Computational Optical Microscopy

869. WE-Heraeus-Seminar

01 - 06 November 2026

at the Physikzentrum Bad Honnef, Germany

WILHELM UND ELSE
HERAEUS-STIFTUNG



Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 869. WE-Heraeus-Seminar:

While optical microscopy has a history of several hundred years and has led to countless discoveries, it is still a very dynamic research area. Innovation in the area is increasingly influenced by computational approaches, in particular inverse problems, coding theory (e.g. sparse coding), optimization, and most recently, large scale machine learning. Continued advances will enable further breakthroughs and innovation exports to other scientific disciplines.

While many techniques still mainly perform an automated post-processing of microscopic images, increasingly employing machine learning, the area of computational microscopy aims at fundamentally merging system design and computation with the goal of improving performance, contrast, speed and the dimensionality of imaging.

A challenge in the development of the field is the wide variety of background knowledge that is necessary to tackle the challenges: physicists often struggle with developing efficient and high-performance algorithms, while not many computer scientists have the in-depth physical understanding to develop novel microscopy schemes. Mathematical developments concern novel simulation and optimization schemes, convergence guarantees, etc. For sustained progress, the development of an interdisciplinary exchange and, in the mid-term, the development of a cohesive subfield is a necessity.

The proposed seminar aims to bring together researchers from the different fields to define and address the challenges in Computational Optical Microscopy lying ahead.

Introduction

Scientific Organizers:

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Introduction

Venue:

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Registration:

Marion Reisinger (WE Heraeus Foundation)
at the Physikzentrum, reception office
Sunday (17:00 – 21:00 h) and Monday morning

Program

Program

Sunday, 1 November 2026

17:00 - 21:00	Registration	
18:30 - 19:30	*BUFFET SUPPER and informal get-together*	
19:30 – 19:45	Scientific Organizers	**Welcome Words**
19:45 – 20:00	Scientific Organizers	**Video "About the Wilhelm and Else Heraeus Foundation"**
From 20:00	Social Evening	

Program

Monday, 2 November 2026

08:00 – 09:00	*BREAKFAST*	
09:00 - 10:00	**Perspective talk** Anne Sentenac	**Super-resolution microscopy using random illuminations**
10:00 - 10:30	Steffen J. Sahl	**Intra-molecular MINFLUX analysis: quantifying interactions and conformations with Ångström precision**
10:30 - 11:00	Rainer Heintzmann	**Generic Inverse Modeling: A Modular Framework for Solving Complex Reconstruction Problems**
11:00 - 11:05	CONFERENCE PHOTO (in front of the main entrance)	
11:05 - 11:30	*COFFEE BREAK*	
11:30 - 12:00	Giuseppe Vicidomini	**Photon-Resolved Microscopy from a Computational Imaging Point of View**
12:00 - 12:30	Lars Loetgering	**Spectral Unmixing Algorithms for Ultra-Multiplex Fluorescence Microscopy**
12:30 - 14:00	*LUNCH*	
14:00 - 14:30	Olivier Haeberle	**Optical diffraction tomography for imaging non-fluorescent samples: state-of-art and perspectives**
14:30 - 15:00	Anand Vijayakumar	**Coded Aperture Correlation Holography: Evolution, Principles, and Future Directions**
15:00 - 15:40	**Poster Flash**	

Program

Monday, 2 November 2026

15:40 - 16:00	*COFFEE BREAK*	
16:00 -17:15	**Poster session 1**	
17:15 - 17:45	Monika Ritsch-Marte	**Simultaneous 3D motion and object reconstruction in acoustically-mediated optical tomography**
17:45 - 18:15	Yessenia Jauregui-Sánchez	**Ptychography: A Coded Optical Imaging Approach**
18:30	*Dinner*	
19:30	**Social evening**	

Program

Tuesday, 3 November 2026

08:00 - 09:00	*BREAKFAST*	
09:00 - 10:00	**Perspective talk** Sylvain Gigan	**Imaging with scattered light: wavefront shaping for fluorescence imaging in complex media**
10:00 - 10:30	Jerome Mertz	**Imaging in thick tissue with backscattered illumination**
10:30 - 11:00	Egidijus Auksorius	**Computational Dynamic Full-Field OCT Microscopy for High-Speed Label-Free 3D and 4D Tissue Imaging**
11:00 - 11:30	*COFFEE BREAK*	
11:30 - 12:00	Zeev Zalevsky	**Computational Imaging of Hemodynamic Brain Activity**
12:00 - 12:30	Sixian You	**Deep label-free lifetime microscopy using single pulse sampling**
12:30 - 14:00	*LUNCH*	
14:00 - 15:30	**Breakout Session 1**	
15:30 – 15:50	*COFFEE BREAK*	
15:50 – 16:30	**Poster Flash**	
16:30 – 18:00	**Poster session 2**	
18:30 – 19:30	*DINNER*	
From 19:30	**Social evening**	

Program

Wednesday, 4 November 2026

08:00 - 09:00	*BREAKFAST*	
09:00 - 10:00	**Perspective talk** Laurent Jacques	**Compressive interferometric acquisition: from lensless imaging to random beamforming in radio astronomy**
10:00 - 10:30	Yoav Shechtman	**3D localization microscopy - spreading the point-spread function**
10:30 - 11:00	Margret Keuper	**tbc**
11:00 - 11:30	*COFFEE BREAK*	
11:30 - 12:00	Antonio Ciarlo	**From Particle Tracking to Autonomous Optical Tweezers**
12:00 - 12:30	Pierre Weiss	**DeepBlur: Blind spatially-varying PSF estimation for fluorescence microscopy**
12:30 - 14:00	*LUNCH*	
14:00 - 18:00	EXCURSION	
18:30 – 20:00	*HERAEUS DINNER*	
From 20:00	**Social evening**	

Program

Thursday, 5 November 2026

08:00 – 09:00	*BREAKFAST*	
09:00 - 10:00	**Perspective talk** Malgorzata Kujawinska	**Metrology aspects of 2D/3D Quantitative Phase Imaging in biomedical applications**
10:00 - 10:30	Lucas Kreiss Roohian	**Multiphoton Imaging with Computational Specificity (MICS)**
10:30 - 11:00	Maria Baczewska	**Quantitative Phase Imaging with Multimodal Support: From Sample Preparation to Cellular Dynamics Analysis**
11:00 - 11:30	*COFFEE BREAK*	
11:30 - 12:00	Gaoliang Dai	**Computational algorithms and AI for enhancing topography fidelity of optical 3D surface measurements**
12:00 - 12:30	Marketa Kubankova	**From Cell Images to Clinical Evidence: Computational Challenges in Imaging Cytometry**
12:30 - 14:00	*LUNCH*	
14:00 - 15:30	**Breakout Session 2**	

Program

Thursday, 5 November 2026

15:30 - 16:00	*COFFEE BREAK*	
16:00 - 16:30	Igor Shevkunov	**Single-Frame Phase Retrieval: Identifiability, Priors, and Quantitative Reliability**
16:30 – 17:00	Andrew Maiden	**Multislice ptychography**
17:00 – 17:30	Stefan Witte	**Computational imaging with table-top extreme ultraviolet sources**
17:30 – 18:00	Manuel Martinez-Corral	**Fourier Lightfield Microscopy. A tool for real time 3D microscopy**
18:30 - 19:30	*DINNER*	
From 19:30	**Social evening**	

Program

Friday, 6 November 2026

08:00 – 09:00 *BREAKFAST*

09:00 - 11:00 **Breakout results**

11:00 - 11:30 *COFFEE BREAK*

11:30 - 12:30 **Panel and poster award**

12:30 - 14:00 *LUNCH*

End of the seminar and departure

Posters

Posters

Muneeb Ali	**Local spectroscopy on thin films using white-light interferometry**
Georg Bergner	**Versatile microscopy setup combining ISM, FLIM, STED, and adaptive optics**
Khadija Boughanbour	**Computational investigation and optimization of perovskite solar cell performance**
Benedict Diederich	**Teaching Computational Microscopy Using An Open, Modular Holography Education Kit**
Filip Dłużewski	**Optical-Tweezer-Driven Sample Rotation for Motion-Robust 3D Refractive Index Tomography**
Eva Ernst	**Skip the picture, keep the signal: Speeding up indirect wavefront sensing in three-photon microscopy**
Areeba Fatima	**Microscopy image upsampling via multi-modal image fusion**
André Felgner	**Do you trust your measurement results? - Strategies for instrument characterization pave the path to reliable measurement data**
Francois Figliolia	**Fourier Transform Acousto-Optic Imaging: Toward Real-Time Imaging of Thick Biological Media**
Leen Ileimi	**Brightfield Snapshot 3D Microscopy: Towards In Vivo Imaging**
Szymon Jastrzebski	**Raman Spectroscopy guided by Optical Diffraction Tomography for biomedical applications**
Gabin Jeatsa Kitio	**Design of a Low-Cost Smart Digital Microscope for Medical Diagnosis in Low-Resource Settings**

Posters

Fatima Zahra Kamli	**Multiscale investigation of La-doped BiVO4: From experimental characterization to DFT modeling**
Nikolai Karner	**Holographic Light Shaping for Precision Photobleaching in Live Cell Membranes**
Augustas Karpavicius	**High-speed fly-scan ptychography with single pulse diffraction**
Syed Muhammad Kazim	**Zero-shot quantitative phase imaging from coded wavefront sensing measurements using optical flow networks**
Rahel Koch	**ADAM vs. PIE: Evaluation and Optimization of Reconstruction Algorithms for Lensless Imaging**
Lucas Kreiss Roohian	**Single-shot Quantitative phase and Polarimetric Imaging (QP^2I) for label-free, non-phototoxic imaging of live cardiomyocytes**
Martyna Mazur	**Full-field swept-source quantitative phase imaging with temporal coherence gating**
Giorgio Meroni	**Quantitative Evaluation of the Efficacy of Holographic Scattering Correction in Two-Photon Microscopy**
Johannes Meyer	**The Missing Scale in Surface Reflectance: Optical Linear Algebra for Reflectance Measurement**
Maciej Mojkowski	**Influence of Optical Aberrations on Lateral and Axial Resolution in Image Scanning Microscopy**
Zhonghui Nie	**Speedy Extreme Ultraviolet Ptychography with a Microchannel plate and Timepix3 detector**
Amir Peiraviminaei	**Robust and Reliable Motion Estimation for Fourier Diffraction Tomography**

Posters

Leonardo Pestana Legori	**Hyperspectral Tomographic Diffractive Microscopy: Development and Applications**
Sobhi Saeed	**Optical-Processing of Raman-Encoded Signals**
Pablo Stickar	**Fourier domain opticalcoherence tomography**
Ondrej Stranik	**Viscope - from virtual to real instrument control**
Sai Deepika Sure	**Single-shot sectioning in 3D I-COACH using cross-correlation and computational reconstruction**
Andrew Voitiv	**Correlation-Enhanced Spontaneous Raman Spectroscopy and Imaging**
Zhiping Wang	**Towards Computational Phase Imaging for Monitoring Light-Based Fabrication**
Wojciech Wroblewski-Wojtkiewicz	**Hybrid coherent/incoherent QPI systems for high throughput phase measurements in biomedical applications**
Li-Yu Yu	**Transcriptomics-informed label-free multiphoton microscopy for mouse brain imaging**
Hossein Zarei Oshtolagh	**Maximum Likelihood Localization Maps**
Haitao Zhou	**Physics-based neural networks for imaging and focusing in scattering media exploiting the memory effect**

Abstracts of Lectures

(in alphabetical order)

Computational Dynamic Full-Field OCT Microscopy for High-Speed Label-Free 3D and 4D Tissue Imaging

Austėja Trečiokaitė[1], Erikas Tarvydas[1], Egidijus Auksorius[1]

[1]*Center for Physical Sciences and Technology (FTMC), Saulėtekio al. 3, LT-10257 Vilnius, Lithuania*

Computational full-field optical coherence tomography (FF-OCT) combines parallel interferometry with digital reconstruction and refocusing [1]. Temporal fluctuations associated with intracellular motion provide label-free dynamic contrast. Our time-domain FF-OCT system achieves approximately 270 nm lateral and 500 nm axial resolution near the surface (Fig. 1 a-c) and dynamic contrast to 120 μm [2], but sequential depth acquisition takes minutes per volume.

Fourier-domain FF-OCT acquires a dynamic 3D dataset in 3.3 s [3]. A spectral Fourier transform reconstructs complex OCT volumes from camera interferograms. Numerical propagation corrects defocus; registration and phase stabilization suppress bulk motion. Temporal standard deviation, frequency-resolved Fourier analysis and singular-value decomposition generate dynamic contrast. GPU acceleration and memory-efficient processing handle datasets approaching 64 GB. Mouse tissue examples (Fig. 1 d-f) illustrate the potential for rapid, label-free 4D imaging.

