EVALUATION REPORT OF PHD DISSERTATION

Thesis: Interaction of fluorescent nanodiamonds with cells investigated by interferometric scattering microscopy techniques

Student: Ing. Kateřina Žambochová

Affiliation: Czech Technical University in Prague, Faculty of Biomedical Engineering Department of Natural Sciences

The dissertation submitted by Kateřina Žambochová, titled "Interaction of Fluorescent Nanodiamonds with Cells Investigated by Interferometric Scattering Microscopy Techniques," represents a significant advancement in the field of interferometric scattering microscopy. Over the past two decades, Interferometric Scattering Microscopy (iSCAT) has transformed our ability to detect and track single biomolecular interactions. Along with mass photometry, the quantitative extension of iSCAT, these developments have led to rapid growth in label-free bioanalytics in both academia and industry, with the number of scientific publications approximately doubling each year. While iSCAT is strengthening its position in biomolecular analytics with single-protein sensitivity due to highly quantitative metrics, biological questions and particularly in vivo applications are largely underexplored due to the high complexity of acquired data. Interferometric imaging in living cells has been unlocked only recently, and Kateřina Žambochová's work is among the pioneering efforts enabling this leap. The impact of her work is supported by two publications with strong international collaboration.

The essence of the thesis lies in combining fluorescence-based imaging and scattering-based imaging of diamond nanoparticles featuring fluorescent defects, mostly nitrogen vacancies. These complementary techniques extend the application range in detection sensitivity and speed while maintaining very high specificity.

The dissertation presents a thorough introduction and review, capturing the underlying physics of the technology and data interpretation, as well as the current state of the art. In the materials and methods section, the PhD candidate presents a detailed optical setup design combining the scattering and fluorescence imaging arms and the synchronization of image acquisition. This section further describes the sample preparation and characterization. The longest section scrutinizes the results of the PhD work, covering two main parts of the research, which closely correspond to her two publications: axial profiling of the interferometric image of subdiffractional particles and cellular imaging with diamond nanoparticles. Among the main results, Kateřina demonstrated in-depth characterization of laboratory samples and the possibility of synchronous tracking of single nanodiamonds of different sizes. By studying the internalization rates and diffusive characteristics, she demonstrated the possibility to carefully pinpoint the fate of each nanoparticle, extrapolating its interaction with cellular structures, interpreting the confined diffusion between cellular compartments, or the probability of nanoparticle uptake in intracellular transport vesicles. These results fulfill the aim of the dissertation as well as the partial objectives.

Kateřina Žambochová's dissertation is well-written, logically structured, and highly informative. Each results section is closed with dedicated short conclusions emerging from the respective publication, while the thesis conclusions and outlook section remains compact. The figures and graphics are visually appealing and effectively convey the core messages and findings. There might be a few labels missing in the figures here and there (e.g., Fig. 31), or in some cases, color bars would help in quantifying the gray-scale iSCAT contrast. While understanding the overall structure of the results section, for the format of a PhD thesis, it would be advantageous to dedicate a separate theoretical section to the simulations preceding the experimental work and discussion. Nevertheless, these small imperfections do not lower the impact and high overall quality of the work.

In summary, Kateřina Žambochová's dissertation represents an important contribution to the field of iSCAT microscopy and single-particle tracking methodology. The work is characterized by its technical rigor, innovative methodologies, and significant impact on our understanding of cellular processes. The dissertation meets the standards of scientific writing and presentation. While there are minor areas for improvement, these do not detract from the overall excellence of the work. Given the depth of the research, the quality of publications, and the potential for future contributions to the field, I recommend the dissertation for acceptance.

Marek Piliarik, Ph. D.
Institute of Photonics and Electronics,
Academy of Sciences of the Czech Republic
Chaberská 1014/57, 18251, Praha 8 - Kobylisy, Czech Republic
piliarik@ufe.cz http://nanooptics.ufe.cz/

Specific questions for discussion during the defense:

1. The combination of fluorescence detection and iSCAT imaging is often challenging due to the large power fluxes required for a high interferometric signal-to-noise ratio. How did you balance the power requirements of these two methods, and what do you identify as the main limitations?
2. The literature often analyzes and interprets the subdiffusive behaviors of partly confined or compartmentalized trajectories using thorough microscopic models. However, superdiffusion often remains underexplored. Did you try to interpret the superdiffusive mean square displacement (MSD) characteristics in terms of forces, velocities, or energy consumption to extract more quantitative information from your unique data?
3. When comparing the simultaneous trajectories of single nanodiamonds acquired in the iSCAT and fluorescence channels in Figures 43 and 44, it is striking that the iSCAT channel is slightly noisier than the fluorescence channel. Is there a quantitative tradeoff in the precision of these two simultaneous methods that needs to be considered? How far can this tradeoff be balanced in favor of one method over the other?