

**Faculty of Physics** - Quantum Optics,  
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**Review of the doctoral dissertation**  
**“Spatiotemporal Light Manipulation**  
**for Nonlinear Microscopy”**  
by **Paweł Szczyrkowski**  
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To the Scientific Council of the Discipline of Physical Sciences, University of Warsaw,

The doctoral dissertation entitled “Spatiotemporal Light Manipulation for Nonlinear Microscopy” by Paweł Szczyrkowski addresses fundamental and applied problems in contemporary nonlinear optical microscopy. The thesis focuses on the design, construction, and experimental validation of advanced optical systems enabling high-resolution imaging, in particular in scattering environments and under nonlinear excitation conditions.

The work is positioned at the intersection of physical optics, nonlinear light–matter interaction, and advanced bioimaging. It combines theoretical analysis of nonlinear and statistical optical phenomena with the development of custom experimental instrumentation. The central theme is the exploitation of optical nonlinearity—both in excitation and detection—to overcome classical resolution limits and to enable imaging in regimes inaccessible to conventional linear methods. Overall, the dissertation constitutes a coherent and well-structured body of research. It demonstrates technical maturity, conceptual clarity, and a strong emphasis on reproducibility, as evidenced by the detailed build guides and alignment procedures included in the appendices.

The main results of the thesis are as follows:

### Chapter 3: Two-Photon Scanning Microscopy (TPSM)

The candidate built a two-photon scanning microscope that was used, in collaboration with biologists, for in vivo imaging of neuronal activity in mice. The setup was further extended to implement variants of TPSM, including Bessel-beam excitation and Image Scanning Microscopy. Paweł Szczyrkowski compares these techniques not only from a specification-oriented perspective, but also with respect to their experimental complexity and usability in realistic biological applications.

#### Chapter 4: Two-Photon Wide-field Temporal Focusing

The candidate designed and constructed a wide-field two-photon microscope based on temporal focusing and implemented super-resolution capabilities using Super-resolution Optical Fluctuation Imaging (SOFI). The combination of these advanced concepts is technically sophisticated and requires a solid understanding of ultrafast pulse shaping, spatiotemporal dispersion, statistical fluorescence analysis, and computational image reconstruction.

#### Chapter 5: Nonlinear Imaging with Speckle Excitation (NISE)

Finally, the candidate presents a novel microscopy technique based on highly nonlinear nanoparticles. These strong nonlinearities enable super-resolution imaging in the absence of ballistic excitation light. It is shown that highly scattering media generate intensity variations in the resulting speckle patterns, which, when combined with a strongly nonlinear optical response, lead to an effectively localized excitation of the nonlinear particles. This result is both conceptually and experimentally significant. The work reflects deep expertise in wave propagation in disordered media, statistical optics, and nonlinear excitation physics, combined with careful experimental validation. The discussion of limitations (twin images, thick scatterers) demonstrates scientific maturity and critical reflection.

Designing, constructing, and experimentally validating three different super-resolution microscopy platforms, and advancing them from the design phase to publication-level results, is a remarkable achievement within a Ph.D. project. The thesis demonstrates a broad understanding of nonlinear and volumetric imaging techniques, as well as exceptional experimental aptitude and flexibility. While Chapters 3 and 4 show that the candidate can quickly master and implement established imaging modalities, Chapter 5 demonstrates the ability to develop creative and original concepts with the potential to push three-dimensional imaging in scattering media into new regimes. In addition, Paweł Szczyrkowski has shown that he can collaborate across disciplines and understands the challenges of translating advanced optical instrumentation into biologically meaningful experiments. Potential questions for the defense are listed below.

I therefore strongly recommend that the doctoral degree be awarded. In my assessment, no major revisions are required. Given the experimental sophistication demonstrated throughout the thesis, and the originality of the NISE concept, I suggest the dissertation to be considered for distinction.

Best regards,



Thomas Juffmann

### Potential Questions for the Defense

1. How can the high nonlinearity exploited in NISE be described using microscopic energy-level diagrams? Which physical processes dominate (e.g., multiphoton absorption, excited-state absorption, avalanche processes), and what are the relevant timescales governing the nonlinear response?
2. What are the practical limitations of combining temporal focusing with SOFI in biological applications? In your view, which concrete biological applications would most benefit from this setup, and what would still need to be optimized?
3. Suppose you aim to perform metabolic two-photon imaging (e.g., NADH/FAD autofluorescence) in live organoids of size 100–200  $\mu\text{m}$ . Which imaging modality would you choose, and why? Would your choice change if the system had to be transferred to a clinical research environment? Please justify your decision in terms of spatial resolution, penetration depth, phototoxicity, photon efficiency, complexity, and usability.