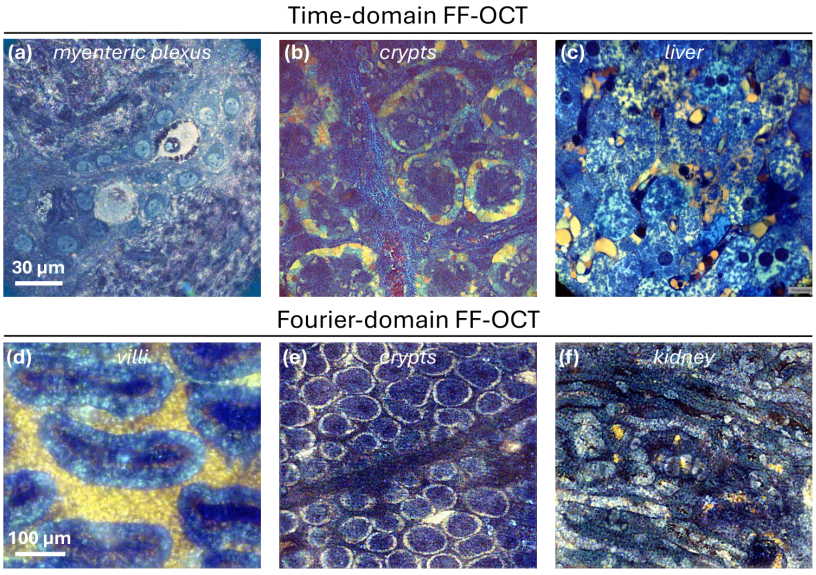


Fig. 1. Time-domain (a-c) and Fourier-domain (d-f) FF-OCT of mouse tissue. Blue-to-red colours encode low-to-high fluctuation frequencies; bands differ between systems.

References

[1] E. Auksorius et al., *iScience* **25**, 105513 (2022).
[2] E. Tarvydas et al., *npj Imaging* **4**, 21 (2026).
[3] A. Trečiokaitė et al., *Opt. Lett.* **51**, 3581-3584 (2026).

Quantitative Phase Imaging with Multimodal Support: From Sample Preparation to Cellular Dynamics Analysis

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Quantitative Phase Imaging (QPI) techniques, such as Digital Holographic Microscopy (DHM) and Holographic Tomography (HT), offer powerful, label-free modalities for evaluating biophysical parameters at the single-cell level. However, obtaining reproducible phase metrics and overcoming QPI's inherent lack of chemical specificity requires systematic design of the underlying experimental and computational protocols. This talk outlines the essential principles and critical considerations for designing robust biological QPI frameworks. Although QPI offers an intuitive, label-free imaging approach, rigorous methodology design is necessary to transform these label-free advantages into reproducible, quantitative measurements. From a pre-analytical perspective, methodology design must strictly account for environmental and biophysical variables that alter endogenous phase contrast. Factors such as substrate optical quality and media stability dictate background reference preservation, while cell culture preparation, most notably live versus fixed conditions [1], introduce direct effects on cellular refractive index and mass density distributions that can mislead inter-laboratory benchmarking. Where optical phase shift measurements require additional biological specificity, protocols should be designed to accommodate correlative workflows, integrating techniques like fluorescence or Raman spectroscopy alongside microfluidics when detailed chemical profiling is needed [2, 3]. Finally, computational pathways must be treated as an intrinsic component of the physical measurement system. Robust framework design requires tailored algorithms for 2D/3D segmentation, advanced field-of-view stitching strategies, and emerging AI-driven analytical tools to handle complex sample morphologies and large-volume acquisitions seamlessly. Addressing these foundational pillars ensures the extraction of reliable, highly accurate phase metrics across scales, from broad population statistics down to heterogeneous single-cell behaviours, paving the way for standardized cell analytics, digital biological repositories, and translational diagnostics.

References

[1] Baczewska, Maria, et al. *Cytometry Part A* 99(4) (2021)
[2] Węgierek-Ciuk, Aneta, et al. *Methods* 238 (2025)
[3] Baczewska, Maria, et al. *Applied Optics* 61(5) (2022)

From Particle Tracking to Autonomous Optical Tweezers

A. Ciarlo[1], M. Selin[1], C. Bustamante[2], G. Volpe[1]

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Video microscopy is widely used to study the motion and dynamics of microscopic objects. Deep learning offers powerful tools for analyzing these images and extracting quantitative information. These methods are also useful in optical-tweezers experiments, where particle tracking is closely linked to calibration, force estimation, and feedback control.

Extracting this information requires accurate and robust particle detection and tracking. This can be difficult under complex imaging conditions, for heterogeneous samples, or when images need to be analyzed in real time.

Therefore, we explored how deep learning can improve particle tracking and how these measurements can be used to control optical-tweezers experiments.

We developed DeepTrack, a deep-learning framework for quantitative microscopy and particle tracking, and SmartTrap, an automated optical-tweezers platform for closed-loop experiments.

DeepTrack provides tools for developing and training deep-learning models for microscopy [1], while SmartTrap combines real-time three-dimensional tracking with optical manipulation and feedback control in an autonomous experimental platform [2]. Together, these tools enable automated experiments on single particles, single cells, and single molecules.

We show how deep-learning-based image analysis can be combined with optical manipulation to make quantitative measurements and control experiments in real time. Combining microscopy, machine learning, and automated control can enable high precision experiments on a previously unattainable scale.

References

[1] B. Midtvedt, S. Helgadottir, *et al.*, **Appl. Phys. Rev. 8**, 011310 (2021)
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Computational algorithms and AI for enhancing topography fidelity of optical 3D surface measurements

Gaoliang Dai*, Min Xu, Nils Koppert, Lena Heinrich, Andre Felgner, Sai Gao, Dorothee Hüser
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Keywords: optical surface texture metrology, white light interference (WLI) microscopy, topography fidelity, surface reconstruction, calibration, hybrid metrology, artificial intelligence (AI)

Accurate characterisation of surface topographies is needed in vast industrial and research fields, ranging from advanced manufacturing, optics, advanced materials, semiconductor to quantum technology. Various optical tools such as phase shifting interference (PSI), white light interference (WLI), confocal, and focus variation microscopes are applied for surface metrology owing to their advantages of fast and noncontact measurements. However, they suffer from measurement errors, particularly when measuring surfaces and structures with large slope angles, complex geometries, diffusely reflecting surfaces. Consequently, their measurement results often include outliers, missing points, and other unexpected topographic features. Comparisons between measurement results of 3D optical microscopes and that of tactile tools frequently reveal significant discrepancies. This issue, referred to as the **topography fidelity** issue, remains as one of the most challenging issues in optical surface metrology. This paper aims to give an overview on a systematic approach currently being studied at the PTB, supported by advanced computational algorithms and AI, for enhancing the topography fidelity.

Surface reconstruction algorithms based on the measured optical signals is usually the most fundamental step in optical surface metrology. Using the WLI as example, surface topography has to be reconstructed pixel-wisely based on the measured correlogram, which typically involves the localization of the coherence envelope and the phase-based refinement. However, measurements on complex structures may deliver correlogram (significantly) deviated from an ideal shape, leading to surface reconstruction errors. To solve this challenge, both classical physic-driven computational algorithms, e.g. Fourier-domain envelope extraction and Frequency-Domain Analysis (FDA), and data-driven AI-based algorithms for surface reconstruction will be introduced.

Reconstructed results of 3D optical microscopes suffer from various geometrical errors such as linearity, flatness, squareness errors and optical resolution limits. Consequently, the characterisation and correction of these errors become essential steps, which demand both appropriated material measures and corresponding computational algorithms. In this talk, some calibration examples will be briefly demonstrated: (i) a marker-based pyramidal 3D standard and a topography-based model fitting algorithm for determining the x-, y- and z- coordinates of markers; (ii) A material measure for characterising two-dimensional (2D) instrument transfer functions (ITF) of areal surface topography measuring tools for determining their resolution limits; and (iii) a material standard with sinusoidal ridge and groove structures comprising series of discrete nominal lateral periods, and a procedure that compares the full geometric shape of the material measure, where reference data consist of selected profiles across the topography accompanied by uncertainty bands, to characterise the topography fidelity of optical tools.

Although the above two approaches well outline the methodologies for characterising and/or improving the topography fidelity of a standalone optical tool, a further approach for improving the topography fidelity is the so-called hybrid metrology. Hybrid metrology aims to apply different measurements/techniques in a combined manner, thus offering potential solutions for highly challenging metrology tasks by complementing the advantages and disadvantages of individual measurements/techniques in the ensemble. This talk will present a novel hybrid microscope, referred to as "hybrid IM+AFM" which combines an interference microscopic (IM) and an AFM measurement mode, recently developed at the PTB. It provides two very promising application potentials: (i) the limited resolution capacity of the IM mode can be complemented by the high-resolution AFM mode; (ii) AFM measurements provide high-fidelity topography data applicable for characterizing and improving IM measurements.

To fully exploit the potential of hybrid metrology, the fusion of measurement data presents significant challenges, as datasets acquired by different techniques may differ substantially in measurement area, sampling density, spatial resolution, accuracy, and spatial-frequency response. Moreover, techniques based on fundamentally different measurement principles, such as tactile and optical methods, may exhibit different sensitivities to surface geometry and material properties and introduce distinct systematic errors and measurement artefacts. Consequently, reliable data fusion requires not only accurate spatial registration and multi-resolution reconstruction, but also a careful consideration of the underlying measurement principles, instrument transfer functions, uncertainty characteristics, and traceability of the individual datasets. The talk will conclude by presenting a preliminary study conducted to address these challenges and demonstrate the potential of data fusion for hybrid surface metrology.

Imaging with scattered light: wavefront shaping for fluorescence imaging in complex media

Sylvain Gigan

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Imaging in scattering media, such as biological tissues, is an extremely challenging problem. Due to the exponential attenuation of ballistic light, and to the seemingly random multiple scattering process. Wavefront shaping has revolutionized this paradim : it allows focusing and imaging in disordered media, exploiting the ability to control multiply scattered light [1]. Many so-called guide-star mechanisms have been investigated to deliver light and image non-invasively at depth. However, the most common microscopy contrast mechanism, fluorescence, remains extremely challenging. I will discuss some of our recent works, exploiting signal processing and machine learning frameworks, to recover images behind scattering layers exploiting linear fluorescence [2-4].

References

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Optical diffraction tomography for imaging non-fluorescent samples: state-of-art and perspectives

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IRIMAS – Université de Haute-Alsace, Mulhouse, France

Fluorescence microscopy has experienced spectacular advances in recent years in terms of diversity and specificity of the markers, acquisition speed, volume of observation, and, above all, resolution. These advances have been recognized, for example, by the awarding of the 2008 Nobel Prizes in Chemistry to Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien “for the discovery and development of the green fluorescent protein, GFP” and of the 2014 Nobel Prize in Chemistry to Eric Betzig, Stefan W. Hell, and William E. Moerner "for the development of super-resolution fluorescence microscopy"

However, when one cannot or does not wish to use fluorescent labels, optical microscopes remain much more limited.
Various label-free methods have been developed to meet the needs of biologists. Second- and third-harmonic microscopy, Raman and CARS microscopy, and microholography have now become recognized tools. Nevertheless, there are still many challenges facing opticians who wish to contribute to these field.

The purpose of this presentation is to rapidly introduce these techniques by placing them in the context of the various model specimens used in biology, then focusing on diffractive microtomography [1].

This imaging method [2-6] belongs to Computational Optical Microscopy techniques: it is based on measurement or reconstruction of the optical field, which is diffracted by the observed sample. Inverting an image formation model [7] then provides a 3D map of the optical index of refraction within the observed sample.

The technique also shows some potential for functionalization, in order to improve chemical sensitivity, structural selectivity, or dynamic discrimination.

References

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Generic Inverse Modeling: A Modular Framework for Solving Complex Reconstruction Problems

Rainer Heintzmann[1,2]

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[2.] Inst. of Physical Chemistry, Friedrich Schiller University Jena, Jena, Germany

Inverse problems arise throughout science and engineering whenever unknown system parameters must be inferred from measured data. While applications often appear highly specialized, many can be formulated within a common computational framework. This talk introduces a generic inverse modeling architecture that decomposes the reconstruction process into four core components: a **forward model** that predicts measurements from a candidate solution, a **statistical comparison** that quantifies agreement between prediction and observation, an **adjoint operation** that efficiently propagates information through the model, and an **optimizer** that iteratively improves the solution.
Building on this structure, we introduce the concept of a **pre-forward model**, a layer preceding the forward model that transforms the optimization variables into physically meaningful parameters. **Automated differentiation** can handle this step seemlessly. This additional stage enables the systematic enforcement of domain-specific constraints, such as positivity, normalization, or fixed-sum requirements, without modifying the underlying optimization machinery. By separating parameterization from physical modeling, the framework increases flexibility, improves numerical robustness, and simplifies the integration of prior knowledge.
The modular design allows a wide variety of inverse problems to be addressed within a unified software and mathematical framework. Example applications include the **deconvolution** of light microscopy data, **wavefront optimization** and focusing through multimode optical fibres, and speckle reconstructions. Through these examples, the talk demonstrates how seemingly disparate imaging problems can be treated using the same generic inverse modeling principles, enabling reusable algorithms, improved maintainability, and accelerated development of new reconstruction methods.



Prof. Dr. Rainer Heintzmann, studied at the Universities of Osnabrück and Heidelberg and worked as a postdoctoral fellow at the Max Planck Institute of Biophysical Chemistry in Göttingen and as a group head at the Randall Division, King's College London. Since 2010 he is Professor of Physical Chemistry at the Friedrich-Schiller University Jena and heads the microscopy research department at the Leibniz Institute of Photonic Technology in Jena.

His research focuses on developing methods for imaging cellular function at high resolution and techniques to measure multidimensional information in small biological objects such as cells, cellular organelles or other small structures of interest. Examples of his developments are structured illumination, image inversion interferometry, optical photon reassignment, and pointillism. He is active in computer-based reconstruction and inverse modelling approaches including deconvolution and released several packages in the programming language **Julia**.

