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04.03.26

Re: Spatiotemporal Light Manipulation for Nonlinear Microscopy,
The PhD Thesis by Paweł Szczypkowski

Dear members of the evaluation committee,

The above thesis presents a coherent body of work cantered on the use of optical nonlinearities for advanced microscopy, with particular emphasis on imaging under challenging conditions such as scattering media. The manuscript spans two-photon microscopy, temporal focusing, fluctuation-based super-resolution, and culminates in a nonlinear speckle-based imaging approach (NISE). Overall, the thesis is technically solid, clearly structured, and reflects substantial experimental effort and independence.
The work demonstrates strong experimental competence. The candidate clearly understands the optical setups in depth and makes a commendable effort to document them in a way that supports reproducibility. The inclusion of detailed setup descriptions, calibration procedures, and workflow documentation (particularly in Chapters 3 and 7) is exemplary and will be genuinely useful to future researchers.
The thesis is written in a didactic and engaging style. While this does not always succeed stylistically—some sections lean toward a “teaser” or presentation format that is unconventional for a thesis—the intent to make the material accessible is evident and largely appreciated. This effort to communicate clearly, rather than merely to demonstrate expertise, is a strength of the manuscript.
At the same time, the manuscript occasionally moves quickly between topics, and in some places the framing tends toward overselling. In particular, certain claims would benefit from a more precise positioning within the existing literature and a more careful distinction between conceptual novelty, technical implementation, and performance gains.
In Chapter 2, the thesis states:
“It took roughly a century from Abbe’s original formula to show, experimentally, that the diffraction limit is not absolute but rather obsolete.” This formulation is problematic. Ernst Abbe was rigorous in clearly stating the assumptions underlying his diffraction limit. The resolution bound he derived is valid within those assumptions—namely, linear, shift-invariant imaging systems operating with incoherent illumination and conventional detection. Modern super-resolution techniques do not invalidate Abbe’s reasoning, rather, they circumvent specific assumptions (e.g., by exploiting nonlinearity, structured illumination, stochastic fluctuations, or prior information).

Thus, it is historically and conceptually inaccurate to suggest that Abbe's limit was shown to be "obsolete." A more precise statement would be that the applicability of the limit has been overstated by the relevant community for a long time, it shall be acknowledged that is not universal, but strictly conditional under the assumptions made. The thesis would benefit from revising this language to reflect the distinction between violating assumptions and disproving a theorem. Given the otherwise careful pedagogical tone, this is an important correction.

The chapters on two-photon microscopy and temporal focusing are well executed. The description of the setup inspired by Benjamin Judkewitz's work is detailed and practically useful. The inclusion of auxiliary explorations (e.g., two-photon image scanning microscopy, Bessel-beam excitation) and the honest assessment of their practical limitations is commendable.

The presentation of super-resolution fluctuation imaging combined with temporal focusing is technically sound. The candidate does a good job of explaining both the physical intuition and implementation details, maintaining a reasonable balance between theory and experiment.

These chapters convincingly demonstrate the candidate's ability to design and optimize advanced optical systems, critically assess performance limits and translate conceptual ideas into functioning platforms.

Chapter 5 introduces a nonlinear speckle-based imaging approach (NISE), in which extreme nonlinear material response effectively promotes the strongest speckle grain above all others, producing a dominant focus. By exploiting the memory effect and scanning the speckle pattern, imaging through scattering media is achieved, with apparent super-resolution compared to linear imaging. The experimental execution appears solid, and the results are clearly presented.

However, here I have reservations regarding the degree of conceptual novelty as framed in the thesis. The work is a creative and well-executed implementation of nonlinear gating within a speckle field, but its conceptual ingredients, specifically the nonlinear contrast enhancement, speckle correlations, and memory-effect-based scanning, are individually well established. The novelty lies in their combination and in the exploitation of unusually strong nonlinearity, rather than in a fundamental shift of imaging principles. I therefore recommend moderating the language to better distinguish between fundamental conceptual innovation, novel combination of known effects and practical performance advances enabled by specific materials. That said, Chapter 5 is likely the most original and ambitious part of the thesis and reflects genuine scientific maturity.

The "inspirational" and presentation-like tone in introductory chapters is unusual for a PhD thesis but not inappropriate. I really like the analogy at the beginning of 4.1. The explicit articulation of goals, accessibility, reproducibility, and scientific communication, is refreshing. However, the manuscript would benefit from slightly more restraint in evaluative language (e.g., "fantastic," "completely new paradigm" or "entirely novel"), more precise claims when discussing limits and breakthroughs, and a clearer separation between intuitive explanation and formal arguments. The occasional rapid transition between topics can make the intellectual thread harder to follow. Some additional connective discussion clarifying how each platform addresses the central depth–resolution trade-off would strengthen the overall coherence.

The thesis demonstrates strong experimental capability, clear understanding of nonlinear optics in microscopy, ability to build and document complex systems, and independent critical thinking (particularly evident in the assessment of less successful experimental directions). The work contributes meaningfully to nonlinear microscopy and imaging through scattering media. While some claims should be reframed with greater conceptual precision, the underlying research quality is high.

In conclusion, I recommend acceptance of the thesis for the award of the PhD degree.

Few minor points:

- In abstract appear previously undefined acronyms.
- Eqn. 2.1 – shouldn't there be the minus sign between sin(a) and sin(b)?
- It would be useful to discuss deconvolution microscopy and its role in super-resolution.
- "in standard approaches, the axial resolution is 2 times the lateral one" – it is frequently even an order of magnitude difference.
- Fig. 3.1, I have seen many similar demonstrations of this, and almost always the saturation makes it impossible to meaningfully judge the effect. I'd recommend including a non-saturated simulation of the case (maybe in log-scale intensity) to give a clearer idea to the followers of this work.
- "we measured neurons stained with thy1-GFP in a brain slice" - thy1 is not a stain, it is gene / protein. In this context, Thy1 is promoter, GFP is the coding sequence. Wherever the Thy1 promoter is active, GFP is expressed.
- Fig. 3.7 – why the reduced FOV?
- "the central amygdala is located around 2 mm beneath the brain surface" – it is deeper, 4-5mm.
- "The proteins that generate this fluorescence signal are called GCaMP and are produced by specific cells after targeted viral infection." – I'd replace "infection" with "transfection". Alternatively, one may use transgenic animal lines expressing GCaMP.

With the best regards,

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