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Optical microscopy has revolutionized the field of biology, enabling researchers to explore the intricate details of biological structures and processes with unprecedented clarity. Over the past few decades, significant strides have been made in tailoring optical microscopy techniques to meet the specific needs of biologists (Schermelleh *et al.*, 2019; Prakash *et al.*, 2022). From sample preparation to hardware designs and software requirements, improvements have been driven by the goal of enhancing imaging capabilities and facilitating quantitative analysis. The papers featured in this issue cover a wide range of topics, addressing various aspects of optical microscopy for bioimaging. From the application of nonlinear micro-spectroscopy techniques to explore the spatial distribution of small gold nanoparticles within multicellular organs background-free (Pope *et al.*, 2023), to the development of a multiple feedback-based wavefront shaping method to retrieve hidden signals (Rumman *et al.*, 2022), and the utilization of artificial intelligence and deep learning algorithms for enhanced phase recovery in inline holography (Galande *et al.*, 2023), each study pushes the boundaries of what is possible with optical microscopy.

Other areas of focus include dark-field microscopy for parallel frequency-domain detection of molecular affinity kinetics (Xie *et al.*, 2022), radioluminescence imaging with nanophosphors (Bai *et al.*, 2022), mesoTIRF for high-resolution imaging of large cell populations (Foylan *et al.*, 2023), and light-sheet microscopy for volumetric imaging with adaptive capabilities (Hong *et al.*, 2022; Keomanee-Dizon *et al.*, 2022). Furthermore, the issue delves into advances in structured illumination microscopy, enabling robust frame-reduced imaging with accelerated correlation-enabled parameter estimation (Qian *et al.*, 2022). It also explores the intersection of optical microscopy and digital pathology, demonstrating the power of generative adversarial networks for H&E-like staining of OCT images of human skin (Tsai *et al.*, 2022). Finally, the issue presents innovative approaches for correlative microscopy workflows in life sciences, leveraging femtosecond laser preparation of resin-embedded samples (Bosch *et al.*, 2023). By collating these diverse contributions, the authors hope to inspire researchers to explore new avenues in optical microscopy for bioimaging.

In the field of ecotoxicology, the application of optical microscopy offers exciting potential for the detection of minuscule metallic nanoparticles within environmentally relevant organisms. A recent improvement in microscopy, known as four-wave mixing microscopy, has provided a window into the inner workings of biological entities. Specifically, it has focused its attention on the hepatopancreas of *Oniscus asellus*, a terrestrial isopod that ingests soil (Pope *et al.*, 2023). After being exposed to 10-nm gold nanoparticles, researchers utilized this technique to capture detailed images of hepatopancreas tubules. Despite the significant challenge posed by light scattering, this approach allowed for the precise observation of the nanoparticle spatial location. The method is able to reveal the ultrafast electron dynamics upon light excitation at the localized surface plasmon resonance of

gold nanoparticles, setting them apart from other metal deposits. This distinctive behavior serves as a key identifier, facilitating the recognition of gold nanoparticles within the complex optical environment of biological tissues.

Rumman *et al.* (2022) presented an optical wavefront shaping method designed to address the challenging light scattering properties encountered in biological samples, which can hinder imaging applications. This method combines the traditional feedback-based wavefront shaping with a switch function that is governed by two distinct signals. It leverages a simple transmission imaging system equipped with two detectors to track speckle patterns while simultaneously tracing hidden signals, such as fluorescent beads. The process begins with the identification of the optimal incident wavefront, a strategic step that enhances light transmission to create a focused area within the scattering medium. This controlled modulation of the wavefront leads to real-time changes in the monitored signals, cleverly pinpointing the positions of obscured objects. As the response of the hidden target becomes clear, a smooth transition occurs, shifting the algorithm's focus toward using this new signal for informed feedback.

Every imaging technique has its own limitations. While optical methods can capture dynamic processes in living systems, they often lack spatial resolution. Electron microscopy offers high spatial resolution but sacrifices temporal information because samples must be fixed before imaging. Correlative multimodal imaging offers a solution, allowing researchers to combine the strengths of both approaches. However, achieving this requires establishing sample preparation protocols compatible with multiple imaging methods. One effective approach involves using light microscopy to capture a fluorescent-based labeled region of interest in a biological soft tissue sample (as demonstrated by Bosch *et al.*, 2022). Subsequently, the sample is fixed and stained with heavy metals for high-resolution imaging at the selected site, revealing fine structural details.