Addressing the Modeling Gap in Computational Microscopy

Ivo Ihrke
University of Siegen, Center for Sensor Systems (ZESS), Germany

Computational microscopes recover images or quantitative sample properties by inverting a model of the optical system, and the result is only as good as that model. Unknown aberrations, approximations in the forward model and differences between simulation and experiment (the modeling gap) lead to artifacts, limited dynamic range and poor generalization. I will present three ways we address this gap. In Fourier ptychographic microscopy, we estimate the field-dependent pupil function directly from the measurement data, without calibration, by exploiting aberration-induced image shifts under single-LED illumination [1]. In quantitative phase imaging with coded wavefront sensing, we replace the approximate analytic forward model with a neural network trained on accurate physics-based simulations, which improves the wavefront dynamic range threefold [2]. Finally, we use a hardware twin of a digital processing pipeline to determine how accurate a simulation must be to train networks that stay robust against real optical aberrations (work in progress).

References

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Compressive interferometric acquisition: from lensless imaging to random beamforming in radio astronomy

Laurent Jacques

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In this presentation, we will examine the properties of a specific image acquisition model encountered in two distinct compressive imaging applications: single-pixel lensless imaging with multi-core fiber (MCF-LI) for biological imaging, and random beamforming radio interferometry (RB-RI) in radio astronomy [1,2]. This common model is based on the collection of rank-one random projections (ROPs) of an "interferometric matrix" encoding a partial Fourier sample of an image of interest. For MCF-LI, the projection of this matrix is provided by programmed random coherent illumination patterns, whereas for RB-RI, it is provided by antenna beamforming, a common technique for phase-shifting antenna signals typically used to focus on a region of the sky. In both scenarios, we will demonstrate theoretically and experimentally that, under certain conditions, the image of interest can be accurately estimated from a number of projections that scales linearly (up to logarithmic factors) with the image's complexity, that is, its level of sparsity.

This work is carried out in collaboration with O. Leblanc, M. Hofer, S. Sivankutty, H. Rigneault, T. Chu, and Y. Wiaux.

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Ptychography: A Coded Optical Imaging Approach

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Advances in optical imaging are partly driven by the need to overcome limitations inherent in conventional systems, including the diffraction limit, optical aberrations, and reduced sensitivity to phase variations [1]. Coded optical imaging (COI) addresses these limitations by encoding information into optical measurements and recovering it through computational processing. It combines optical system design with algorithm development to improve image quality, resolution, or functionality beyond what the corresponding conventional system can achieve [2]. COI includes approaches such as single-pixel imaging [3], Fourier ptychographic microscopy [4], computational holography [5], and lensless imaging [6].

Among the techniques classified under COI, ptychography is a computational imaging method that addresses the longstanding phase retrieval problem using iterative algorithms. In a typical configuration, a localized probe is scanned across overlapping regions of a sample, and the resulting far-field diffraction intensities are recorded. The overlap introduces data redundancy: adjacent illuminated regions encode correlated information that provides additional constraints for recovering both the amplitude and phase of the sample. This redundancy improves reconstruction stability in the presence of noise and experimental uncertainties [7]. Ptychographic methods have been applied across several scientific fields, reflecting the close interaction between optical design, data acquisition, and computational reconstruction.

In this context, this contribution examines the computational reconstruction process that underpins ptychography as a coded optical imaging approach. It describes ongoing work toward implementing and evaluating iterative phase-retrieval algorithms, focusing on how algorithmic choices affect convergence and the recovery of amplitude and phase from simulated diffraction measurements.

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Multiphoton Imaging with Computational Specificity (MICS)

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Inflammatory bowel disease (IBD) is diagnosed by invasive biopsy, with stained tissue sections graded by trained experts. This process is invasive, time-intensive and carries subjective bias. We present Multiphoton Imaging with Computational Specificity (MICS), a deep-learning framework for label-free optical histology using natural contrast of SHG and autofluorescence. At the cellular level, MICS classifies 2D image patches into distinct cellular populations underlying tissue inflammation. At the 3D tissue level, MICS classifies IBD directly from multiphoton image stacks of ex vivo biopsies, at an accuracy on par with the inter-observer agreement between trained experts. Transfer learning of this ex vivo tissue model to a unique in vivo data set, acquired with a custom-built multiphoton endomicroscope, successfully distinguishes between healthy, acute tissue-damage colitis, and chronic immune-induced colitis with 75% accuracy in previously unseen test samples. Gradcam and occlusion maps tie predictions to metabolic and structural features for interpretable and insightful feedback. Thereby, MICS demonstrates direct inflammatory phenotyping from label-free in vivo optical histology.

From Cell Images to Clinical Evidence: Computational Challenges in Imaging Cytometry

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Imaging cytometry can quantify morphological and functional variation across thousands of individual cells while retaining information that is lost in population-averaged measurements. Yet acquiring large numbers of cell images is not sufficient to produce a clinically useful measurement. The central computational challenge is to transform variable cell-level observations into robust, interpretable and generalizable evidence at the level of an individual patient.

I will discuss this challenge using real-time deformability cytometry as a case study. This technique combines microfluidic cell deformation, high-speed brightfield imaging and multidimensional analysis to quantify the mechanical and morphological properties of individual cells. Applications to blood, inflammatory and infectious conditions, and freshly dissociated tumour tissue demonstrate the potential of label-free imaging cytometry for rapid analysis of clinical samples.

These applications also reveal a fundamental challenge: acquiring large numbers of cell images does not automatically produce clinically meaningful information. Biological heterogeneity, technical variation and limited ground truth complicate the interpretation of image-derived phenotypes, while models developed in one cohort or experimental setting may not transfer to another.

I will discuss how computational approaches could help bridge the gap between cell-level measurements and robust patient-level conclusions. These challenges provide opportunities for closer co-design between imaging physicists, computational researchers and biomedical users.

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Metrology aspects of 2D/3D Quantitative Phase Imaging in biomedical applications

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Quantitative Phase Imaging (QPI) refers to a number of label-free 2D/3D microscopy techniques that provide contrast by quantifying the phase shifts in the wavefront when light propagates through a transparent specimen [1]. The commercial and research landscape of phase microscopy and tomography is constantly growing with multiple of solutions including off-axis and lensless digital holographic microscopes, common path interferometry solutions, ptychography/Fourier ptychography microscopy (PM/FPM) or Transport of Intensity Equation (TIE) based microscopes. Each of these systems have their own advantages and limitations which may strongly influence the measurement capabilities. Additionally many of these systems use AI tools to support data analysis at different stages of their processing.

The utility of phase measurement in biomedical applications depends on a measurement system performance itself (hardware+software), the sample complexity, its preparation protocols and the data processing/analysis algorithms. This creates a lot of uncontrolled degrees of freedom and the interplay between these factors cannot be reliably determined, although the results provided by any 2D/3D QPI system should be comparable within the measurement uncertainty associated with the instrument under test. For uncertainty determination and proper QPI systems intercomparison we need to provide adequate reference materials (phantoms) and detailed measurement protocol which would be accepted and implemented by both the producers and users of QPI.

In this talk I will present an overview of QPI systems from the metrology point of view and discuss the interlaboratory comparison of QPI systems. This will include (i) description of numerical and physical (produced by means of the two-photon polymerization) phantoms that strive to mimic biomedical specimens, ultimately providing tool for validation and benchmarking a variety of QPI systems [2,3], (ii) measurement and data intercomparison protocols and example of their implementation (iii) progress towards creating digital twins of 2D/3D QPI systems.

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Spectral unmixing algorithms for ultra-multiplex fluorescence microscopy

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Ultra-multiplex fluorescence microscopy plays a key role in future personalized medicine, but poses the challenge how to disentangle overlapped fluorescence spectra. We give an overview of algorithmic developments for spectral unmixing used in fluorescence microscopy.

Multislice ptychography

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Ptychography is an increasingly popular form of coherent diffractive imaging (CDI), where a deceptively simple optical system yields rich data containing an unprecedented diversity of information about the specimen being imaged [1,2]. Among the most useful aspects of this information is axial resolution, i.e. depth sensitivity. Multislice ptychography unlocks access to this third dimension, recovering a stack of 2D images representing axial sections of the specimen [3]. In recent years, it has become highly popular among electron microscopists because it avoids experimentally tricky specimen rotation (tomography) while naturally modeling multiple scattering of the electron beam, see e.g. [4,5]. Here, I will outline the multislice method, describe its various implementations across different forms of microscopy, and share recent results [6]. Finally, I will address open questions concerning the robustness, regularization, and uniqueness of the reconstruction algorithms.

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Fourier Lightfield Microscopy. A tool for real time 3D microscopy

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Lightfield Microscopy (LMic) is based on the insertion of a microlens array (MLA) at the image plane of an optical microscope and allows the simultaneous capture, in a single image, of the spatial and angular information of the field emitted by microscopic samples. This enables the computational reconstruction of the 3D structure of the samples. The main limitation of this technique is its low spatial resolution, which results from the trade-off between the amount of spatial and angular information captured by the system, and the lack of spatial invariance, which prevents the use of deconvolution tools.

To overcome these drawbacks, Fourier Lightfield Microscopy (FLMic) was proposed. In this new architecture, the lenslet array is inserted into the Fourier plane to enable the capture of multiple 2D orthographic images of the 3D sample, each from a different perspective. One advantage of FLMic is that the images are captured with improved resolution compared to the previous technique. But its main feature is its inherent spatial invariance, which allows the application of tailored computational tools for rendering the 3D sample with optimized resolution.

This talk will present the optical fundamentals of FLMiC, explain the computational tools, and discuss current and potential applications.

Imaging in thick tissue with backscattered illumination

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Almost all phase-contrast microscopes operate in transmission geometries, making them unsuitable for imaging in thick, complex media such as brain tissue. I will discuss various techniques for imaging phase (and absorption) in arbitrarily thick tissue using strategies that exploit incoherent backscattering [1,2]. The strategies highlight some fundamental differences between transmission versus reflection imaging. In addition, I will discuss methods to control backscattered illumination based on a remarkable phenomenon called the “memory effect” [3], which can be further exploited to provide phase contrast in thick media in cases when the backscattering is coherent (i.e. speckle-like). Most of the applications I will discuss relate to blood-flow imaging using optical backscattering, though I will also discuss generalizations to other forms of waves, such as acoustic backscattering [4].

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Simultaneous 3D motion and object reconstruction in acoustically-mediated optical tomography

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Medical University of Innsbruck

Large bio-samples such as organoids and cancer spheroids are often too opaque for single-sided optical imaging. Multiangle illumination enables 3D refractive-index tomography, but requires rotating the sample. Because heavy particles cannot be held by optical tweezers without weak trapping or damage, we use standing MHz ultrasound waves to levitate and re-orient samples in a microfluidic chamber, avoiding “missing-cone” artifacts. Since the acoustically driven motion is not known a priori, the object’s 3D position and orientation at each acquisition must be reconstructed jointly with the unknown object volume itself. This is achieved via GPU-accelerated stochastic gradient descent with a physics-informed forward model. We demonstrate this with OCT of a levitated zebrafish larva and ODT of cancer cells and spheroids

Intra-molecular MINFLUX analysis: quantifying interactions and conformations with Ångström precision

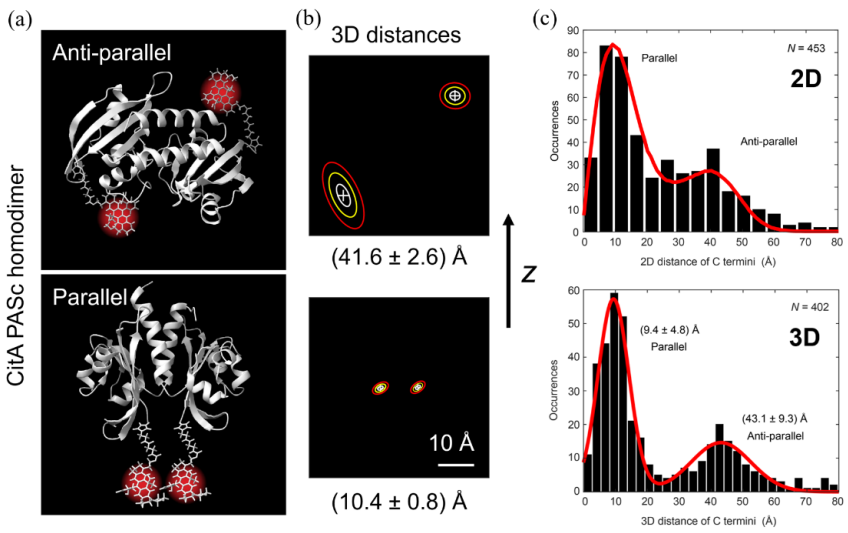
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The demonstration of intra-molecular MINFLUX resolution capabilities [1] has extended single-molecule fluorescence analysis to the direct position-based probing of macromolecular conformation. Photon-efficient 2D or 3D MINFLUX localization protocols [2], together with photoactivatable fluorescent dyes, enable the independent detection and Ångström-precise relative coordinate registration of dye-labeled protein sites even in the 1-to-5-nanometer distance range [1]. This has allowed to quantify intra-molecular details such as, for example, equilibria of distinct conformations in enzymes, or the ability to measure small protein conformational changes. Recent results in small molecular systems uncover a further new frontier of what can be probed with MINFLUX and Ångström measurement precision, reflecting the continued vigorous development in the field of single-molecule localization over the last decade [3].

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Direct conformational analysis with MINFLUX. (a) Two identical subunits of the bacterial citrate sensor (CitA PASc) in antiparallel (top) and parallel (bottom) arrangement. (b) Optical 3D position measurements detect the two states of the dimer formed by the subunits, by very precisely measuring the distances between the fluorescent molecules at the protein ends (C-termini). The ellipses show the measurement uncertainty of the two positions (1, 2 and 3 sigma). (c) Independently measured distributions of 2D (projected) and 3D distances. The set of 3D distances allows to clearly assign parallel and antiparallel dimer measured at (9.4 ± 4.8) Å and (43.1 ± 9.3) Å, respectively. Data from Sahl et al. Science 2024.

