INTERFEROMETRIC SCATTERING MICROSCOPY

26 – 28 MAY 2020

ERLANGEN, GERMANY

This workshop aims to discuss the emergence, current state and future potential of interferometric scattering microscopy. A hands-on tutorial session will familiarize the participants with the experimental nuances of this powerful technique.

Confirmed Speakers:

Paola Borri
Ji-Xin Cheng
Wonshik Choi
Christian Eggeling
Naomi Ginsberg
Seok-Cheol Hong
Chia-Lung Hsieh
Vinothan Manoharan
Alexandre Persat
Rafael Piestun
Marek Piliarik
Gabriel Popescu
Alexander Rohrbach
Kheya Sengupta
Selim Ünlü
Michael Unser

Organizers: Philipp Kukura (University of Oxford) and Vahid Sandoghdar (MPL)

Registration and Information:
http://bit.ly/iSCAT-Mpl
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MAX-PLANCK-INSTITUT für die Physik des Lichts

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INTERFEROMETRIC SCATTERING MICROSCOPY

INTERFEROMETRIC SCATTERING MICROSCOPY

Invited Speakers

Stroboscopic quasiparticle tracking with an iSCAT probe

Naomi S. Ginsberg
University of California, Berkeley

iSCAT has been employed very powerfully to identify and track small particles in a wide range of contexts. We describe our recent work to instead track photoexcited species in a wide range of semiconducting and composite materials—electrons and holes, bound electron-hole pairs, heat, and sound—at the nanometer scale. Because these *quasiparticles* often travel nanometers over very short time scales, we follow how these different forms of energy travel through materials stroboscopically. In other words, a confocal impulsive light pulse first generates a localized population of excited species, and an iSCAT probe light pulse is used to interferometrically detect how these species modify their index of refraction locally, generating differential contrast. By progressively delaying the probe from the pump, we obtain the mean squared expansion of the initial distribution as a function of time. Doing so enables measurement of quasiparticle diffusivity and, more importantly, identifies deviations from bulk diffusive behavior due to material heterogeneity. This heterogeneity can be caused by material morphological features such as polycrystallinity/grain boundaries, surface specific properties, or structural anisotropy. It can also be due to structural variations that affect the energy landscape of the quasiparticles more microscopically, leading to subdiffusion. I will show various recent examples of our approach, *stroboSCAT*, that illustrate the mapping energy flow in four dimensions of spacetime with few-nanometer lateral precision and direct correlation to material morphology via iSCAT on its own.

Watching RNA viruses self-assemble

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Nearly 60 years ago, Caspar and Klug [1] coined the term "self-assembly" to describe the formation of a virus from its constituent parts (Cold Spring Harbor Symposia on Quantitative Biology 1962). But we still don't understand how this process occurs even in the simplest viruses, positive-sense RNA viruses. Such viruses consist of proteins that form a highly-ordered protective shell (called a capsid) around the viral RNA. Viral particles can self-assemble spontaneously in a mixture of RNA and coat protein in a buffer, in the absence of any host factors. The yield and fidelity of the assembly is particularly remarkable in viruses with a triangulation number of 3 or higher, in which case some of the proteins must find their way to 5-fold coordinated sites and others to 6-fold coordinated sites on the same shell. To understand how such systems assemble, we use interferometric scattering microscopy [2] to measure the kinetics of individual assembling viral particles (MS2 bacteriophage) on time scales ranging from 1 ms to 1000 s. By comparing the scattered intensity to that of the wild-type virus, we infer the mass of the protein shell growing around the RNA as a function of time [3]. We find that individual particles grow to nearly full size in a short time following a much longer delay period. The distribution of delay times suggests that the assembly follows a nucleation-and-growth pathway. I will discuss how such a pathway might allow the virus to assemble so robustly.

1. Caspar DLD, Klug A (1962) Physical Principles in the Construction of Regular Viruses. *Cold Spring Harbor Symposia on Quantitative Biology*, 27:1–24. https://doi.org/10.1101/SQB.1962.027.001.005

2. Ortega-Arroyo J, Kukura P (2012) Interferometric scattering microscopy (iSCAT): new frontiers in ultrafast and ultrasensitive optical microscopy. *Physical Chemistry Chemical Physics*, 14(45):15625–15636. https://doi.org/10.1039/C2CP41013C

3. Garmann RF, Goldfain AM, Manoharan VN (2019) Measurements of the self-assembly kinetics of individual viral capsids around their RNA genome. *Proceedings of the National Academy of Sciences*, 116(45):22485–22490. https://doi.org/10.1073/pnas.1909223116

Fluctuating dynamics of single disassembling microtubules

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Dynamic instability of microtubules is a stochastic process that underpins a large variety of essential intracellular motion, including the movements driving cell division, morphogenesis and cell motility. The tubular shaft of the microtubule consists of laterally-connected chains of tubulin dimers (protofilaments). Static electron micrographs suggest structural transition of straight protofilaments into curled ones accompanied by breaking of the lateral tubulin bonds at the tip of a disassembling microtubule. However, the dynamics of the disassembly is not understood, and no method has been able to capture the underlying structural transitions in real-time.

We combine two optical microscopy techniques to decipher the conformation changes of protein oligomers preceding the microtubule disassembly. We explore fluctuation in the scattering anisotropy at the very tip of disassembling microtubules and associate its momentary changes with conformation switching at the level of single protofilaments. Additionally we track the detailed trajectory of single disassembling tubulins from the microtubule shaft using scattering labels confirming the fluctuating character of disassembling microtubules explaining the thermally driven mechanisms of the dynamic instability of microtubules.

Visualizing and understanding bacterial extracellular structures using iSCAT

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The surface of bacteria is covered with structures that allows single cells to physically interact with their environments. For example, single cells swim with flagella, and attach to and move on surfaces with pili. Both flagella and pili are micrometer-long protein polymers only a few nanometer-thick extending from the cell body. These structures have been traditionally studied by means of genetic manipulation combined with macroscale motility assays, but little is known about their dynamics and spatial localization. This is mainly due to the difficulty in visualizing these nanometer-wide structures. We solved this shortcoming by imaging pili and flagella in live cells at high spatial and temporal resolution by iSCAT. iSCAT offers an unprecedented dynamic view of these extracellular structures, enabling detailed biophysical characterization of their activity in a native environment. We leverage this microscopy technique in studies of bacterial motility and mechanosensation that depend on the dynamics of flagella and pili. In particular, we were able to demonstrate that the pathogen Pseudomonas aeruginosa synchronizes pili extension and retraction with attachment of the tip with a surface. In summary, iSCAT fills an important technological gap in the field of microbiology.

Dissecting molecular membrane organization with combined fluorescence and scattering microscopy

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Molecular interactions are key in cellular signaling. They are usually ruled by the organization and mobility of the involved molecules. We present a combined fluorescence and scattering microscopy study to determine such information and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS), spectral detection, and interferometric scattering (iSCAT). We highlight how these approaches can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts.

Coherent brightfield microscopy: iSCAT microscopy in transmission

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Coherent brightfield (COBRI) microscopy, the iSCAT microscopy in transmission, measures the forward scattering signal by recording its interference with the transmitted non-scattered light. The COBRI microscopy shares many common features with the reflective iSCAT microscopy, including the exquisite sensitivity, the phase retrieval for 3D localization, and the high-speed, long-term observation. In addition, the transmission geometry of COBRI provides a unique advantage for measurements in live cells by avoiding the complication of the fluctuating background signal originated from the reflective interfaces, e.g., the cell membrane. In this talk, I shall start with the concept of the COBRI microscopy and benchmark its performance. I will introduce the pupil function engineering that allows us to tune the imaging sensitivity of COBRI over a wide range. I will present a few examples of biophysical studies of high-speed 3D tracking of single nano-sized particles in live cells, including the virus particles, cell vesicles, and gold nanoparticles. Finally, the perspective of label-free visualization of molecular dynamics in live cells will be discussed.

Interferometric Scattering Microscopy to Characterize Nanometric Objects and Subcellular Structures.

