Stroboscopic quasiparticle tracking with an iSCAT probe

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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).