Super-resolution microscopy using random illuminations

Anne Sentenac

Structured Illumination Microscopy (SIM) and Imaging Scanning Microscopy (ISM) are widespread solutions for improving the resolution while maintaining the speed and low phototoxicity required for live imaging. Their main issue is that they require a strict control of the illumination patterns (periodic grid or focused spots). Unwanted pattern distortions caused by the sample itself or slight misalignments, drifts or aberrations in the optics result in a loss of resolution and image artefacts [1].

A method called Random Illumination Microscopy (RIM) has been recently proposed to relax the constraint on the illuminations' control. RIM forms a super-resolved image from low-resolution images obtained under (200) random [2] or (20) pseudo-random [5] illumination patterns. RIM does not require the knowledge of the patterns but only that of their auto-correlation. The latter being very robust to scattering, aberrations and misalignements, RIM has been shown to produce robust super-resolved imaging of live and fixed biological samples in difficult configurations, while keeping a temporal resolution adapted to live-imaging [2-4]. I will describe the principles of RIM (including its mathematical foundations), detail its different implementations, from fluorescence microscopy [2-4] to Coherent Anti-Stokes Raman Scattering imaging [6] and present super-resolved images of a wide range of biological samples that (hopefully) will convince you of the interest of RIM.

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3D localization microscopy - spreading the point-spread function

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In the recent decade, deep learning has revolutionized image analysis in many fields including microscopy. Our group has developed neural-net based tools for decoding and encoding information specifically in localization microscopy, for applications such as 2D, 3D and spatiotemporal super-resolution imaging as well as single particle tracking.

In this talk I will present and discuss selected methods of interest, as well as ways to make them accessible, specifically in the context of 3D localization microscopy by point-spread function engineering. I will describe our efforts in lowering adoption barriers including ‘one-click’ image reconstruction for single molecule localization microscopy and 3D printing of diffractive optical elements. I will also discuss our recent efforts in minimizing the simulation-to-experiment gap in the context of localization microscopy, which is crucial for simulation-based training.

Single-Frame Phase Retrieval: Identifiability, Priors, and Quantitative Reliability

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Recovery of a complex optical field from a single intensity measurement is a nonconvex, generally non-unique inverse problem. In lensless in-line holography, missing detector-plane phase, finite detector support, noise, and forward-model uncertainty produce ambiguities not resolved by data fidelity alone. Reconstructions are therefore jointly determined by the measurement and priors from calibration, regularization, learned estimators, and numerical optimization [1-3].

We investigate compact lensless quantitative phase microscopy for longitudinal live-cell measurements, using a learned sensor-plane phase estimate to initialize model-based iterative refinement. Phase targets and living cells reveal complementary failure modes: physics-based iterations can stagnate at data-consistent but object-inaccurate solutions, while learned estimates may reproduce plausible morphology with systematic phase bias. The hybrid method improves convergence but remains sensitive to calibration and domain mismatch.

We examine identifiability under noise and perturbations of propagation distance, illumination geometry, wavelength, detector response, and specimen class. Central questions are whether detector-plane residuals imply object-plane accuracy; how phase uncertainty propagates to dry mass, growth rate, and treatment-response metrics; and whether temporal constraints suppress transient phenotypes.

Validation is organized via measurement consistency, calibration sensitivity, reproducibility across initializations and priors, agreement with traceable phase standards, and stability of downstream biological observables. Benchmarks should combine calibrated targets, dynamic specimens, controlled noise and aberrations, and imposed model mismatch. We seek falsifiable criteria for when a single hologram supports quantitative biological inference.

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Photon-Resolved Microscopy from a Computational Imaging Point of View

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Fluorescence laser-scanning microscopy traditionally integrates photons in space and/or time into intensity values, discarding much of the information potentially encoded in their individual spatial and temporal signatures. The emergence of single-photon avalanche diode (SPAD) arrays is changing this paradigm. By preserving precise spatial and temporal signatures of individual photons, these detectors generate multidimensional datasets in which information about the specimen is encoded across detector position, photon arrival time, and scanning coordinates. The challenge is therefore to computationally decode these photon signatures into otherwise inaccessible information about the specimen. In this talk, I will present photon-resolved microscopy as a computational imaging framework in which photon detection, physical image formation, and reconstruction are jointly exploited.

I will first discuss image-scanning microscopy as an inverse problem, showing how the detector position of each photon encodes information about both its lateral and axial origin in the specimen. Within a maximum-likelihood framework, these spatial signatures are jointly decoded to achieve super-resolution, high signal-to-noise ratio, and optical sectioning from a single-plane acquisition [1]. I will then show how temporal tagging can generate statistically independent noise realizations of the same specimen, enabling data-driven regularization without imposing explicit assumptions on the specimen. The temporal signature of each photon can also be exploited to encode the fluorescence decay [2]. Extending the forward model to photon arrival times enables the reconstruction of super-resolved, optically sectioned fluorescence lifetime maps and multi-species images based on lifetime contrast [3]. Beyond image reconstruction, I will discuss photon-resolved microscopy in the context of single-molecule localization microscopy, where the computational problem shifts from reconstructing an image to estimating the position of individual emitters from their photon signatures.

Together, these examples illustrate a broader perspective in which photon-resolved microscopy is not simply a detector upgrade, but a route towards microscopes in which physical encoding and computational decoding are designed and exploited as a single imaging system.

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Coded Aperture Correlation Holography: Evolution, Principles, and Future Directions

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Coded Aperture Correlation Holography (COACH) was introduced as a new paradigm in incoherent digital holography that integrates principles of incoherent holography with coded aperture imaging.[1] Originally developed to overcome some of the limitations associated with wave propagation-based reconstruction methods, COACH introduced the use of coded phase masks and point spread holograms, enabling three-dimensional imaging through correlation-based reconstruction rather than numerical wave propagation. This shift removed the restriction to quadratic phase modulation and enabled the use of arbitrary coded phase masks for holographic imaging and reconstruction.[2]

In this presentation, I will discuss the evolution of COACH from its original implementation based on self-interference and coded phase modulation to later interferenceless architectures, computational reconstruction methods, and multidimensional imaging.[3] Particular emphasis will be placed on the scientific questions that motivated these developments and on the broader directions that emerged from COACH, including super-resolution imaging, microscopy, endoscopy, spectral and polarization imaging, aperture engineering, and post-recording tuning of imaging characteristics.[4] The talk will focus on the motivations that drove the development of different COACH architectures, the lessons learned during their evolution, and the conceptual transitions that led from interferometric to interferenceless implementations. Rather than providing a comprehensive review of published results, the presentation will focus on the motivations, conceptual developments, and unresolved challenges that have shaped the evolution of COACH. Finally, I will discuss several open questions and future research opportunities. The future evolution of COACH and its role within the broader landscape of incoherent digital holography and computational imaging will be discussed.

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DeepBlur: Blind spatially-varying PSF estimation for fluorescence microscopy

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Optical aberrations degrade fluorescence microscopy images, blurring fine structures and biasing downstream quantification, yet most microscopists cannot measure or correct them. These aberrations introduce space-varying point spread functions (PSFs) that depend on the sample and drift over time. Standard PSF measurement requires calibration beads, which are incompatible with live imaging. Here we present a neural network that estimates a smoothly-varying PSF directly from a single aberrated biological image without calibration targets. Using a deformable mirror, we validate this on 1,400 experimentally aberrated real-world image pairs, demonstrating accurate PSF estimation across diverse aberrations and noise levels. The estimated PSF can be fed to any PSF-dependent reconstruction method; we demonstrate this with a deconvolution network (RAM) and random-illumination super-resolution. This approach consistently restores sub-diffraction structures and improves downstream tasks, including cell segmentation. Our open-source tool, trained on physical simulations, is available as a Napari plugin and within the DeepInv library.

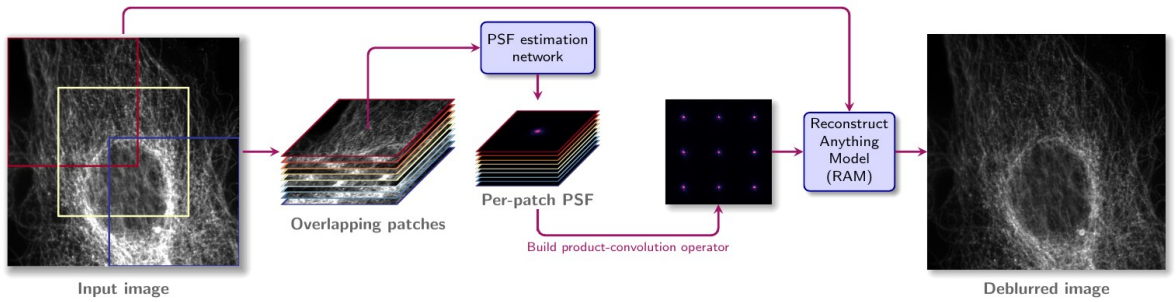


Figure 1: Principle of DeepBlur

Computational imaging with table-top extreme ultraviolet sources

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High-harmonic generation (HHG) driven by ultrafast lasers is an effective way to produce broadband coherent extreme ultraviolet (EUV) radiation with a table-top setup. With spectral coverage in the range of 10-50 nm, such sources open up the prospect of spectroscopic nanoscale imaging of integrated circuits and biological structures. Given the limitations of high-resolution optical components for EUV radiation, lensless imaging concepts such as ptychography are essential for efficient HHG-based imaging. Major steps forward have been made in recent years, including demonstrations of wavelength-scale resolution [1], quantitative phase imaging [2], and material-sensitive microscopy [3,4]. But to establish computational EUV imaging as a practical application-ready technology, important challenges remain.

One such challenge is the ability to image three-dimensional structures in a reflection geometry, which is particularly relevant for semiconductor metrology. Extending the ptychography forward model to 3D reflection imaging is complex, and creating the measurement diversity needed for 3D reconstructions rapidly leads to large datasets and slow measurements. Another challenge is measurement speed, as ptychography involves recording large datasets through sequential image acquisition with full-frame cameras.

To address these challenges, we are focusing our research efforts on more efficient measurement schemes that optimize the retrieved information for a given geometry. On the hardware side we are developing ptychography using novel detector systems and structured illumination, while simultaneously implementing improved forward models through automatic-differentiation-based algorithms. I will discuss these ongoing efforts, recent advances, and the main challenges ahead.

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Deep label-free lifetime microscopy using single pulse sampling

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Lifetime probes molecular contrast in a way largely orthogonal to intensity measurements, however at the cost of accumulating hundreds, if not thousands of pulses per measurement. Here we demonstrate how to synergize hardware and algorithms to achieve single-pulse label-free fluorescence lifetime microscopy in deep and living biosystems.

Computational Imaging of Hemodynamic Brain Activity

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I will present a new methodology for computational imaging of hemodynamic brain activity based upon processing of images coming from a non-contact photonic speckle patterns-based sensor. The sensor senses nano-vibrations such as those associated with hemodynamic processes in our brain. This technology is based upon illuminating a biological surface with a laser and then using a camera with its special optics to perform temporal and spatial tracking of secondary speckle patterns in order to have nano metric accurate estimation of the nano-movements of the back reflecting surface. The capability of sensing those movements in nano-metric precision allows connecting the movement with remote bio-sensing and with medical diagnosis capabilities.

The proposed technology was already applied for remote and continuous estimation of vital bio-signs (such as heart beats, respiration, blood pulse pressure and intra ocular pressure), for molecular sensing of chemicals in the blood stream (such as for estimation of alcohol, glucose and lactate concentrations in blood stream, blood coagulation and oximetry) as well as for sensing of hemodynamic characteristics such as blood flow to various regions of the brain. It was also used in medical trials for remote medical diagnosis of various diseases such as cardio-pulmonary diseases.

The capability of sensing the hemodynamic activity when applied on the brain and when combined with advanced machine learning and AI based algorithms allows us to detect various insights regarding brain activity, for example, how to detect which one out of the five senses related cortices of the human brain is stimulated in a given moment. In addition, the sensing methodology can remotely classify the objects that a subject is looking at and thus they stimulate his visual cortex (visual cortex shape recognition), it can perform remote decoding of inner speech by directing the sensor to brain's Broca area and finally it can also extract motor cortex related hemodynamic activity which as a results can be used to operate an electrical motor that will be actuated with various types of motions only by thinking about those motoric motions.

Abstracts of Posters

(in alphabetical order)

Local spectroscopy on thin films using white-light interferometry

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White-light interference microscopy enables non-destructive, full-field measurement of surface topography and optical properties. The data acquisition process involves translating the sample along the optical axis while sequentially capturing interference images at regular intervals to construct a complete interferometric stack. Subsequent Fourier-based analysis of this stack yields quantitative three-dimensional reflectance maps over a large field of view. These three-dimensional reflectance maps are called hyperspectral cubes. This hyperspectral cube has both spatial and spectral information about the samples under investigation. Thus, the use of a Linnik-type interferometer with Fourier transform processing provides the spectral response on different areas of the sample.

In this study, we performed local spectroscopy on a thin layer of TiO_2 applied to a B270 glass substrate and a thin layer of ZrO_2 on a silicon substrate using a vertically oriented Linnik-type interferometer. The wavelength range of the reflectance response is 400–900 nm. We also compared the reflectance spectra obtained from this method with those measured using a Cary 5000, a commercial reflectance spectrometer.

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Versatile microscopy setup combining ISM, FLIM, STED, and adaptive optics

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We report on the development of a scanning microscope setup that targeting high resolution in deep tissue. It utilizes the resolution enhancement of image scanning microscopy (ISM) and stimulated emission depletion (STED). Imaging in deep tissue with high resolution requires adaptive optics to compensate substantial aberrations. The setup provides precise photon timing required for fluorescence-lifetime imaging microscopy (FLIM).