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It is of great interest to visualize nano-scale objects in bioscience. Fluorescence microscopy, most powerful bioimaging tool to date, bears limitations in measurement time, time resolution, and sample preparation. To circumvent such problems, a new scattering-based method named iSCAT (interferometric scattering microscopy) has been recently developed. Since this technique relies on scattering signal from a target nanoparticle and detects the interference of the signal with a constant reference, it is less prone to the aforementioned limitations. Here, we report our recent achievements: First, utilizing polarized scattering from an anisotropic scatterer, we determine the orientation of gold nanorods without sacrificing the bright-field image of the entire view-field, in contrast to dark-field imaging [1]. Second, we successfully track the position of nanoparticles along the axial dimension and image the sample at different depths by adopting the remote-focusing (RF) technique. Our RF-iSCAT approach would significantly expand the utility of iSCAT [2]. Lastly, we show our iSCAT imaging of biological cells and multi-protein focal adhesion complexes. The iSCAT technique enables us to visualize subcellular structures with remarkable spatial, temporal details and sharp contrast even without labeling [3]. In summary, the iSCAT technique would be an indispensable tool in visualizing the nanoscopic biological world.

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References

[1] J.-S. Park, I.-B. Lee, H.-M. Moon, J.-H. Joo, K.-H. Kim, S.-C. Hong*, and M. Cho*, *Chem. Sci.*, **9**, 2690 (2018). J.-S. Park, I.-B. Lee, H.-M. Moon, J.-S. Ryu, S.-Y. Kong, S.-C. Hong*, and M. Cho*, *submitted*.
[2] I.-B. Lee, H.-M. Moon, J.-H. Joo, K.-H. Kim, S.-C. Hong*, and M. Cho*, *ACS Photonics*, **5**, 797 (2017).
[3] I.-B. Lee, H.-M. Moon, J.-S. Park, K. Zambochova, S.-C. Hong*, and M. Cho*, *under revision*.

Label-free deep optical imaging using a time-gated reflection matrix

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Optical microscopy suffers from the loss of resolving power when imaging target objects embedded deep within complex scattering media. With the increase of target depth, the signal wave is attenuated exponentially due to multiple light scattering, and strong multiple scattering noise obscures the signal wave. In this talk, I will present imaging methods based on the recording of a time-gated reflection matrix, which goes beyond the conventional confocal detection scheme. In essence, we record the amplitude and phase of the backscattered waves not only at the confocal position but also at other non-confocal positions.

In the image reconstruction process, we made use of the non-confocal signals to enhance the imaging depth beyond the conventional limit. Specifically, we developed a unique algorithm termed ‘closed-loop accumulation of single scattering (CLASS)’ that makes the preferable choice of the non-confocal signals containing the object information and coherently add them to the confocal signals. We could correct the sample-induced aberrations in illumination and imaging paths separately without the need for guide stars and even in the presence of multiple light scattering. We performed in vivo and label-free volumetric imaging underneath an intact mouse skull and visualized myelinated axons with the ideal diffraction-limited spatial resolution.

Mind the gap

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The boundary between a cell and its environment or another cell is important - chemical talk goes on across this boundary, and forces are exerted. But the gap between cell boundaries is difficult to image, especially because it is dynamic. The cell membranes that line this gap are known to 'jiggle' and 'twitch', that is to say fluctuate, with at least some energy consumption. The twitching impacts the process of formation of bonds between the two surfaces and across the gap. In fact, this process is already complicated due to the fact that reaction across two 2D surfaces involving mechanosensitive biological bonds may not follow familiar laws of physical-chemistry such as the law of mass action. However, adhesion mediated by such bond formation is a very important process in biology – all multicellular organisms for example, depend on membrane-to-membrane adhesion *via* bio-chemical bonds for structural integrity. While the physics of a free, soft, thermally fluctuating membrane is well known, combining this with chemistry in two dimensions is challenging. Another challenge is measuring and describing the fluctuations especially when they are cell-driven and active. I will present new techniques to measure membrane shape fluctuations [1] and show that these may be a means for living cells to modulate the interaction with their environment [2].

[1] C. Monzel, D. Schmidt, C. Kleusch, D. Kirchenbuechler, U. Seifert, A.-S. Smith, K. Sengupta and R. Merkel. Nature communications. 6, 8162 (2015).

[2] Susanne Fenz, Timo Bihr, Daniel Schmidt, Rudolf Merkel, Udo Seifert, Kheya Sengupta, and Ana-Suncana Smith. Nature Physics, 13, 906–913 (2017).

Four-wave mixing interferometry: A new tool for imaging and tracking single plasmonic nanoparticles in 3D background-free

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Imaging and tracking single nanoparticles using optical microscopy are powerful techniques with many applications in biology, chemistry, and material sciences. Despite significant advances, imaging single small nanoparticles in a scattering environment as well as localizing objects with nanometric position precision in 3D remains challenging.
Here, I will present a four-wave mixing interferometry technique recently developed by us, whereby single gold nanoparticles in the 10-40nm diameter range are imaged background-free inside highly heterogeneous biological environments, owing to their specific nonlinear plasmonic response. The set-up enables correlative four-wave mixing/confocal fluorescence imaging, opening the prospect to study the fate of nanoparticle-biomolecule-fluorophore conjugates and their integrity inside cells [1].
Beyond imaging, the technique enables to track single particles with nanometric position localisation precision in 3D from rapid single-point measurements at 1 ms acquisition time, by exploiting the optical vortex field pattern in the focal plane of a high numerical aperture objective lens [2]. The localisation precision in plane is found to be about 15nm, consistent with the photon shot-noise, while axially it is limited to about 3nm by the nano-positioning sample stage, with an estimated photon shot-noise limit of below 1 nm. Through phase-sensitive polarisation-resolved detection, the technique can measure particle asymmetries of only 0.5% ellipticity, corresponding to a single atomic layer of gold, as well as particle orientations. This method opens new ways of unravelling single-particle trafficking within complex 3D architectures.

[1] Giannakopoulou et al., Nanoscale 12, 4622 (2020). DOI: 10.1039/c9nr08512b
[2] Zoriniants et al., Phys. Rev. X 7, 041022 (2017). DOI: 10.1103/PhysRevX.7.041022

Rotating coherent scattering (ROCS) microscopy – 100 Hz live cell imaging at 150nm resolution.

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Thermal and active cellular forces drive biological structures. These movements become the faster the smaller the biological structures because of less friction and steric hindrance. This is an important aspect for superresolution microscopy, since unfortunately the resolution of smaller structures requires more photons and time. Therefore, novel concepts enabling smart trade-offs between temporal and spatial resolution have to be developed.
Here, we present a variant of an oblique illumination super-resolution microscopy based on rotating coherent scattering (ROCS). The technique generates thousands of high contrast images without post-processing at frame rates of more than 100 Hertz and without labeling. The sample is scanned over all azimuthal illumination angles within a single camera exposure time and allows for variant illumination and detection modes such as bright-field, dark-field or total internal reflection (TIR). Thus, structures as small as 150 nm become separable through local destructive interferences. Within one rotation, multiple speckles do not disappear, but change to the object itself thereby improving contrast and resolution. ROCS is applied to different cells revealing unexpected dynamic biological processes.

Computational Interferometric Microscopy for High Resolution Visualization of Biological Nanoparticles

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Biological nanoparticles such as viruses and exosomes are important biomarkers for a range of medical conditions, from infectious disease to cancer. Biological sensors that detect whole viruses and exosomes with high specificity, yet without chemical labeling, are promising because they generally reduce the amount and complexity of sample preparation and may improve measurement quality by retaining information about nanoscale biological structure. However, label-free optical biological nanoparticle imaging often experiences two specific challenges: (i) the weak contrast due to low refractive index difference and exceptionally small size and (ii) the inability to resolve nanoscale features. Advances in interferometric scattering microscopy have successfully overcome weak signal limitations and enabled direct detection of low-index biological nanoparticles. We have demonstrated label-free identification and visualization of various viruses in multiplexed format in complex samples in a disposable cartridge and detection of exosomes. However, interferometric scattering microscopy falls short of providing sufficient spatial resolution to study the morphology of individual nanoparticles, especially in a high-throughput manner. Recently, we combined wide-field interferometric microscopy with computational imaging to demonstrate a two-fold resolution improvement. With computational imaging combingin multiple images acquired under asymmetric illumination, we clearly resolve ~150 nm weakly-scattering features in visible light (420 nm) over a wide field-of-view of >100μm, enabling sub-wavelength imaging of more than 10^4 nanoparticles at once. Therefore, high-throughput and high-resolution imaging and characterization of a broad size range of biological nanoparticles will be possible without any labelling.

