of improving spatial resolution and ensuring accurate reconstruction of the speckle patterns. This approach significantly reduces the time complexity to a more manageable level. The

implementation of this algorithm was done using C++ programming language with optimizations such as OpenMP for multi-threading and SIMD for enhanced performance. The implementation was wrapped using Cython to create a Python module named "Sparse Subpixel Convolution" for easy integration into Python scripts.

With the optimized implementation, the thesis demonstrates that the proposed algorithm can generate a large number of frames with high-resolution images and multiple particles in a sample within a minute, showcasing a significant performance increase compared to previous approaches.

The thesis explores various classification models, including the Multi-Layer Perceptron (MLP) with 2000 neurons, which achieves only 16% accuracy on the validation dataset. The Two-dimensional ResNet, a deeper convolutional neural network, performs better with up to 78% accuracy. Considering the temporal dimension, the Three-dimensional ResNet achieves a comparable accuracy of 78% with fewer parameters (719,008) than the Two-dimensional ResNet. The Two-plus-one-dimensional ResNet further improves efficiency by splitting convolutions, resulting in an accuracy of 81% with increased capacity.

The thesis addresses the task of particle localization within image sequences. The U-net architecture is modified by incorporating the 2D ResNet as a backbone. The model achieves outstanding performance, with an accuracy of 99.931%. By effectively utilizing temporal information, the model accurately localizes particles within the field of view.

However, it is worth noting a few minor issues that were observed in the thesis. Specifically, there were instances of inconsistency in font sizes used in the figures, which could potentially affect the visual presentation and clarity of the content. Additionally, certain figures, such as images 2.1, 2.2, and 2.12, lacked labelled axes, which may make it slightly challenging to interpret the visual information provided. Addressing these issues would further improve the overall quality and readability of the thesis.

In conclusion, Vítězslav Lužný's thesis on the analysis and reconstruction of dynamic speckle patterns in iSCAT microscopy makes a significant contribution to the field. The thesis stands out for its originality and creativity, evident in various aspects of the research. From integrating deep neural networks (DNNs) to exploring novel techniques and adapting neural network architectures, the author consistently demonstrates innovative thinking. The development of a simulation module and the successful adaptation of DNN architectures showcase the author's dedication and expertise. This work opens up exciting possibilities for advancements in speckle pattern analysis using DNN-based approaches.

Případné otázky k obhajobě:

[Nahradíte vlastním textem]

1. On page 22, you mentioned the classification of each image sequence into 8 classes. However, I respectfully believe that having just one class to indicate the presence or absence of particles would be sufficient, especially considering the feasibility of particle counting. I kindly request clarification regarding the reasoning behind your choice of this classification approach.
2. As mentioned on page 30, "Our U-net achieves an accuracy of 99.931%, which corresponds to 2.826 pixels incorrectly classified per sample on average." However, considering that „the average particle count in the field of view (FOV) is around 3.3“, it implies that even if the model missed all the particles, it would still show an accuracy of 99.85%. In cases where the class distribution is highly imbalanced, such as the scenario you described, accuracy alone may not be the most suitable metric to

evaluate model performance. This is because a high accuracy can be achieved even if the model predominantly predicts the majority class and fails to identify the minority class. This raises the question of whether there is a better way to calculate accuracy for such problems. Can you please provide some alternative metrics?

3. As highlighted on page 33 of the thesis, there is an acknowledgement that the demonstrated performance on simulated data might not directly translate to real-life experiments. This raises an important question: How can we bridge the gap and ensure the applicability of the findings in real-world scenarios?

Předloženou práci

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uznat jako práci bakalářskou/diplomovou.

Předloženou práci hodnotím stupněm: **Výborně**

Datum a místo: 19.06.2023, Praha

Podpis: 