Computational investigation and optimization of perovskite solar cell performance

Khadija Boughanbour

Abstract

This study investigates the photovoltaic performance of perovskite solar cells using the SCAPS-1D simulation tool. The numerical results were first validated by comparison with available experimental data, showing good agreement and supporting the reliability of the proposed simulation approach.

The study then focused on improving device performance by systematically analyzing key parameters, including the absorber-layer thickness, carrier concentration, and the configuration of bilayer electron transport layers (ETLs). Different TiO_2/ZnO configurations were investigated to enhance charge transport and reduce performance losses within the device.

Following the optimization of these parameters, the best-performing structure achieved a power conversion efficiency (PCE) of 29.26%, using an absorber thickness of 800 nm combined with a TiO_2/ ZnO bilayer ETL. These results highlight the potential of carefully engineered multilayer structures for improving the efficiency of perovskite solar cells.

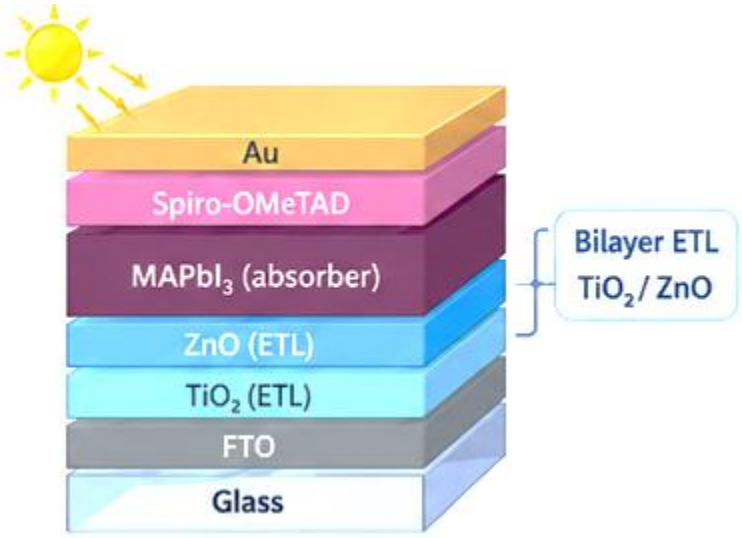


Figure 1:Structure solar cell

Teaching Computational Microscopy Using An Open, Modular Holography Education Kit

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Abstract

In many cases it is advisable to teach students the principles of light-matter interaction, but typically this is done with a single teacher or professor performing the experiment in front of many students. This leaves everyone else without the hands-on experience of, for example, aligning a system such as a Michelson or Mach-Zehnder interferometer to gain an intuitive sense of what a wavelength really means. With the openUC2 system, we have established an open, modular framework built on modular cubes containing, for example, lenses, mirrors, lasers, or cameras. These modules can be combined with one another to form arbitrarily complex optical systems. Together with the Educational Chair at the University of Münster, we have created a curriculum that assembles a set of physics experiments addressing optical holography, teaching the concepts of interference and the wave propagation of light.

We start with simple experiments explaining diffraction at a single or double slit, progressing to a grating, and then move on to building an inline lensless holography setup using either a point source (an LED) or a single-mode laser. Here we explain the different strategies of incoherent versus coherent illumination and their respective pros and cons. This is paired with a computational reconstruction device built from a Raspberry Pi and a camera, where hologram processing is performed on-device, including background reduction via adaptive masking. Using the same cubes, the so-called HoloBox can also be configured as a Michelson interferometer or a Mach-Zehnder interferometer, the latter convertible into an off-axis holography microscopy setup, where the digital Fourier transform and beam propagation are explained. Inside Jupyter Notebooks running on the Raspberry Pi, students can code their own reconstruction using Python as the interface.

The box comes with teaching material for teachers and professors who want to explain these phenomena and give students the tools to explore them independently. It is accompanied by fully open-source documentation and building instructions following the Diátaxis documentation style, with tutorials, how-tos, references, and explanations. With this we hope to establish an open platform where students can learn and interact with the system. The entire box is also part of a digital twin, enabling students to first simulate the setups online using the openUC2 OptiKit configurator, and then replicate them in real life using the cubes.

Optical-Tweezer-Driven Sample Rotation for Motion-Robust 3D Refractive Index Tomography

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Optical diffraction tomography reconstructs the three-dimensional refractive index (RI) distribution of transparent microscopic objects from holographic measurements taken at many illumination or sample orientations. Rotating the sample, instead of scanning the illumination, gives dense and continuous angular sampling with a single, fixed illumination direction. However, it usually requires mechanical mounting and assumes a precisely known, stable rotation. We present TomoTweezers, a holographic tomography platform in which the sample is rotated contact-free about the optical axis by three optical traps. The traps are positioned and steered through holographic wavefront shaping on a phase-only spatial light modulator, so no mechanical stage or capillary mounting is required. Combined with oblique illumination, this rotation samples the object's spatial-frequency spectrum over the full azimuthal range. Optically trapped samples drift, wobble and reorient during manipulation, so their pose cannot be taken from the trap control alone. We therefore developed an iterative reconstruction framework in which the six-degree-of-freedom trajectory of the sample (rotation, tilts, lateral and axial displacement) is recovered from the holograms themselves, jointly with the RI volume, using differentiable forward models from Ewald-sphere mapping to a non-paraxial multiple-scattering model. We validate pose recovery on simulated data with known ground truth. We present reconstructions of a 3D-printed calibration phantom with resolution-target features and of biological cells.

Keywords: holographic tomography, optical trapping, refractive index imaging, motion correction, inverse problems

Acknowledgements

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Skip the picture, keep the signal: Speeding up indirect wavefront sensing in three-photon microscopy

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Three-photon microscopy (3PM) enables imaging deep within scattering biological tissue. However, the tissue itself introduces optical aberrations that degrade the achievable power density in the focal volume and thus image quality. Adaptive optics (AO) compensates for these aberrations, commonly via image-based modal wavefront sensing followed by correction with adaptive elements — but in 3PM such approaches are limited by long image acquisition times needed for sufficient signal.

To find a robust and fast AO strategy, we performed a Fisher information analysis of the influence of individual aberration modes on the image. The results show that for three-photon imaging, aberration information is predominantly encoded in the cumulative fluorescence intensity rather than the spatial structure of the acquired image.

Building on this result, we refrain from taking resolved images for the sake of speeding up the wavefront sensing process. To maximize the photon flux and protect the sample from damage and photobleaching, we employ holographic beam splitting to distribute the excitation power across multiple foci. Using a fast MEMS phase modulator, we demonstrate fast and robust aberration measurement and compensation in brain organoids.

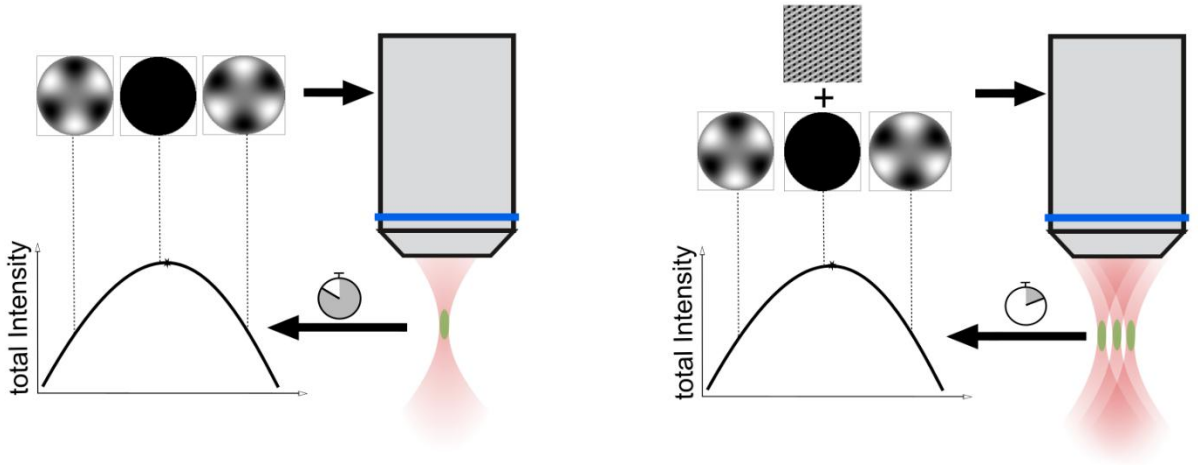


Figure 1: Concept of speeding up modal wavefront sensing. The left image shows the standard procedure of image-based modal wavefront sensing. The right image visualizes our method to speed up the process by measuring signal from multiple foci in parallel.

Microscopy image upsampling via multi-modal image fusion

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In the context of microscopy-based imaging systems, reduced data acquisition time has been a key requirement especially when the exposure time is critical for the samples. A common strategy for speeding up image acquisition is to sparsely sample the field of view and reconstruct the unobserved information using computational methods. Traditionally, these reconstruction approaches formulate an optimization problem based on the forward model, with a suitable cost function and prior assumptions used to recover the underlying image. Recently, neural network-based methods have emerged as powerful alternatives, often achieving substantial improvements in reconstruction quality and computational efficiency. However, their performance can depend on large and representative training datasets introducing vulnerability to out of distribution hallucinations. These limitations motivate the development of reconstruction frameworks that can use information intrinsic to the individual sample being imaged, thereby reducing reliance on external training datasets [1].

We move away from single-sensor measurements and propose an upsampling approach that fuses data acquired from two different modalities for the same sample. One of the modalities captures the images in higher resolution than the other. The algorithm design focuses on using latent space representations of the higher resolution data which is used in an instance-based learning pipeline that predicts the upsampled version of the second image. The framework is general and can potentially be applied to different microscopy platforms that allow imaging modalities of two kinds. We present our results for fluorescence lifetime imaging microscopy (FLIM) images and Brillouin microscopy images.

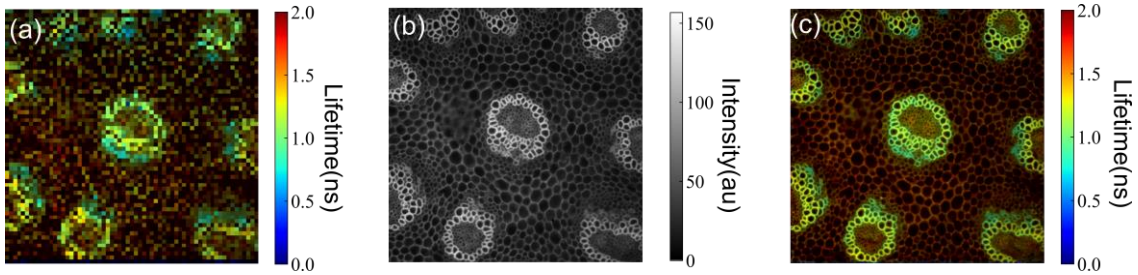


Figure: (a) Low resolution (128 x 128) FLIM image, (b) High resolution (512 x 512) intensity image and (c) Upsampled FLIM image via proposed algorithm.

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Do you trust your measurement results? - Strategies for instrument characterization pave the path to reliable measurement data

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Advances in computer-aided evaluation have fundamentally transformed how measurement data is analyzed and interpreted compared to just a few years ago. As a logical next step, artificial intelligence (AI) and machine learning models will enable sophisticated simulations that help explain - and potentially even correct - specific behavioral characteristics of measurement devices. However, because these models rely on real-world measurement data that inevitably contain imperfections, the validity and explanatory power of the resulting simulations can be severely limited. It is precisely at this critical juncture that the user could retain control: by actively engaging with the data, they can identify and optimize the relevant training datasets in advance.

This publication aims to raise critical awareness against the blind trust often placed in raw measurement datasets. In this context, we investigate the causes and specifically highlight that different measurement techniques (e.g., tactile versus optical) give rise to distinct, often system-dependent systematic errors. We analyze some of the major effects in real datasets within the framework of current national (VDI) and international (ISO) standards and aim to augment these conventional procedures with novel approaches and methods.

Furthermore, we sensitize users to the detection of erroneous measurements—ranging from localized, isolated anomalies to multi-level systematic flaws. We aim to identify the errors that arise and subsequently classify and categorize them based on the extent of their potential impact on the measurement results. This is currently subject of ongoing research projects. Finally, this allow to draft effective strategies for prevention and subject the measured datasets to straightforward diagnostic tests.

Ultimately, while this approach cannot eliminate or correct all errors entirely, it significantly improves the quality of our underlying measurement data, results, and training datasets - thereby establishing a robust foundation for enhanced reliability and, ultimately, also for better AI models.

FOURIER TRANSFORM ACOUSTO-OPTIC IMAGING: TOWARD REAL-TIME IMAGING OF THICK BIOLOGICAL MEDIA

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Acousto-optic imaging (AOI) aims at probing locally the optical contrast of biological media at depths (~cm) where multiple scattering of light by inhomogeneities limits microscopy. Yet, imaging of absorbing objects embedded in highly scattering media remains challenging since the detectable signal which is suitable for image reconstruction is weak. Significant improvements were made possible by the joint use of (i) a newly developed and characterized high peak-power laser diode source and (ii) the Fourier Transform Acousto-Optic Imaging (FT-AOI) technique. Here, we highlight that using a 9 W high-peak power while maintaining an average power below 1 W proved the ability of the overall setup to probe highly scattering media in real time.