Phase imaging with computational specificity (PICS)

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Quantitative phase imaging, has gained significant interest, especially in the past decade, because of its ability to study unlabeled cells and tissues. As a result, QPI can extract structure and dynamics information from live cells without photodamage or photobleaching. However, in the absence of labels, QPI cannot identify easily particular structures in the cell, i.e., it lacks specificity. This represents the major limitation of QPI when applied to biomedicine.

Recently, deep learning techniques have been translating from consumer to scientific applications. For example, it has been shown that AI can map one form of contrast into another. Significantly, it has been demonstrated that the neural network can learn from label-free (bright field, phase contrast, DIC) and ground-truth fluorescence images to predict where specific fluorophores would bind in an unlabeled specimen.

Inspired by this prior work, we applied deep learning to QPI data, generated by SLIM and GLIM. These methods are white-light and common-path and, thus, provide high spatial and temporal sensitivity. Because they are add-on to existing microscopes and compatible with the fluorescence channels, these methods provide simultaneous phase and fluorescence from the same field of view. As a result, the training data necessary for deep learning is generated automatically.

We present a new microscopy concept, where the process of retrieving computational specificity is part of the acquisition software, performed in real-time. We demonstrate this idea with various fluorescence tags and operation on live cells as well as tissue pathology. This new type of microscopy can potentially replace some commonly used tags and stains and eliminate the inconveniences associated with phototoxicity and photobleaching. Phase imaging with computational specificity (PICS) has an enormous potential for biomedicine.

References (complete list at: http://light.ece.illinois.edu/index.html/publications/articles)

1. Kandel, M. E., C. Hu, G. Naseri Kouzehgarani, E. Min, K. M. Sullivan, H. Kong, J. M. Li, D. N. Robson, M. U. Gillette, C. Best-Popescu and G. Popescu (2019). "Epi-illumination gradient light interference microscopy for imaging opaque structures." Nature Communications 10(1): 4691.
2. Park, Y., C. Depeursinge and G. Popescu (2018). "Quantitative phase imaging in biomedicine." Nature Photonics **12**(10): 578.
3. Nguyen, T. H., M. E. Kandel, M. Rubessa, M. B. Wheeler and G. Popescu (2017). "Gradient light interference microscopy for 3D imaging of unlabeled specimens." Nat Commun **8**(1): 210.
4. Kim, T., R. J. Zhou, M. Mir, S. D. Babacan, P. S. Carney, L. L. Goddard and G. Popescu (2014). "White-light diffraction tomography of unlabeled live cells." Nature Photonics **8**(3): 256-263.
5. Popescu, G. (2011). Quantitative phase imaging of cells and tissues. New York, McGraw-Hill.

Sparsity-based techniques for solving the inverse scattering problem

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Inverse-scattering is a challenging problem encountered in many fields such as coherent microscopy. While classical reconstruction methods were relying on linear approximation of the forward model, recent works have shown the benefit of combining advances physics (nonlinear models) and sparsity. In this talk, we present a reconstruction algorithm that deploys the nonlinear Lippmann-Schwinger model together with sparsity-based regularizations. Then, we show the ability of the method to provide high-quality reconstructions for difficult configurations in coherent light microscopy (high contrasts, few illuminations).

INTERFEROMETRIC SCATTERING MICROSCOPY

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Contributed Talks
May 27th

REVEALING THE DYNAMICS OF CYTOSKELETAL NON-MOTOR PROTEINS USING INTERFEROMETRIC SCATTERING MICROSCOPY

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KEYWORDS: iSCAT, interferometric scattering microscopy, single-particle tracking, single-molecule detection, high-fidelity tracking

Advances in fluorescence-based super-resolution microscopy and nanoscopic tracking techniques helped to reach a spatial resolution below 10 nm with temporal resolution limited to milliseconds timescales [1]. To push the spatiotemporal resolution of fluorescent-based techniques further, we are employing interferometric scattering microscopy (iSCAT). The sensitivity of iSCAT was previously proven in detection experiments of small scattering nanoparticles as well as unlabeled single proteins with sub-nanometer spatial and sub-millisecond time resolution [2, 3].

Here we use a high frame rate of iSCAT microscopy in combination with a novel labeling method to track the motion of a single labeled anaphase spindle elongation protein 1 (ASE1) on a microtubule. We achieved a spatial resolution better than 2 nm in all three dimensions, and a temporal resolution of 22 μs. With such spatiotemporal resolution, we discover that the ASE1 movement is not, as often believed, directed along a microtubule protofilament and features a more stochastic pattern on the microtubule lattice (Fig 1). We distinguish different diffusion characteristics of the axial motion and the perpendicular motion of ASE1. We reveal clear statistical signatures of confined motion and are able to detect sub-millisecond confinements in the trajectories, having the periodicity corresponding to the size of a single tubulin dimer. Our results of high-fidelity ultrafast tracking shade new light not only on the unknown choreography and mechanisms of diffusive motion of microtubule-associated proteins but also on the interaction of the ASE1 protein with the microtubule lattice. We were able to reconstruct the energetic landscape of microtubule.

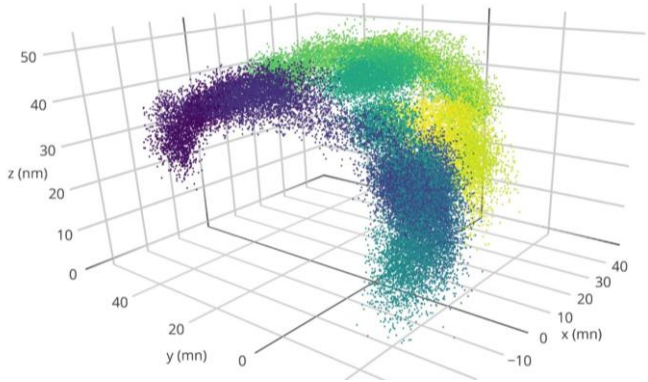

Figure 1 A three-dimensional trajectory of the labeled ASE1 dimer reveals microtubule shape and tubulins position. Time is color-coded from yellow to blue.

[1] J. Schnitzbauer, M. T. Strauss, T. Schlichthaerle, F. Schueder, and R. Jungmann, "Super-resolution microscopy with DNA-PAINT," *Nat. Protoc.*, **12**(6), 1198–1228 (2017).
[2] M. Piliarik and V. Sandoghdar, "Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites," *Nat. Commun.*, **5**, 4495 (2014).
[3] S. Spindler et al., "Visualization of lipids and proteins at high spatial and temporal resolution via interferometric scattering (iSCAT) microscopy," *J. Phys. Appl. Phys.*, **49**(27), 274002 (2016).

Ultrasensitive protein detection

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Proteins are the physical and biochemical building blocks of the cell and are fundamental to biological function. Thus, the field of proteomics has emerged over the course of last decades with the aim of systematically identifying the entire set of proteins within a given tissue or cell via established methods such as cryo-EM, and mass spectrometry. In addition, several optical sensing techniques, e.g. using plasmon resonances or microresonators have been explored.

Here, we present the progress in the use of interferometric detection of scattered light (iSCAT) for sensing at the single protein level [1]. The iSCAT signal arises from the interference between the protein's weak scattered field and a reference field [2]. The amplitude of this interference is determined by the magnitude of the scattered field and is thus linearly proportional to a protein's polarizability and mass. In this work we report on measurements on protein samples, spanning the record range of 21-250 kDa [3].