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Brightfield Snapshot 3D Microscopy: Towards In Vivo Imaging

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Three-dimensional (3D) microscopy is essential for resolving complex biological structures, yet conventional microscopes inherently acquire two-dimensional (2D) intensity measurements. In brightfield microscopy, 3D imaging is commonly achieved by axial scanning, which limits acquisition speed and may introduce motion artifacts in vivo. Snapshot alternatives avoid scanning but suffer from resolution tradeoffs and reduced field of view or sensitivity. This work aims to enable snapshot 3D brightfield microscopy, by optically encoding depth information through point-spread-function (PSF) engineering, traditionally used in fluorescence microscopy, and computationally decoding it from a single 2D measurement. The focus is on capillary-like structures in biological environments.

For the optical encoding, we formulate a Fourier imaging model under partially coherent illumination to guide PSF engineering. A novel PSF, termed Tripod, is designed to enhance depth encoding for filament-like structures. The optical system is implemented experimentally by adapting the illumination and introducing a phase mask in the Fourier plane. Volumetric information is then reconstructed from a single 2D image using a physics-informed deconvolution framework that incorporates the engineered PSF and the imaging forward model.

Simulations demonstrate that 3D structure can be reconstructed from a single 2D image using the proposed Tripod PSF. Experimentally, the engineered PSF produces the expected depth-dependent features under partially coherent illumination. Reconstruction of experimental single-shot measurements shows qualitative agreement with reference z-stacks of the imaged structures, while quantitative evaluation is ongoing.

Together, these results demonstrate an optical-computational framework for snapshot 3D brightfield microscopy without axial scanning. This work extends PSF engineering, which was traditionally explored in fluorescence microscopy, to partially coherent brightfield imaging and enables volumetric reconstruction of filament structures from a single measurement. Future work will focus on integrating the optimized phase mask into a compact system and evaluating it for in vivo capillary imaging.

Raman Spectroscopy guided by Optical Diffraction Tomography for biomedical applications

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Spontaneous Raman spectroscopy (SRS) is an optical technique that enables the analysis of a sample's chemical composition. It is the only technique that provides access to the spectral region known as the "fingerprint" region, which is of crucial importance in biological research [1]. However SRS has a critical limitation in measurements of living cells, which are dynamic structures. Due to very weak SRS signal the measurement time is long (reaching a few seconds per measurement point) and therefore chemical mapping of cells, as well as time-lapse measurement mode are very difficult, if not impossible. The solution to both these problems lies in employing a pilot technique for SRS measurements that can precisely locate specific cellular organelles in 3D space, while simultaneously relaying updated coordinates of the cell and its organelles, thus allowing for time-lapse imaging of living, moving cells. Here we present the concept of the method and opto-mechatronic design of multimodal imaging system in which the selected pilot technique is optical diffraction tomography (ODT). ODT is a label-free technique, which provides three-dimensional tomographic reconstructions of the cellular refractive index/dry mass distribution [2]. In ODT-SRS system, the tomographic measurement is performed first, followed by automated volumetric segmentation based on the resulting tomographic reconstruction to automatically generate digital markers for organelles. Subsequently, an SRS measurement is performed only at the locations indicated by the markers. This dual-modal approach will enable the rapid, non-invasive acquisition of information regarding the precise morphology of a living cell (via ODT) and the chemical composition of its organelles (via SRS). It will also be possible to conduct time-lapse measurements while tracking the cell and its organelles in space. Ultimately, the newly developed method and system will be used for automated, non-destructive screening and analysis of biological cells and tissues. The proof-of-concept of the ODT-SRS system is demonstrated through the investigation of the effects of Yokukansan on neuroblastoma cells [3].

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Design of a Low-Cost Smart Digital Microscope for Medical Diagnosis in Low-Resource Settings

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Abstract

Access to reliable microscopy remains a major challenge in low-resource settings, where conventional diagnostic microscopes can be expensive, difficult to maintain, and dependent on specialized equipment and trained personnel. This work presents the design and development of a low-cost smart digital microscope for accessible biomedical imaging and medical diagnosis in resource-constrained environments [1]. The proposed system combines an affordable optical configuration, a digital camera, embedded computing, and image-processing techniques to convert microscopic observations into digital images suitable for visualization, analysis, storage, and remote consultation. The prototype is developed using widely available and low-cost components, with the objective of reducing equipment costs while providing adequate image quality for basic biomedical applications. Experimental evaluation was performed using biological specimens [2], including onion epidermal cells and blood smears. The results demonstrate the capability of the proposed system to acquire, process, and visualize microscopic images digitally. The integration of embedded computing provides a foundation for intelligent image enhancement, quantitative analysis, and future artificial-intelligence-assisted medical diagnosis.

This work demonstrates the potential of combining low-cost optical instrumentation, digital microscopy, and embedded intelligence to develop accessible diagnostic technologies for low-resource environments. In the long term, the proposed platform could be extended toward automated image analysis, remote diagnosis, and secure processing of biomedical images [3].

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Multiscale investigation of La-doped $BiVO_4$: From experimental characterization to DFT modeling

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Abstract:

La-doped monoclinic $BiVO_4$ [1] samples were synthesized by solid-state reaction method and studied using experimental and theoretical approaches. X-ray diffraction (XRD) was used to confirm the monoclinic phase and examine changes in the unit-cell volume with La concentration from 0 to 20%. Scanning electron microscopy (SEM) was used then to characterize the morphology of the samples. Density functional theory (DFT) [2] calculations were performed using Quantum ESPRESSO [3] to calculate the structural parameters and compare the theoretical unit-cell volumes with the experimental results. The effect of La doping on the structural properties of $BiVO_4$ was then analyzed.

Keywords:

XRD, DFT, SEM, Doping, Solid-state reaction, Quantum ESPRESSO.

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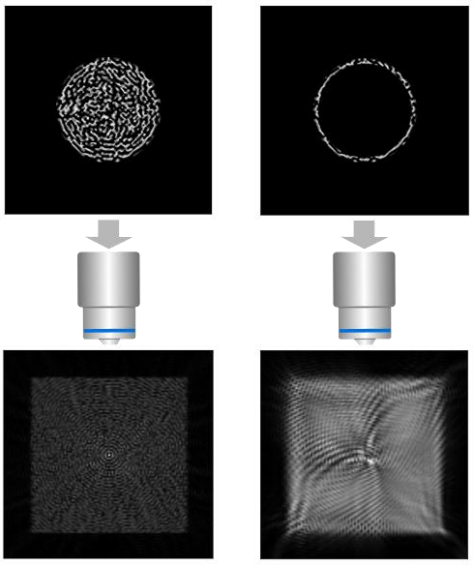
Holographic Light Shaping for Precision Photobleaching in Live Cell Membranes

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Quantifying protein oligomerization in live cell membranes is essential for understanding cell signaling, but classical microscopy techniques cannot resolve this at the required scale without disrupting the sample. The TOCCSL (Thinning Out Clusters while Conserving the Stoichiometry of Labeling) [1] method addresses this by photobleaching a defined membrane area and tracking single molecules as they diffuse back in. However, TOCCSL currently relies on simple, static bleaching geometries, which introduce different artifacts, for example partial bleaching close to the bright/dark transition zones [2].

To overcome these limitations, we aim to generate dynamic, tailored, and speckle-free bleaching patterns through holographic light shaping, with high contrast and illumination confined to total internal reflection angles. Since high-NA optics are used during bleaching, an accurate computational forward model of the optical wave propagation is required to reliably translate simulated patterns into experimentally realizable geometries. This work focuses on developing such a physical forward model - combining analytical and neural network approaches - together with the numerical generation of various bleaching patterns designed to reduce artifacts and enable more precise quantification of membrane protein oligomerization.



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High-speed fly-scan ptychography with single pulse diffraction

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Ptychography is a powerful computational optical imaging technique that enables quantitative reconstruction of both the complex object and illumination field from a series of highly overlapping diffraction patterns. However, its application to dynamic systems has traditionally been limited by long measurement times and low acquisition throughput. These limitations arise primarily from sequential sample scanning, finite exposure times, and detector readout across the large number of diffraction patterns required.

Here, we address these bottlenecks by implementing a high-speed, synchronized fly-scan ptychography system that combines continuous sample motion with single-pulse diffraction measurements using an event-based Timepix3 detector. A high-flux picosecond laser, continuously scanning sample stage, and detector are synchronized through pulse picking, enabling the acquisition of individual diffraction patterns from ~15 ps pulses at kilohertz repetition rates.

With this approach, we achieve acquisition times of 166 ms for a 0.95 mm² field of view and 30 ms for a 0.13 mm² region. Computational reconstruction of the diffraction data yields diffraction-limited imaging at a temporal resolution of 33 frames per second and a space-bandwidth product throughput of 2.3 Gpix/h. In addition to the time-resolved object reconstruction, the computational reconstruction simultaneously retrieves the complex illumination field, providing time-resolved access to the wavefront and sensitivity to beam-pointing instabilities and spatiotemporal variations in the illuminating pulses.

This work demonstrates how high-speed computational ptychography can overcome the temporal limitations of conventional ptychographic imaging and enable dynamic imaging on millisecond timescales. Future work will extend the approach to an in-house high-harmonic source for high-resolution imaging at extreme ultraviolet wavelengths.

Zero-shot quantitative phase imaging from coded wavefront sensing measurements using optical flow networks

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Coded wavefront sensing (CWFS) is an inexpensive, high-resolution quantitative phase imaging method that can be readily integrated into standard laboratory microscopes. However, conventional optical flow-based phase retrieval methods simplify the CWFS forward model by neglecting wave-optical effects such as diffraction to make the optimization tractable. This reduces the range of recoverable wavefront curvatures and limits the allowable phase mask-sensor distances.

Our goal is to leverage the forward model without explicit calibration to enhance the QPI capabilities of CWFS. To this end, we introduce a wave-optical CWFS simulator that generates a synthetic dataset of 18,500 samples for supervised training of state-of-the-art optical flow neural networks, implicitly embedding the physics of the forward model into the network through data. We show that this Deep CWFS approach yields nearly fivefold and 12 dB improvements in root mean squared error and peak signal-to-noise ratio, respectively, on synthetic data and achieves a threefold expansion of the range of recoverable curvatures across practical mask-sensor distances.

Despite being trained exclusively on simulated data, the 3D object composition and wave-optical modeling to create synthetic training data enable Deep CWFS's zero-shot generalization capability, producing high-fidelity phase maps of several biological cell specimens. These are validated by quantitative comparisons on artificial cell phantoms, a microbead cluster, and real HEK293 cells which are simultaneously imaged using digital holographic microscopy. Moreover, Deep CWFS's robustness to variations in microscopes and phase masks is experimentally verified by imaging specimens with two phase masks and several microscope objectives in this work.

ADAM vs. PIE: Evaluation and Optimization of Reconstruction Algorithms for Lensless Imaging

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Diffuser-based lensless imaging (Fig. 1a) enables high-resolution microscopy of transparent specimen using a compact and cost-effective setup without conventional imaging optics. As a consequence of this hardware simplification, the amplitude and phase of the sample must be reconstructed from multiple coded intensity measurements by solving a high-dimensional, nonlinear inverse problem (Fig. 1b). We optimize and compare two reconstruction methods: a forward-model-based optimization framework using stochastic gradient descent (Adam) and a refined version of the Ptychographic Iterative Engine (PIE) [1]. Both methods are evaluated using simulated and experimental lensless microscopy data with respect to reconstruction accuracy, convergence behavior, and computational complexity, employing a problem-adapted error metric. Our results show that, with a suitable loss function and learning-rate schedule, stochastic optimization achieves higher reconstruction accuracy and faster convergence than PIE for moderate batch sizes. In contrast, PIE is more robust for small batch sizes and requires less computation per iteration. Furthermore, we demonstrate that incorporating a priori diffuser knowledge significantly improves reconstruction quality for both methods while reducing the required number of measurements and computational effort.

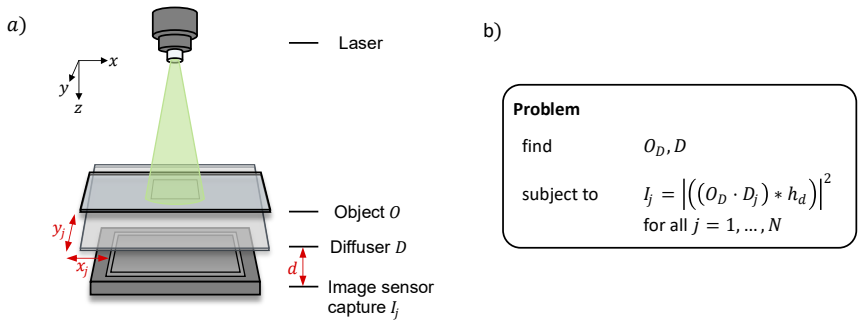


Figure 1. a) General setup scheme as presented in [2]. A laser illuminates the transparent sample O, positioned above a thin diffuser D that can be laterally displaced by (x_j, y_j). The resulting modulated intensity I_j is repeatedly recorded by the underlying image sensor. b) Formulation of the problem for the simultaneous reconstruction of the object field at diffuser level O_D and the diffuser D. Here, D_j represents the j-th lateral shift position of D, and h_d denotes the point spread function associated with the diffuser-to-sensor distance d.

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Single-shot Quantitative phase and Polarimetric Imaging (QP²I) for label-free, non-phototoxic imaging of live cardiomyocytes

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Quantitative phase imaging (QPI) provides label-free maps of optical path length difference caused by refractive index and thickness of a sample. Quantitative polarimetry is the equivalent for anisotropic properties, recovering retardance and fast-axis orientation of birefrigent samples. With polarization-sensitive cameras, both modalities can now be obtained from a single shot at full camera frame rate. We present a dual-mode system that switches between quantitative phase and quantitative polarimetric imaging, using the polarization camera in a conventional DIC microscope. Our new QPI reconstruction uses a total-variation-regularized phase solver that avoids the streak artifacts of conventional line-integrations. We apply QP²I to live, contracting cardiomyocytes derived from induced pluripotent stem cells. While QPI captures cell morphology and nuclear thickness, polarimetry selectively reveals the contractile myofibrillar architecture, resolving individual sarcomeres at sub-microradian sensitivity. Compared to conventional fluorescence, our approach eliminates photobleaching and phototoxicity constraints, enabling sustained observation of contractile dynamics.

Full-field swept-source quantitative phase imaging with temporal coherence gating

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The requirements for quantitative phase imaging (QPI) are continuously growing, which has naturally led scientists to search for novel solutions. Currently, transmission optical coherence tomography (tOCT) is emerging as a compelling alternative to conventional QPI techniques, primarily because of its temporal coherence gating ability. However, state-of-the-art tOCT approaches [1,2] still lack the capability for direct complex amplitude retrieval, which greatly limits their broad applicability. To address this critical gap, we introduce quantitative transmission OCT (qtOCT) [3], a framework for direct 2D QPI using a full-field swept-source Mach-Zehnder-based optical system. By combining the highly coherent complex amplitudes captured across the entire 75 nm swept bandwidth and performing an inverse Fourier transform, we computationally synthesize a narrow temporal coherence gate. This data-processing step enables the temporal sectioning of the arriving photons and discards the delayed photons, which do not contribute to the correct ballistic phase of the object. The non-object tOCT signals can originate from parasitic system reflections or multiple-scattering events, and effectively filtering them out offers two fundamental advantages of qtOCT: 1) powerful signal-to-noise ratio (SNR) improvements and 2) a clear pathway toward imaging thick, highly scattering biological tissues or organoids. In addition, qtOCT is compatible with optical setups operating in on-axis and off-axis configurations, including, e.g., standard digital holographic microscopy (DHM) systems equipped with a swept-source laser. It is also worth noting that our coherence-gated 2D approach can be extended into 3D refractive index (RI) tomography via variable-angle illumination and tomographic reconstruction algorithms. The resulting 3D quantitative RI maps provide detailed, volumetric characterization of samples in their native state while preserving core advantages of the 2D implementation.

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Quantitative Evaluation of the Efficacy of Holographic Scattering Correction in Two-Photon Microscopy

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Optical microscopy plays a central role in the quantitative investigation of biological systems; however, its performance is severely limited by the achievable imaging depth in optically turbid samples. Refractive index inhomogeneities within biological tissues give rise to scattering and wavefront distortions, progressively degrading image quality and spatial resolution with increasing imaging depth. Adaptive optics has become a key strategy for compensating these effects. Among the available approaches, *Dynamic Adaptive Scattering compensation Holography* (DASH) has emerged as a promising technique for iteratively reconstructing and compensating scattered wavefronts through the combination of dynamic wavefront shaping and holographic phase-stepping interferometry [1].
In this work, we present an experimental quantitative evaluation of DASH-based scattering correction, focusing on its performance as a function of imaging depth. Experiments are performed on lab-grown human epidermal tissue models [2], where the method enables the recovery of fine morphological features of cellular nuclei. The effectiveness of the correction is quantitatively assessed through Fourier-domain analysis of the acquired images, revealing the recovery of high spatial frequency components after the correction. The experimental characterization is complemented by numerical simulations of the correction process using synthetic scattering phase masks, generated through a model that exploits the optical properties of the scattering medium.

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The Missing Scale in Surface Reflectance: Optical Linear Algebra for Reflectance Measurement

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The bidirectional reflectance distribution function (BRDF) is the standard descriptor of surface appearance in radiometry, rendering and industrial inspection, yet it plays almost no role in optical microscopy. The two communities are separated mainly by length scale: goniometric measurements use millimetre-sized spots and average away the microstructure that microscopy is designed to resolve.

We recently proposed a mechanically scan-free BRDF acquisition scheme based on a co-aligned projector-camera pair and a single lens (see Fig. 1). The lens images both projection centres onto the same surface point, establishing a bijection between projector pixels and incident directions and, by reciprocity, between reflected directions and camera pixels. The resulting light transport matrix is therefore a permuted representation of the tabulated BRDF. More importantly, each projected pattern and captured image realize one matrix-vector product optically; an Arnoldi iteration with non-negative illumination patterns can thus recover a rank-K approximation using 2K exposures, independent of angular resolution.

This pupil-conjugation condition is scale-free. Replacing the macroscopic lens with a high-NA microscope objective retains the same formulation. The concept thereby becomes a motionless microscopic goniometer with substantially increased angular coverage. At these scales, reflectance changes character. Comparing spot size with surface correlation length suggests ensemble-averaged, spatially non-stationary regimes.

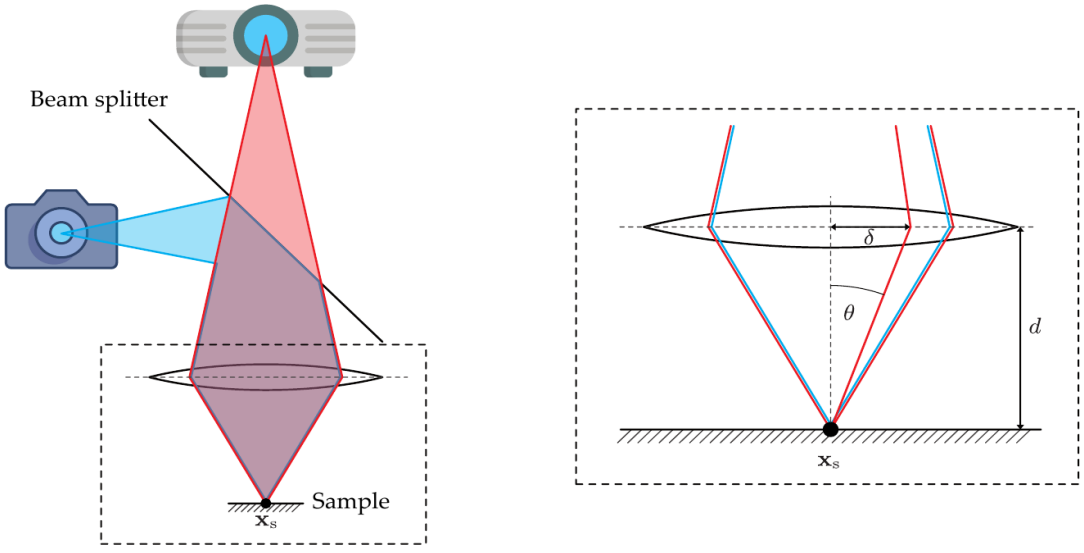


Figure 1. Pupil-conjugate BRDF measurement principle.

Influence of Optical Aberrations on Lateral and Axial Resolution in Image Scanning Microscopy

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Image scanning microscopy provides a better resolution and higher signal-to-noise ratio compared to confocal microscopy. Detecting a larger fraction of emitted fluorescence photons by a SPAD-array detector, instead of rejecting most of it, allows techniques like pixel reassignment to improve the resolution by a factor up to $\sqrt{2}$. This method assumes that the signal is not distorted, whereas both the optical system (misalignment, imperfect optics) and sample refractive-index variations can cause aberrations such as spherical, coma or astigmatism. These aberrations dominate when focusing deep into thick, scattering tissue, exactly where adaptive-optics correction is being introduced into ISM. It is not clear how much of the ISM advantage survives, or whether pixel reassignment still works when the wavefront is no longer flat.

This contribution investigates how optical aberrations affect lateral and axial resolution in ISM. Using a forward model with a SPAD-array detector and Poisson noise, we quantify the change in reconstructed resolution as a function of aberration type and amplitude. The main question is whether pixel reassignment provides robustness against aberration degradation in ISM, the same way it does in confocal. Single Zernike modes are applied with increasing amplitude to a simulated sample, and we compare three cases: the whole array summed, a single central pixel (confocal) and ISM with pixel reassignment. We use Fourier ring correlation (FRC) as a resolution metric, since confocal and ISM do not collect the same number of photons: the pinhole blocks most of the light while the SPAD array retains it. A purely geometrical measure ignores this difference, whereas FRC depends on noise, so the photon budget is included in the resolution value itself. FRC is also measured on the reconstructed images, so it takes the reassignment step into account. We also treat the lateral and axial responses separately, since ISM enhances lateral resolution but contributes mainly to optical sectioning along the axis.

The expected outcome is a map showing which aberrations limit ISM most, and how ISM and confocal differ when the wavefront is distorted.

Speedy Extreme Ultraviolet Ptychography with a Microchannel plate and Timepix3 detector

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Ptychography is a lensless imaging method in which high-resolution images are reconstructed from a set of coherent diffraction patterns. The adoption of a coherent table-top EUV source (i.e., high harmonic generation) greatly improves ptychography's spatial resolution when compared to visible sources. However, the low efficiency of the HHG source results in long measurement times. Here we introduce the combination of a microchannel plate (MCP) and an event-driven Timepix3 camera to speed up conventional ptychography. The high gain of up to 10^{10} from the MCP guarantees enough photon flux, and a ns-scale readout speed of Timepix3 paves the way for the single shot measurement.[1] The stochastic amplification process of MCP and the dynamical response of Timepix3 are firstly modelled numerically and calibrated experimentally, which are used as prior information in image reconstruction. Using MCP and Timepix3, we are working towards diffraction-limited imaging resolution in EUV ptychography with orders of magnitude improvements in measurement time, making EUV ptychography more compatible with industrial applications.

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Robust and Reliable Motion Estimation for Fourier Diffraction Tomography

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In Fourier diffraction tomography, unknown specimen motion must be accurately estimated to obtain high-quality reconstructions. Recent works, (Quellmalz et al., [1,2]), showed that this motion can be estimated directly by comparing pairs of complex field measurements. However, chaining estimates from consecutive frame pairs lets individual errors accumulate. Rather than estimating this motion from a single chain of measurements, our approach is to consider different trajectories taken across different frame separations into one consistent motion estimate, making the result less sensitive to any single unreliable measurement. We test this approach on simulated data with noise and jittery motion, comparing it against existing methods.

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Hyperspectral Tomographic Diffractive Microscopy: Development and Applications

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Tomographic diffractive microscopy (TDM) provides label-free three-dimensional imaging by reconstructing the complex refractive-index distribution of a sample from optical fields acquired under different illumination angles [1]. However, at a single wavelength, chemically distinct structures may have similar optical properties. To address this limitation, this study aims to develop hyperspectral TDM, which uses wavelength-dependent dispersion and absorption to enable material discrimination, as demonstrated by spectroscopic microtomography [2].

For this purpose, we designed and built a TDM system at IRIMAS using a supercontinuum laser with a tunable spectral filter and a quadriwave lateral shearing interferometry (QWLSI) wavefront sensor [3]. The sensor enables self-referenced acquisition, allowing us to recover the transmitted wavefront without a separate reference arm. At each selected wavelength, measurements acquired at different illumination angles are combined through Fourier-domain aperture synthesis and reconstructed using the Rytov approximation [4]. The aim is to recover the three-dimensional complex refractive-index distribution as a function of wavelength.

Preliminary multi-wavelength acquisitions have allowed us to reconstruct a 5 µm silica bead at 633 nm (red), 532 nm (green), and 488 nm (blue), using 400 illumination angles [4]. These results are an initial step towards hyperspectral operation, with material discrimination remaining an aim of the study. Work is ongoing to improve the quality of the reconstructions, as well as optimise the acquisition pipeline.

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Optical-Processing of Raman-Encoded Signals

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The development of all-optical labeling and processing represents a frontier in the creation of intelligent photonic sensors for real-time biomedical diagnostics. In this study, we leverage supercontinuum generation in a highly nonlinear optical fiber as a feature-extraction layer within an extreme learning machine (ELM) framework. To demonstrate the core physical principle, synthetic Raman spectra of proteins, lipids, and nucleic acids were encoded onto femtosecond pulses using amplitude modulation with a 0.2 dB depth, emulating the signal characteristics of coherent anti-Stokes Raman scattering (CARS). These encoded pulses were then injected into a nonlinear fiber, where the resulting supercontinuum served as a high-dimensional nonlinear transformation of the input data. After sampling the output spectra and selecting the most informative wavelength windows, we train a weight matrix via linear regression to map the signals to their dominant biochemical component. Using only 150 readouts, we achieved a classification accuracy of 90.3%. These results establish a viable proof-of-concept, paving the way for the development of ultra-fast, all-optical diagnostic systems.

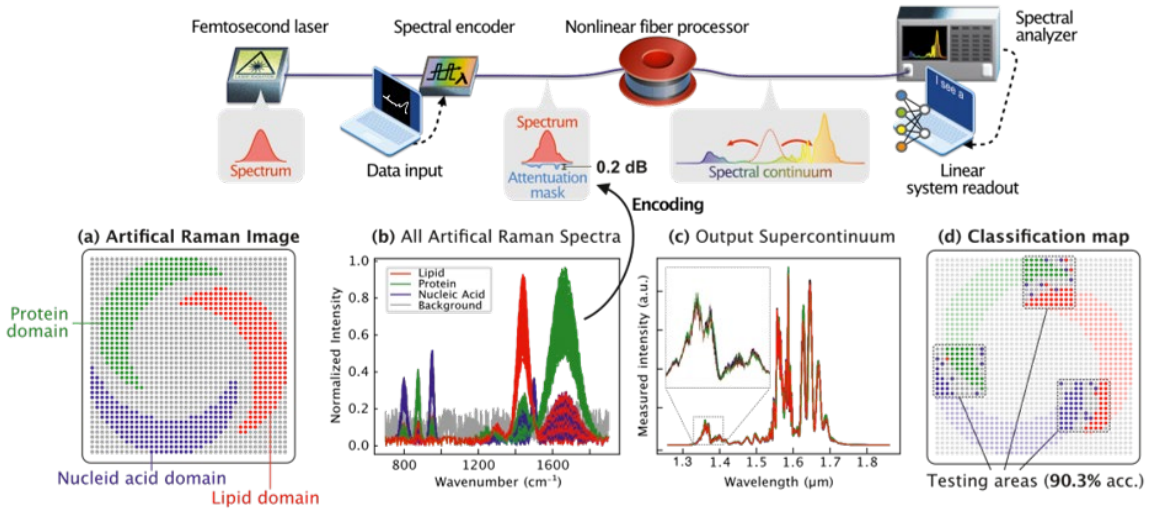


Figure 1. Dataflow of the optical classification system. (a) Synthetically generated hyperspectral image data with chemically dominant regions. Each pixel in the 40×40 image corresponds to a Raman spectrum, shown in panel (b). (c) Measured output spectra of each pseudo-Raman-encoded-signal. (d) Classification results, the pixels in the marked areas were used as test data while the other pixels of the image were used as training data.