References

[1] Piliarik, M., Sandoghdar, V. Direct Optical Sensing of Single Unlabelled Proteins and Super-Resolution Imaging of Their Binding Sites. *Nat. Commun.* 5, 4495 (2014).

[2] Lindfors, K, Kalkbrenner, T. Stoller, P. Sandoghdar, V. Phys. Rev. Lett. 93, 037401 (2004).

[3] Dahmardeh, et al, in preparation.

Electric-Double-Layer-Modulation Microscopy

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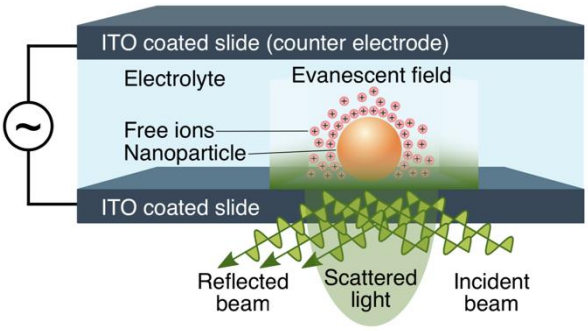

The electric double layer (EDL) formed around charged nanostructures at the liquid-solid interface determines their electrochemical activity and influences their electrical and optical polarizability. We experimentally demonstrate that restructuring of the EDL at the nanoscale can be detected by dark-field scattering microscopy. We refer to this intensity change in the elastic light scattering as the potentiodynamic optical contrast (PDOC). The temporal response of the PDOC is influenced mostly by the physical adsorption of counter-ions with an optical polarizability that is different from the neutral mixture. We demonstrate this effect by quantifying the temporal relaxation of the PDOC, which is directly related to the charging time of the EDL. Temporal and spatial characterization of the scattering signal demonstrates that the PDOC is proportional to the accumulated charge of polarizable ions at the interface and its time derivative represents the nanoscale ionic current. The material-specificity of the EDL formation is used in our work as a label-free contrast mechanism to image nanostructures and perform spatially resolved cyclic voltammetry at ion current levels of a few attoamperes, corresponding to the exchange of only a few hundred ions. This imaging technique provides important additional information such as spatial resolution, sensitivity to surface heterogeneity, local ion accumulation, and the possibility of studying deposits, possibly down to single biomolecules.

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Label-free single protein detection with iSCAT: From purified samples to living cells

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The functionality of the human body is maintained via a complex interplay of various different cell types that constantly communicate with each other by a range of different signaling molecules. As an example, the immune response is regulated via proteins like antibodies or cytokines that are secreted by one cell and recognized by another, which reacts to the signal. In order to understand and potentially treat possible malfunctions, it is necessary to study the key elements of those processes in more detail, like the secretion of signaling molecules by a cell.

Conventional methods to study cellular secretion mostly rely on the use of large cell ensembles (immunoassays) although new single-cell studies have been reported. Furthermore, established methods rely on the ensemble analysis of the secreted analytes, often via fluorescence labeling. iSCAT was shown to be able to detect single small proteins in a completely label-free fashion at high spatio-temporal resolution [1, 2]. Applied to cell secretion, it can reveal important insights into the dynamics of this process at the single cell level and in real-time.

In a first application, we demonstrate the suitability of iSCAT to study cell secretion on single LAZ-388 cells, an Epstein-Barr virus-transformed B cell line [3,4]. We find that LAZ-388 cells secrete proteins in a wide molecular weight range from 100 to 1100 kDa. By introducing functionalized cover glasses to specifically bind IgG antibodies, we could identify IgG as one significant part of a LAZ-388 secretome. We show the capabilities of iSCAT to analyze dynamical changes in the secretional behavior of LAZ-388 cells in real time by introducing stress via the continuous rise of the buffer pH value which causes a distinct change in the secretion rate. This makes iSCAT a valuable tool for the detailed study of cellular response to external stimuli, as posed by organisms like antigen-presenting cells or viruses.

References

[1] M. Piliarik & V. Sandoghdar, “Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites” Nat. Comm. **5**, 4495 (2014).

[2] Young et al., “Quantitative mass imaging of single biological macromolecules” Science **360**, 423-427 (2018)

[3] M. P. McDonald, A. Gemeinhardt, K. König, M. Piliarik, S. Schaffer, S. Völkl, M. Aigner, A. Mackensen, and V. Sandoghdar, "Visualizing Single-Cell Secretion Dynamics with Single-Protein Sensitivity," Nano Lett. **18**, (2018).

[4] A. Gemeinhardt, M. P. McDonald, K. König, M. Aigner, A. Mackensen, and V. Sandoghdar, "Label-Free Imaging of Single Proteins Secreted from Living Cells via iSCAT Microscopy," J. Vis. Exp. (2018).

Implementation of an i-SCAT setup for imaging dynamic processes in perovskite nanostructures

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Metal halide perovskite nanocrystals have emerged as promising materials for highly efficient opto-electronic devices such as solar cells or LEDs due to their unique opto-electronic properties. To date, the low stability of these nanophotonic systems hinders them from industrial applications as they are prone to degrade under influence of environmental factors such as moisture, heat and light. Neither their growth nor their destabilization processes are well understood, limiting the targeted synthesis of stable and application tailored nanostructures.[1] The advent of interferometric scattering microscopy (i-SCAT) makes in-situ imaging of these nanoscopic processes accessible.[2]

In our group, an i-SCAT microscope (reflection mode; wide-field detection) was implemented following protocols published by the groups of *Kukura*[3] and *Sandoghdar*[4] and first i-SCAT images of an ordinary cover glass have been taken (Fig. 1a). After conducting several tests to calibrate the system, the smoothness of the images was improved considerably, as shown in Fig. 1b. The corresponding statistics showed a decrease in standard deviation to 2.2×10^{-4} (140 ms effective exposure time; 10.5 ke^- full well depth; 5×5 binning; 333× magnification).

Subsequently, first images of polymer micelle protected 27 nm $MAPbI_3$ perovskite nanocrystals[5] in toluene were obtained (Fig. 1c). Here, toluene harbors different challenges for i-SCAT imaging due to a refractive index that nearly matches the index of glass. Following these preliminary experiments, the dynamical assembly and disassembly processes of several perovskite nanostructures including nanocubes will be investigated.

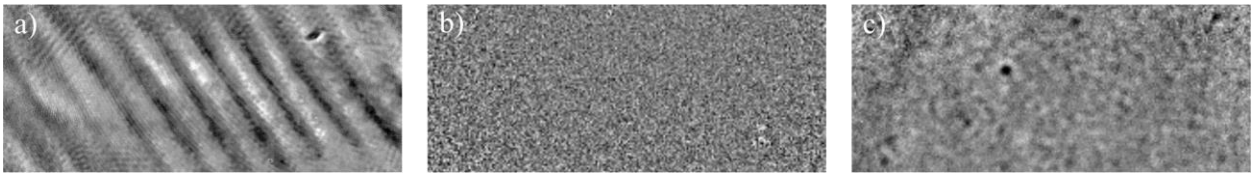

Figure 1. a) Background before system calibration (effective exposure time = 140 ms). b) Background after system calibration (effective exposure time = 140 ms). c) Preliminary results of $MAPbI_3$ NCs in toluene.

References

[1] J.-K. Sun et al., *J. Am. Chem. Soc.* **2018**, *140*, 11705.
[2] a) M. Delor et al. *Nat. Mater* **2020**, *19*, 56; b) P. K. Nayak et al., *Nat. Commun.* **2016**, *7*, 13303.
[3] J. Ortega Arroyo et al. *Nat. Protoc.* **2016**, *11*, 617.
[4] A. Gemeinhardt et al. *JoVE* **2018**, e58486.
[5] V. A. Hintermayr et al., *Nano Lett.* **2019**, *19*, 4928.