Fourier domain optical coherence tomography

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Optical coherence tomography (OCT) is a high-resolution imaging technique, which works by measuring the time-of-flight of light echoes from turbid scattering media. This is accomplished by creating an interference pattern between light propagating in the sample arm and light propagating in the reference arm of a Michelson interferometer. In Fourier-domain OCT, a depth scan is computed by performing an inverse Fourier-transformation of the interference spectrum.
In this contribution, we present a mathematical description of Fourier-domain OCT, deriving a numerical recipe for the computation of a tomogram.

Viscope - from virtual to real instrument control

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Abstract

In this work, we present a Python-based framework for instrument control called *Viscope* [1]. The main advantage of this framework is that the development of data acquisition procedures and their debugging are carried out without the need for any physical instrument. This approach speeds up code development and minimizes the risk of instrument damage.

The core idea is that a virtual sample and its interaction with all instruments in the system is defined via a forward model. All these calculations are encapsulated in a class called the 'virtual microscope'. For each class controlling a real instrument, an equivalent class with the same methods and parameters — controlling a virtual instrument — is generated. These virtual instruments communicate their state to the 'virtual microscope' class so that the response to other instruments can be calculated and updated.

In the *Viscope* framework, classes for each type of physical instrument (e.g., camera, stages, laser), together with classes for data processing, are defined. These classes have the ability to run their methods, each in a separate thread, and to communicate with other classes using integrated flags. During the development and debugging of the code, virtual instrument classes are used. After successful testing, it is only necessary to swap the virtual classes for the real instrument classes in the code, which should then run with the physical instruments.

The *Viscope* framework also contains classes for a graphical user interface (GUI). Each instrument class has a dedicated window for the adjustment of parameters (e.g., values, text) or the presentation of data (e.g., images, graphs). These GUIs make use of PyQt and the multi-dimensional data viewer Napari.

The *Viscope* (and its extensions) are applied for hyperspectral imaging, label-free plasmonic biosensing, fluorescence lifetime image scanning microscopy, ApoTome fluorescence microscopy or holographic MinFlux localisation microscopy.

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Single- shot sectioning in 3D I-COACH using cross-correlation and computational reconstruction

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Abstract: Imaging thick three-dimensional objects remains a fundamental challenge for many applications in biomedical imaging and microscopy [1] because out-of-focus light from multiple depth planes degrades image contrast and obscures fine structural details. Although confocal microscopy [2] and optical coherence tomography provide excellent optical sectioning capability, their reliance on point-by-point scanning limits imaging speed while increasing system complexity and cost. In this work, we present a single-shot optical sectioning technique based on cross-correlation within the framework of interferenceless coded aperture correlation holography (I-COACH). Unlike the previously reported approach [3], which requires three camera recordings projecting into the complex space, the proposed method achieves depth-selective imaging from a single camera exposure with effective background noise suppression. In the proposed single-shot approach, a real-valued system-to-object response (SOR) is captured from the I-COACH system and cross-correlated with a corresponding real-valued synthetic point-spread function (PSF). The amplitude of the resulting correlation is used to obtain the reconstructed image, from which the image plane corresponding to the desired object depth is extracted to yield the final sectioned image with suppressed background noise and reduced contributions from out-of-focus planes. The proposed method is experimentally demonstrated using objects located at different axial positions, including individually reconstructed depth planes and their combined reconstruction. The performance of the single-shot approach is compared with the previously reported three-shot SLICE method [3] in terms of reconstruction quality and background-noise suppression. The reconstructed images are quantitatively evaluated using peak signal-to-noise ratio (PSNR) values with respect to the corresponding reference images. The results demonstrate that the proposed single-shot SLICE approach can provide depth-selective imaging from a single camera recording while retaining the optical-sectioning capability of the previous three-shot method. By eliminating the need for multiple camera exposures and complex-valued SOR construction, the proposed approach simplifies the reconstruction procedure while maintaining effective rejection of out-of-focus information. The single-shot, non-scanning operation and computational cross-correlation framework make the proposed technique suitable for fast three-dimensional imaging and optical sectioning applications.

Keywords: Optical sectioning, Depth of field, coded aperture imaging, 3D imaging, cross-correlation and computational reconstruction.

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Correlation-Enhanced Spontaneous Raman Spectroscopy and Imaging

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Spontaneous Raman spectroscopy is a label-free and minimally-invasive modality of optical microscopy for the molecular fingerprinting and chemical analysis of materials. The strengths of spontaneous Raman are in its experimental simplicity and material non-intrusiveness, which also comes with weaknesses in low signal levels and susceptibility to parasitic background sources. Typically, coherent Raman effects and techniques are employed to overcome this signal limitation but at the cost of experimental complexity and, often, loss of chemical quantitation—the quantitative determination of relative chemical concentrations [1].

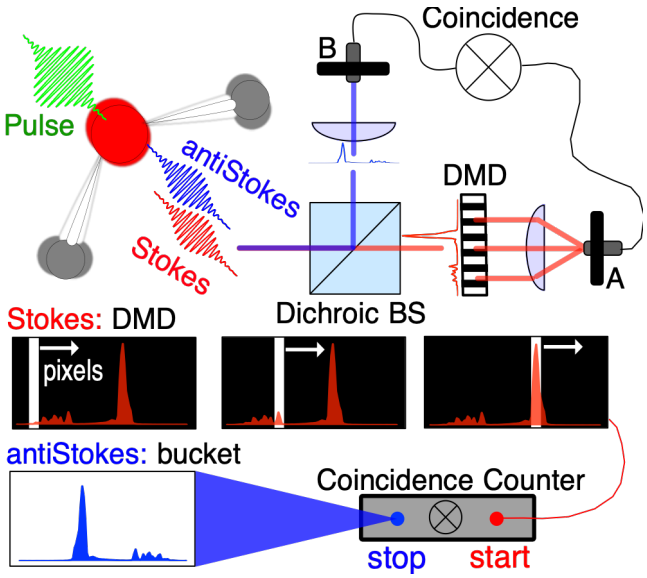


Figure 1. The concept of heralded SaS spectroscopy, including bucket detection of antiStokes (aS) photons and raster scanning Stokes (S) photons with a digital micromirror device (DMD).

In this work, we show how the intrinsic quantum correlations in spontaneous Raman scattering can be exploited to enhance the chemical and quantitative capabilities of Raman spectroscopy, using commercial single-photon detectors and common structured-light technologies. By heralding spectrally-resolved Stokes photons with their correlated antiStokes photons, originating from shared quanta of molecular vibrations under a single-beam laser excitation [2], we demonstrate chemical-specific and noise-suppressed spontaneous Raman spectroscopy. Our results establish these spontaneous Raman correlations as a promising resource for applications in robust chemical imaging and microspectroscopy. We present our latest progress in applying this methodology to point-scanning confocal Raman imaging.

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Towards Computational Phase Imaging for Monitoring Light-Based Fabrication

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Light-based fabrication enables the creation of complex structures by controlling the light distribution inside photosensitive materials. However, the refractive-index changes that occur during fabrication are often small and can be difficult to observe with conventional intensity imaging, limiting direct monitoring of the process. Access to phase information could therefore provide a useful way to visualize and characterize optical changes during fabrication.

Computational phase imaging is sensitive to small refractive-index variations and offers a potential route toward non-invasive process monitoring. In this work, we use multi-angle LED illumination to acquire a limited number of intensity measurements and investigate computational strategies for recovering phase information from sparse measurements. By combining physical forward models with computational reconstruction, we study the trade-off between the number of illumination angles, reconstruction quality, and acquisition speed. Numerical simulations are used to evaluate reconstruction behavior under different illumination conditions, while experiments are performed on phase objects and light-fabricated samples.

The overall goal is to assess whether few-frame computational phase imaging can provide sufficiently robust phase information for monitoring light-based fabrication processes. We particularly investigate optical changes associated with polymerization and phase reconstruction in printed structures, with the longer-term aim of enabling faster feedback during fabrication.

Hybrid coherent/incoherent QPI systems for high throughput phase measurements in biomedical applications

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Recently quantitative phase imaging (QPI) in biological applications is realized by a variety of methods and systems. However, each individual method exhibits distinct trade-offs. Non-interferometric techniques like Fourier ptychographic microscopy (FPM) offer high-resolution synthetic aperture imaging in relatively large field of view (using small NA) but cannot accurately reconstruct low-frequency phase information [1]. To retrieve low frequency term, interferometric anchoring is required. The most popular way is implementation of off-axis digital holographic microscopy (DHM), which enables direct, single-shot phase retrieval, but is limited in resolution for low NA imaging and suffers speckle noise and environmental sensitivity [1]. Recently, a grating based shearing DHM (SDHM) was proposed, which offers common-path stability and operates under partially coherent illumination (low speckle noise), yet requires numerical phase integration and a more complex optomechanical implementation.

We present the implementation of two hybrid coherent/incoherent QPI systems, namely FPM/DHM and FPM/SDHM, working within a unified optomechatronic platform, In the system the respective interferometric anchor populates the central aperture of the Fourier space, while angle-varied illumination from a programmable LED ring and an adaptive FPM algorithm synthesize an extended frequency bandwidth to broaden the imaging resolution.

We systematically evaluate both hybrid systems using calibrated phase targets and 3D phase phantoms replicated via two-photon polymerization [2]. Our comparative analysis examines the practical trade-offs between reconstruction fidelity, system alignment, algorithmic complexity and speckle suppression establishing benchmarks for high-throughput QPI applications.

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Transcriptomics-informed label-free multiphoton microscopy for mouse brain imaging

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Understanding the spatial molecular landscape is crucial for unraveling the cellular mechanisms of neurological disorders. Although current spatial transcriptomics (ST)[1,2] technologies are transformative, they require destructive tissue sectioning, preventing live or longitudinal analysis. Deep-tissue, multimodal label-free nonlinear microscopy (SLAM)[3,4] offers a non-invasive alternative, capturing rich morphological and metabolic contrasts in real-time, yet it lacks direct molecular specificity. To bridge this gap, we introduce an integration pipeline to align SLAM mouse brain images with online ST datasets, followed by an AI-driven representation learning framework[5] designed for associating rich morphological signals in SLAM images with gene enrichment analysis. Our model translates multimodal label-free images into a unified latent space to identify both handcrafted and deep optical signatures associated with distinct compartments and microenvironments. By correlating these representations with spatially resolved gene expression profiles, we link molecularly defined biological pathways to cellular morphology, metabolism, and extracellular matrix features that are resolvable in SLAM. This approach promises to enrich existing spatial biology studies with complementary endogenous contrasts as a lens to phenotypes in native tissue environments, offering a new route to pathological diagnosis in live and longitudinal studies.

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Maximum Likelihood Localization Maps

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We present a non-iterative method for single-molecule localization microscopy (SMLM) that directly constructs maximum-likelihood localization maps (MLLMs) while accounting for Poisson-distributed photon statistics and the point-spread function (PSF). The method evaluates the spatial likelihood distribution of possible emitter positions via direct computation, avoiding iterative parameter fitting. Rather than representing each detected emitter solely by an estimated coordinate, MLLM retains the emitter's spatial likelihood distribution. These likelihood maps can be accumulated directly to form a reconstructed SMLM image without a separate rendering step based on localized coordinates. The computational formulation enables efficient implementation suitable for processing large SMLM datasets. We evaluate MLLM using simulated and experimental SMLM data and obtain localization and reconstruction performance comparable to conventional fitting-based approaches. The non-iterative formulation provides a computationally efficient framework with potential applications in real-time and high-throughput localization microscopy.

Physics-based neural networks for imaging and focusing in scattering media exploiting the memory effect

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Imaging and focusing light through scattering media is central to applications ranging from deep-tissue microscopy to autonomous driving and remote sensing. d'Arco et al. [1] recently introduced a physics-based two-layer neural network that learns to focus light non-invasively through a diffuser. Because both layers are fully connected, however, the number of parameters scales with the number of input and output modes, which restricts the approach to sparse targets.

Here we lift this limitation by embedding the optical memory effect as a prior in the network architecture. Within the memory-effect range, tilting the incident wavefront translates the speckle pattern in the target plane, so the medium acts as a shift-invariant operator. We use this property to replace the two fully connected layers with Fourier-transform layers, whose computational cost no longer depends on the density of the sample. The trained network simultaneously reconstructs the hidden object and retrieves the transmission matrix of the illumination path, which directly provides the wavefront needed to focus through the medium. Multi-photon contrast (e.g., two-photon fluorescence) is modeled by raising the intensity to the corresponding power in the forward model, making the method applicable to both linear and nonlinear fluorescent signals.

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