Multicolor high-speed tracking of single biomolecules with silver, gold, and silver-gold alloy nanoparticles

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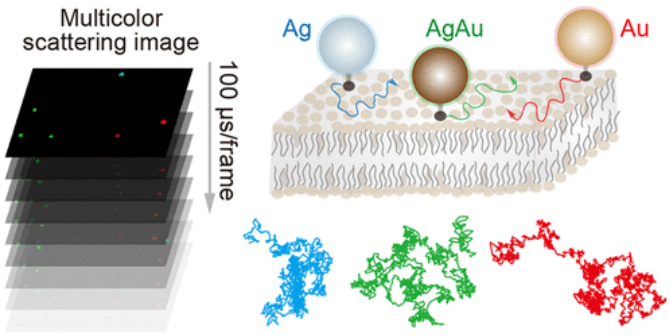
Gold nanoparticles have been used as imaging probes to track the motions of single biomolecules. To investigate behaviors of various biomolecules simultaneously, increase of the color palette is necessary. Here we developed a multicolor high-speed single-particle tracking system using silver, gold, and silver-gold alloy (5:5 composition ratio) nanoparticles. The peak wavelengths of the plasmon resonances for 30 nm silver, 30 nm silver-gold alloy, and 40 nm gold nanoparticles were around 410, 460, and 530 nm, respectively, and we constructed multicolor total internal reflection dark-field microscope with multiple lasers at 404 nm for silver, 473 nm for silver-gold alloy, and 561 nm for gold nanoparticles. By the use of a spectrophotometer in the detection optics, scattering images at each wavelength were projected onto different portions of a single two-dimensional detector. High-contrast images of silver, silver-gold alloy, and gold nanoparticles were simultaneously obtained in different color channels. After correction of positional shifts among different color channels by affine transformation, a maximum shift less than 17 nm was achieved. Furthermore, an additional 649 nm laser enabled the detection of plasmon coupling by transient dimer formation of two nanoparticles. With this system, diffusional motions of phospholipids in a supported membrane and stepping motions of kinesins along microtubules were successfully observed with a localization precision of 2 nm and a time resolution of 100 μs at each channel.



Reference

Ando J, Nakamura A, Yamamoto M, Song C, Murata K, Iino R, Multicolor high-speed tracking of single biomolecules with silver, gold, silver-gold alloy nanoparticles, ***ACS Photonics*** 6, 2870-2883 (2019) DOI: 10.1021/acsphotonics.9b00953

Multi-pass microscopy for signal to noise enhancement in interferometric scattering

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Interferometric scattering techniques for the detection of unlabeled nanometric particles operate at the shot-noise limit. Increasing signal to noise is achieved by averaging more and more frames of a recorded data set. This sets high demands on the stability of the setup, or on the camera well depth and photostability of the system of interest.

Here I will discuss multi-pass microscopy [1,2] as a means to increase the signal-to-noise ratio at constant photon budget. I will show the design of a multi-pass iScat system, and will present first data demonstrating the detection of gold nanoparticles.

I will also present a proof of concept experiment[3] that demonstrates how one could in principle deal with background inhomogeneity using wavefront shaping. Technical limitations will be discussed.

1. Juffmann, T., Klopfer, B. B., Frankort, T. L. I. I., Haslinger, P. & Kasevich, M. A. Multi-pass microscopy. *Nat. Commun.* **7**, 12858 (2016).
2. Nimmrichter, S., Chen, C.-F., Klopfer, B. B., Kasevich, M. A. & Juffmann, T. Full-field cavity enhanced microscopy techniques. *J. Phys. Photonics* **1**, 015007 (2019).
3. Juffmann, T., de los Ríos Sommer, A. & Gigan, S. Local Optimization of Wave-fronts for optimal sensitivity PHase Imaging (LowPhi). *Opt. Commun.* **454**, 124484 (2020).

Tracking lipid diffusion on GUVs with gold nanoparticles of different size

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Single-particle tracking is an established method that can reveal essential information about cellular structures [1] or the organisation of protein recruitment. Generally, fluorescent molecules are used as labels; however, then the tracking procedure suffers from photophysical effects. To circumvent photophysical effects one can use non-fluorescent labels, such as gold nanoparticles (GNPs). Here we use interferometric scattering microscopy (iSCAT) to track lipids labelled with GNPs on giant unilamellar vesicles (GUV) [2], and show how the size of the GNP influences the measured diffusion.

[1] Taylor et al. Nature Photonics 13, no. 7 (July 2019): 480–87.

[2] Stein et al. Frontiers in Physiology 8 (2017).

Lipid Bilayer Thickness Measured by Quantitative DIC Reveals Phase Transitions and Effects of Substrate Hydrophilicity

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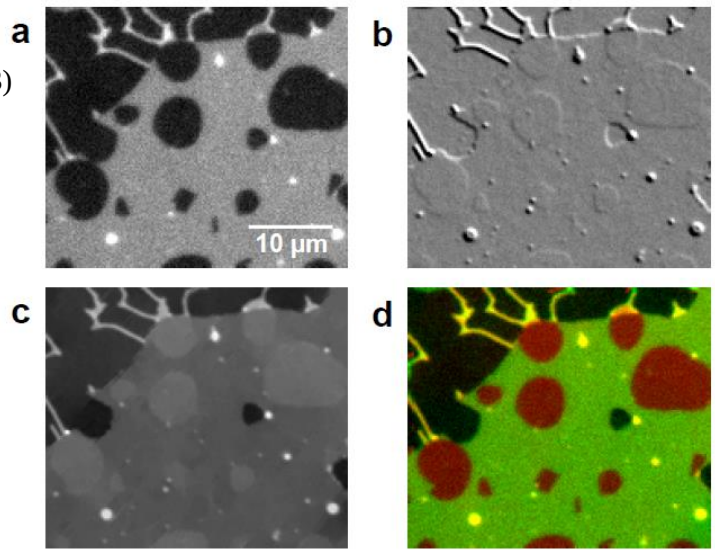
Supported lipid bilayers are one of the most prolific model systems used to study the biophysical properties of phospholipid membranes [1]. They are commonly imaged using fluorescence microscopy, which suffers from limitations including fluorophore saturation and photobleaching, and artefacts from insertion of the label. Alternatively, quantitative differential interference contrast microscopy (qDIC) is a phase technique that allows label free imaging of lipid bilayers, as well as the extraction of quantitative information about the layer thickness with high sensitivity [2]. Combining Wiener deconvolution reconstruction [2] with an energy minimization scheme [3], adapted to layered structures, we retrieve bilayer images with 0.1nm height resolution and low image reconstruction artefacts.

We investigate [4] the influence of the substrate on the thickness of fluid-phase 1,2-dioleoyl-snglycero-3-phosphocholine (DOPC)-supported lipid bilayers (SLBs) and find a thinning of up to 10%, depending on substrate hydrophilicity, local bilayer coverage, and ionic strength of the medium. With fluorescently labeled lipid bilayers, we also observe changes in the bilayer thickness depending on the choice of fluorophore. Furthermore, liquid-ordered (L_o) domains in bilayers, formed from DOPC, cholesterol, and sphingomyelin, are measured, and the corresponding thickness change between the liquid-ordered and liquid-disordered phases is accurately determined (see figure). Again, the thickness difference is found to be dependent on the presence of the fluorophore label, highlighting the need for quantitative label-free techniques.

[1] Langmuir **22**, 3497 (2006).
[2] Biophysical Journal **105**, 1414 (2013)
[3] Scientific Reports **6**, 30420 (2016)
[4] Langmuir **35**, 13805 (2019)



DOPC/SM/Chol/H-DOPE SLB with Lo domains excluding the fluorescent labels, shown in (a) background subtracted fluorescence (m =0 pe, M = 109 pe), (b) qDIC contrast (m = -0.02, M = +0.02), (c) qDIC phase (m = 1.6 mrad, M = 34.5 mrad), and (d) a composite image showing the phase (red) and fluorescence (green).

Direct optical visualisation of single-particle phase transformations in lithium ion battery materials

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Charging and discharging rates in lithium ion batteries are limited by ion transport into and through electrode materials. With a rising demand for high-rate batteries, a detailed understanding of how ion transport kinetics are affected by material morphology at the nanoscale is essential. Here, we introduce interferometric scattering microscopy (iSCAT) to the field of battery characterisation. iSCAT is an optical, non-invasive and label-free imaging technique which sensitively measures the polarisability of individual active particles in an electrode, with the potential for nanometre localisation precision. We benchmark our technique with micron-sized particles of $LiCoO_2$, a well-studied cathode material. *Operando* experiments demonstrate that the iSCAT intensity for a single particle changes dramatically but reversibly during (de)lithiation, with substantial variations between individual particles. Additionally, the solid solution regime, biphasic regime and lithium ordering transition are clearly distinguished during charging and discharging, allowing us to extract the phase propagation velocities. During delithiation, we observe shrinking-core behaviour in the biphasic regime, whereas upon lithiation, phase fronts propagate from one nucleation point. This indicates that the rate-limiting process in each case is ion diffusion and charge transfer respectively. Our results demonstrate that iSCAT can provide fundamental insights into the ion transport dynamics of battery materials, highlighting the need to transition towards a nanoscopic understanding of battery materials.

References:

1. J. Ortega Arroyo *et al*., Interferometric scattering microscopy and its combination with single-molecule fluorescence imaging, *Nature Protocols*, 2016, 11, 617-633.
2. J. N. Reimers and J. R. Dahn, Electrochemical and In Situ X-Ray Diffraction Studies of Lithium Intercalation in LixCoO2, *Journal of The Electrochemical Society*, 1992, 139, 2091-2097.
3. Neel Nadkarni *et al.*, Modelling the Metal–Insulator Phase Transition in Li_xCoO_2 for Energy and Information Storage, *Advanced Functional Materials*, 2019, 29, 1902821

iPSF: The point spread function in iSCAT Microscopy

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Interferometric scattering (iSCAT) microscopy is a powerful label-free technique well suited for the sensitive detection and tracking of nanoscale matter such as individual viruses, colloidal nanoparticles, proteins and single molecules [1]. Recent efforts to track the nanoscale mobility of nanoparticles in challenging speckle environments and over extended axial ranges [2] has necessitated a quantitative description of the point-spread function in iSCAT (iPSF). We present a robust vectorial diffraction model for the iPSF in tandem with experimental measurement and rigorous FDTD simulations and how it differs from conventional intensity-based PSFs in the diffraction-limited focal region [3]. We examine the iPSF under various imaging scenarios to understand how aberrations due to the experimental configuration render its complex ring patterns which encode information about the nanoparticle. We show that the lateral feature of the iPSF can be used to achieve precise and nanometric three-dimensional localization either by means of a fit to the model or calibration-free unsupervised machine learning to break the previous limitation of 100 nm unambiguous height determination. Furthermore, we demonstrate three-dimensional localization over an extended axial range on the order of 10 μm and envisage PSF engineering could be employed to push the range further, permitting deep tissue imaging. We discuss the origin of the remarkably long range and high resolution of iSCAT particle tracking in three dimensions [3].

References

[1] R W Taylor and V Sandoghdar. Interferometric scattering microscopy: seeing single nanoparticles and molecules via rayleigh scattering. *Nano Letters*, 2019.

[2] R W Taylor, R Gholami M, V Rauschenberger, A Giessl, A Schambony, and V Sandoghdar. Interferometric scattering microscopy reveals microsecond nanoscopic protein motion on a live cell membrane. *Nature Photonics*, 13(7):480–487, 2019.

[3] R Gholami M, R W Taylor, M Kaller, S Spindler, and V Sandoghdar. The point spread function in interferometric scattering microscopy (iscat). *To be submitted*, 2020.

INTERFEROMETRIC SCATTERING MICROSCOPY

INTERFEROMETRIC SCATTERING MICROSCOPY

Contributed Talks
May 28th

PiSCAT: A Python package for iSCAT image analysis

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Detection and tracking of nanoscale objects in interferometric scattering (iSCAT) microscopy [1, 2] offers unique opportunities for research in many areas such as physics, biophysics, chemistry and material science. Some of the advantages of iSCAT are the ability to record very long trajectories, very high three-dimensional spatial and temporal resolution, and a remarkable sensitivity for detecting small nano-objects. While the construction of an iSCAT microscope is fairly simple, the analysis of the data can become complex. Thus far, researchers have used a combination of several libraries and packages in home-made data acquisition and analysis programs. In this work we present "PiSCAT", as a unified package based on open-source python libraries for efficient and advanced processing of iSCAT images and videos. The package reads different image formats and offers tools to perform state-of-the-art analysis in various applications such as unlabeled protein detection or 3D tracking of nanoparticles [2]. In future, we aim to design a GUI (Graphical User Interface) for an efficient and user-friendly application and to develop PiSCAT further to run on a CPU or GPU in parallel.

References

[1] K Lindfors, T Kalkbrenner, P Stoller, and V Sandoghdar. Detection and spectroscopy of gold nanoparticles using supercontinuum white light confocal microscopy. *Physical Review Letters*, 93(3):037401, 2004.

[2] R W Taylor and V Sandoghdar. Interferometric scattering microscopy: seeing single nanoparticles and molecules via rayleigh scattering. *Nano Letters*, 2019.

Picometer-resolved universal single-lens interferometer unveiling ultraprecise frugal devices

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Abstract:

The application of precision interferometers is generally restricted to expensive and smooth high-quality surfaces. Here, we offer a route to ultimate miniaturization of interferometer by integrating beam splitter, reference mirror and light collector into a single optical element, an interference lens (iLens), which produces stable high-contrast fringes from in situ surface of paper, wood, plastic, rubber, unpolished metal, human skin, etc. A self-referencing real-time precision of sub-20 picometer (~λ/30000) is demonstrated with simple intensity detection under ambient conditions. The principle of iLens interferometry has been exploited to build a variety of compact devices, such as a paper-based optical pico-balance, having 1000 times higher sensitivity and speed, when compared with a high-end seven-digit electronic balance. Furthermore, we used cloth, paper, polymer-films to readily construct broadband acoustic sensors possessing matched or higher sensitivity when com-pared with piezo and electromagnetic sensors. Our work opens path for affordable yet ultra-precise frugal photonic devices and universal micro-interferometers for imaging.

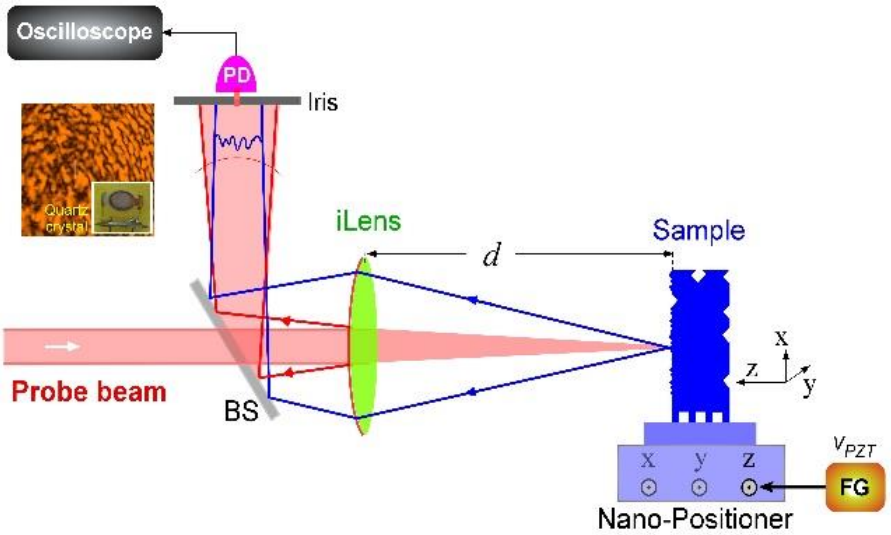

Fig. 1: Schematics of iLens interferometer. An iLens produces high-contrast structured fringes (left image) from a surface using a low-power He-Ne probe laser (10mW, λ= 632nm). The z-position of the sample from the iLens, d(t), is controlled by a piezo-positioner driven by V_{PZT} from a function generator (FG).

Keywords: Interferometry, picometer, rough surface, devices

References:

[1] **P. Munjal**, & Kamal P. Singh, "A single-lens universal interferometer: towards a class of frugal optical devices", *Applied Physics Letters*, **115**(11), 2019.

[2] **P. Munjal**, & Kamal P. Singh, "Optically probing picometer resolved photo-dynamics of solid surfaces", *URSI Radio Science Bulletin*, **2019**(370), 12-16, 2019.

Point Spread Function Engineering in Real Time for iSCAT

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Optical processing of images is commonly used in microscopy to optimise the collected signals by increasing contrast or decreasing noise. Fourier plane processing is a common method to select specific image properties and, while powerful, the application of optical processing to iSCAT has typically been limited to static apodising filters. Adaptive Fourier filtering can be performed using Spatial Light Modulators (SLM), computer controlled high resolution devices capable of applying any type of filter in real-time.

We demonstrate here the application of real time SLM processing applied in iSCAT microscopy to obtain a 6-fold increase of the signal-to-noise ratio. We investigate a range of optical processing methods, and demonstrate that this optical pre-processing reduces the molecular weight threshold of detectable label-free proteins and polymers.

Sub-millisecond tracking of single lipids on cell membranes through ISCAT microscopy

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The study of lipid dynamics on cellular surfaces is a topic of great interest in membrane Biophysics. One of the prevalent models for lipid diffusion is the compartmentalized diffusion model, whereby the cellular membrane is subdivided by the actin cytoskeleton in smaller areas. The diffusion of lipids is therefore heterogeneous, with periods of confinement alternating with free diffusion. The study of single particle trajectories is particularly suited to highlight the peculiarity of this diffusion motion. Interferometric Scattering microscopy has proven particularly successful at these tasks in similar situations, and therefore is ideally suited to pursue this objective. For this purpose, we have therefore delivered headgroup-biotinylated lipids to an epithelial cell line, PtK2, which has been widely used in the study of lipid membrane diffusion. These lipids have been labelled with gold nanoparticles of two different sizes (20nm and 40nm diameter), and their diffusion was tracked on the cell surface. In order to properly analyse the trajectory data thus obtained, we have adopted a novel analysis approach, in which no prior knowledge of the diffusion mode of the particle is required, and that adequately addresses influence of localization errors in the case of high-framerate SPT measurements. Our results show that the motion of a majority of the tracked lipids is compartmentalized. Moreover, we show that the gold nanoparticles strongly affect the long-range diffusion behaviour of the lipids, by slowing them down, whereas it appears the compartment diffusion is less affected by the particle size. Finally, we found out that the magnitude of the short-range diffusion is much smaller than that predicted in the literature, most likely due to the way the localization uncertainty is included in the analysis. The work we present should therefore serve as a starting point to address the issues relating to membrane lipid diffusion with a fresh approach, with the aid of ISCAT, and also in conjunction with other single particle tracking-capable techniques.

Correlative iSCAT and fluorescence highlight a positive feedback between bacterial mechanosensation and motility

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The bacterial pathogen *Pseudomonas aeruginosa* senses surface contact using type IV pili. These pili are thin filaments (5 nm in diameter) composed of polymerized proteins. They extend and retract from the cell body thanks to dedicated molecular motors in order to act as active probes. Upon mechanical contact, pili activate a two-component signaling pathway called the Chp system. In response, *Pseudomonas* upregulates virulence genes thereby infecting upon contact with its host.

Pseudomonas also extends, attaches and retracts pili to power twitching motility, a surface related motility mechanism important for exploration and taxis toward favorable niches. Thus, pili act as means for displacement as well as transducers of mechanical signals to gene expression.

However, it is unclear if, and how, this mechanoresponse feeds back into the regulation of pili activity and twitching. Indeed, such a feedback would strengthen the commitment for an infectious state of the bacterium.

To answer this question we observed simultaneous pili activity and biological processes. Fluorescence microscopy pipelines are gold standards in visualization of intracellular biological process. However, pili visualization is somehow more challenging as their size prevents their observation in conventional label-free microscopy.

Here we combined the powerful interferometric scattering microscopy technique to fluorescence in order to assess simultaneous pili activity and intracellular protein localization in living *Pseudomonas aeruginosa* cells. We correlate pili number to fluorescently labeled motors and regulatory proteins involved in pili biogenesis and mechanosensation. Our findings show that protein localization profiles change on surfaces, suggesting that segregation of specific proteins to a certain location in time is crucial in pili dynamics regulation.

Altogether, we provide a powerful tool to visualize both intracellular and extracellular events with high temporal and spatial resolution in living organisms.

Interferometric scattering microscopy reveals microsecond nanoscopic protein motion on a live cell membrane

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The plasma membrane of the living cell is organized in an intricate and dynamic manner across and to understand how this organization arises and the consequent cellular function it creates is topic of intense investigation. It is understood that the membrane is compartmentalised into nanoscale domains and regulated by mechanisms ranging from confinement by the actin cytoskeleton down to protein-protein interactions. It is of great interest to investigate how these mechanisms lead individual membrane molecules to inevitable cellular function, but to do so is challenging owing to the need to visualise the mobilities of individual molecules in the plasma membrane.

Here we present our work wherein we use the high sensitivity of iSCAT microscopy to track the mobility of membrane proteins in 3D to nanometric precision and microsecond temporal resolution. Using the epidermal growth factor receptor protein (EGFR) as a model candidate, we show that one can investigate to new levels of detail the role of individual proteins in important biological processes such as membrane diffusion and endocytosis whilst under physiological conditions [1]. We can also harness the 3D nature of iSCAT tracking over extended axial ranges to visualise the labelling of the membrane in situ as well as reconstruct the membrane topology the probe travelled over were hence the probe acts as a cellular nano-rover, shown in Figure 1. We will discuss the possibilities for iSCAT single-particle tracking in the live cell, describe the main challenges, and provide a perspective on what the future holds for this promising technique.

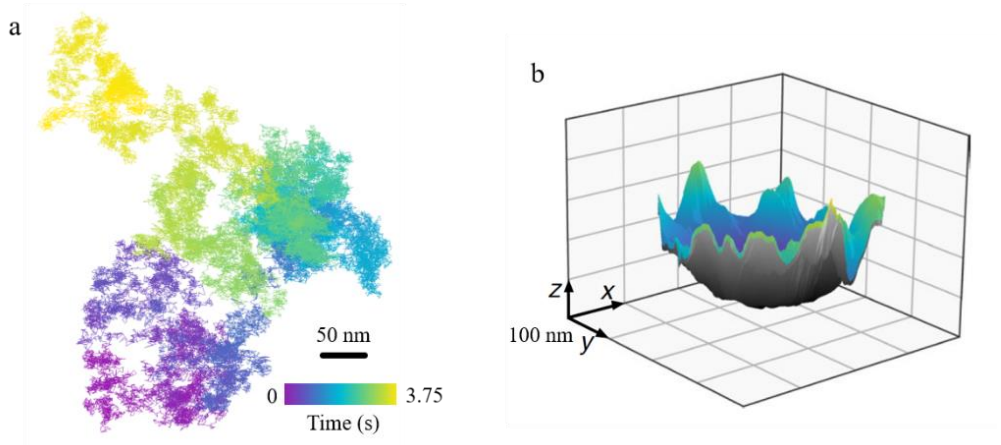

Figure 1: (a) High-speed tracking of EGFR diffusion on the live cell membrane, recorded at 20,000 fps. (b) Surface interpolation of a trajectory of confined mobility revealing a bowl-pattern in 3D recalling an endocytotic clathrin pit.

[1] R.W. Taylor, R. G. Mahmoodabadi, V. Rauschenberger, A. Giessl, A. Schambony and V. Sandoghdar, *Interferometric scattering microscopy reveals microsecond nanoscopic protein motion on a live cell membrane*. Nature Photonics, 13, 480–487, 2019.

Tunable subdiffusion and ergodic-nonergodicity transition of gold nanoparticles

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Anomalous diffusion usually occurs in complex systems, from disordered semiconductors to turbulence and living biological cells [1, 2, 3]. The complexity of these systems often renders them out of reach, and the precise mechanism leading to anomalous dynamics, e.g. trapping or viscoelastic medium, is commonly not known exactly [4]. This is compounded by the fact that anomalous phenomena are generally not controllable. In the case of anomalous subdiffusion, amplitude fluctuations are described by a nonlinear mean-square displacement, $\langle \Delta x^2 \rangle \propto t^{\alpha}$, with $\alpha < 1$ [5]. Moreover, they prevent time averages to converge to the corresponding ensemble average, thus ergodicity is (weakly) broken [6], and the system lies beyond the reach of ordinary statistical mechanics. In this regime, an "average" behavior cannot be simply inferred from an individual trajectory, in contrast to the ergodic trapping phase where single particle tracking or ensemble measurements are interchangeable.

In this work, we experimentally investigate subdiffusion and nonergodicity of single charged gold nanoparticles (GNPs) diffusing under the effect of an electrostatic potential. We use wide-field interferometric scattering microscopy (iSCAT) [7] to record individual trajectories of the Brownian particles (Fig. 1 a). The analysis of individual trajectories allowed us to determine the potential depth ($\beta = U_0/k_BT$) for each particle, while the ensemble analysis revealed that the transition from trapping to normal diffusion (Fig.1 b) is not sharp, rather occurs via an intermediate subdiffusive regime. Furthermore, we have also shown the transient behavior between ergodic and nonergodic dynamics of a system in a simple setting.

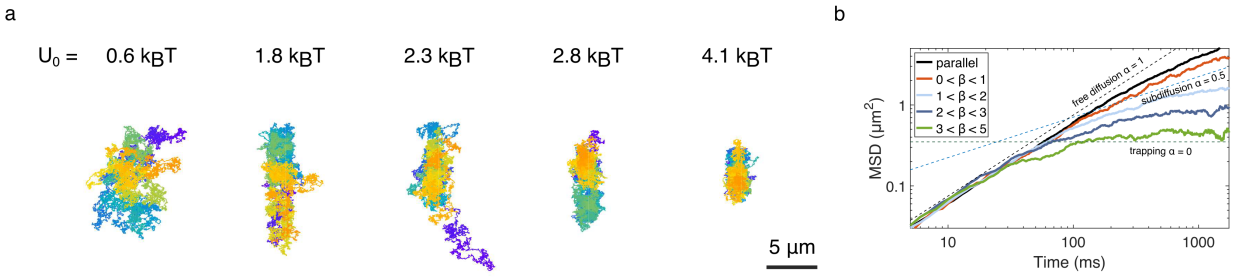

Figure 1. (a) Sample experimental trajectories for various potential depths. (b) Ensemble averaged mean-square displacement (MSD) shows the tunability of the subdiffusion on the ensemble level with the diffusion exponent $\alpha = (3 - \beta)/2$. The dashed lines show the values of $\alpha = 1$, 0.5 and 0 in order of decreasing slope.

[1] I. Golding and E. C. Cox, Phys. Rev. Lett. **96**, 098102 (2006).
[2] J.-P. Bouchaud and A. Georges, Phys. Rep. **195**, 127–293 (1990).
[3] S. Marksteiner, K. Ellinger and P. Zoller, Phys. Rev. A **53**, 3409 (1996).
[4] I. M. Sokolov, Soft Matter **8**, 9043 (2012).
[5] Y. Meroz, I. M. Sokolov, and J. Klafter, Phys. Rev. Lett. **110**, 090601 (2013).
[6] A. Rebenshtok, and E. Barkai, Phys. Rev. Lett. **99**, 210601 (2007).
[7] R. Taylor and V. Sandoghdar, Nano Lett. **19**, 4827 (2019).

Interferometric plasmonic microscopy

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Interferometric plasmonic microscopy (iPM) is the extention of interferometric scattering microscopy (iSCAT) into the plasmonic field. Using surface plasmons as the illumination source has provided unique advantages, including field enhancement in longitudinal directions, easier tuning between reference and scattering beams, and quantitative phase imaging. We have successfully applied the iPM technology for imaging single bioparticles and biomolecules, such as exosomes and DNA molecules.

LABEL-FREE SINGLE MOLECULE QUANTIFICATION OF DNA BY MASS PHOTOMETRY

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Abstract:
A primary advantage of label-free detection methods over fluorescent measurements is its quantitative detection capability, since an absolute measure of material facilitates kinetic characterisation of biomolecular interactions. Mass photometry based on interferometric scattering microscopy (iSCAT) is a novel, label-free imaging and quantification approach, having demonstrated accurate mass measurement of single biomolecules in solution. Here, we show that mass photometry is equally applicable to nucleic acid detection and mass quantification. Individual ds-DNA molecules binding to a cover glass surface produce strong interferometric contrast, with a detection limit on the order of 60 base pairs. Characterisation of standard DNA ladders yields mass distributions with well-resolved peaks, exhibiting a clear correlation between interferometric contrast and the number of base pairs resulting in 2 base pair accuracy and 13 base pair precision. Combined with the capabilities of mass photometry to study soluble proteins, these results furthermore pave the way towards label-free, single molecule, solution-based quantification of protein-DNA interactions.

Mass Photometry of Membrane Proteins and Membrane Mimetic Systems

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Integral membrane proteins (IMPs) are indispensable in cell function and signalling. However, they are inherently challenging to study because they require a lipid-like environment, which is difficult to maintain *in vitro*. As such, studying IMPs generally requires use of membrane mimetic systems. We explore the application of mass photometry (MP) – single-molecule mass measurement by interferometric scattering in solution - to IMPs and membrane mimetic systems at the single particle level. We apply MP to membrane mimetic systems including amphipathic vehicles, such as detergent micelles and amphipols, and lipid and native nanodiscs, characterizing the particle size and the sample purity and heterogeneity. We differentiate between empty and occupied mimetic systems and study IMP heterogeneity and multimer formation. Using methods established for cryogenic electron microscopy, we eliminate detergent background, enabling high-resolution studies of membrane protein structure and interactions in detergents. Demonstrating the power of this approach, we find evidence that, when extracted from native membranes using styrene-maleic acid nanodiscs, the potassium channel KcsA is present as a dimer of tetramers – in contrast to results obtained using detergent purification and lipid nanodiscs. Finally, using lipid nanodiscs and NMR, we show that MP can help distinguish between functional and non-functional KcsA nanodisc assemblies, as well as determine the critical factors for optimal lipid nanodisc formation. This approach thus opens the door to the routine use of native preparations of membrane proteins for *in vitro* functional studies, structural characterization by cryoEM, and *in vitro* studies of purity, structure and interactions more generally.

Quantifying binding affinities, kinetics and stoichiometry of biomolecular complexes with mass photometry

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The characterisation of biomolecular complexes and their interactions is essential for understanding the function and regulation of cellular processes. Many of the existing biosensor techniques, despite their maturity, are often hampered by the requirement for surface immobilisation, introduction of tags and the inability to differentiate and quantify co-existing species. Here, we show that mass photometry based on interferometric scattering microscopy (iSCAT), can determine the binding affinities, kinetics and stoichiometries of biomolecular complexes in a label-free fashion in solution at the single molecule level. We illustrate these capabilities by quantifiying antibody-antigen and antibody-receptor interactions ranging in strength over 4 orders of magnitude (K_D= 0.1 – 1000 nM) and showing the simplicity of differentitating between co-existing species. The ease and speed of use (< 1 min), low sample consumption (< picomole protein/run), minimal sample and assay preparation in combination with clear avenues to high-throughput and data analysis makes mass photometry a powerful new method for characterising biomolecular interactions and dynamics *in vitro*.