When merging imaging techniques at different scales, precision is crucial. Carving out a precise volumetric region of interest from the overall sample volume before high-resolution imaging is essential for the success of the correlative workflow. This process relies on accurately targeting the region of interest and transforming the sample's physical and chemical properties to meet the requirements of downstream imaging. The femtosecond laser (fs laser) device plays a pivotal role in this process (Bosch *et al.*, 2023). Its ability to finely sculpt biological tissues into geometries optimized for synchrotron x-ray or volume electron tomography applications simplifies the preparation of samples for correlative multimodal imaging. Moreover, its capacity to carve out an unprecedented number of targeted samples in a high packing density from a single parent specimen substantially improves throughput and efficiency.

The pursuit of a cost-effective solution has driven a determined search for the one-shot reconstruction of inline holograms, particularly in situations with limited resources (Galande *et al.*, 2023). However, this endeavor has been hampered by the emergence of twin image artifacts, an unwanted outcome caused by the propagation of the conjugated wavefront, carrying incomplete phase information. The presence of these artifacts in reconstructions has posed a significant challenge, obstructing the realization of this promising imaging technique. Previous efforts to address this problem have mainly focused on deep learning techniques that require substantial amounts of carefully curated training data under stringent environmental and system stability conditions. A recent contender in this domain is the deep image prior (DIP), an approach that incorporates the physical principles of hologram formation into deep neural networks, without the need for prior training. Nevertheless, the fusion of DIP with the task of fitting the model output to a single measured hologram has inadvertently introduced interference-related noise, degrading the quality of reconstructions.

An untrained deep neural network is enhanced by explicit regularization through denoizing, aptly named RED (regularization by denoizing). This combination acts as a powerful solution to eliminate twin image artifacts and mitigate noise from the reconstructed output. Here, the alternating directions of multipliers method (ADMM) is used, a strategic facilitator that seamlessly combines DIP and RED into a robust process for single-shot phase recovery.

To refine TIRF imaging, scientists have long grappled with the need for high numerical aperture (NA) objectives to create the necessary evanescent wave. However, this pursuit has come with a trade-off because high-NA objectives often limit the field of view. In the 1980s, Axelrod proposed the prism-TIRF configuration as an intriguing alternative. It had the potential to bypass the high-NA requirements, allowing the use of larger-field objectives and expanding the observable cellular regions. Nevertheless, this approach had its own challenges, primarily related to limited light throughput, which hindered its practical use.

Recent improvements, however, have led to a prism-based TIRF illuminator designed to work in harmony with the Mesolens, a unique objective lens known for its remarkable combination of NA and magnification (Foylan *et al.*, 2023). The Mesolens offers an expansive imaging field, surpassing traditional TIRF objectives by a significant margin, effectively overcoming the long-standing field of view limitation. Furthermore, the optical throughput of this integrated system has been significantly improved, representing substantial progress in TIRF imaging capabilities. The modality's ability to handle multiple wavelengths enhances its versatility, as demonstrated by the comprehensive imaging of a large cell population within a single composite frame. The integration of the prism based TIRF illuminator and the Mesolens not only addresses the historical constraints of TIRF imaging but also introduces a transformative tool for mesoscopic exploration.

In the field of molecular affinity kinetics detection, a new method employs single nanoparticle plasmon sensors (known as NanoSPR) in the frequency domain. This approach referred to as versatile multiplex NanoSPR holds promise for unraveling complex molecular interactions. To improve both the precision and speed of detection, a technique called single-color imaging NanoSPR (SI-NanoSPR) has been introduced (Xie *et al.*, 2022). SI-NanoSPR leverages the combined capabilities of light scattering from multiple gold nanorod sensors, capturing the temporal behavior under the guidance of a total internal reflection dark-field microscope.

The core of this methodology lies in the analysis of the power spectral density in the frequency domain, which, much like a skilled interpreter, deciphers the intricate fluctuations in the signal. It extracts a characteristic frequency known as "fc," which serves as a distinctive signature of the molecular affinity kinetics under examination. This frequency becomes the guiding star for measuring previously elusive affinity interactions. The potency of this technique is demonstrated through its application in discerning the kinetics of two distinct

aptameric affinity systems coexisting within the same microscopic field of view. The equilibrium dissociation constants (KD values) derived from this analysis closely align with those obtained through traditional methods, affirming the reliability and robustness of the NanoSPR approach.

Bai *et al.* (2022) aimed to improve the sensitivity and precision of positron emission tomography (PET) and single photon emission computed tomography (SPECT) probes commonly used in clinical settings. Traditional techniques, such as Čerenkov luminescence imaging (CLI) and radioluminescence imaging (RLI), have long struggled with limited signal strength and clarity. The study focuses on a new method known as painted radioluminescence imaging (PRLI), which involves the use of a painted scintillator to significantly enhance both signal strength and imaging resolution for the widely utilized 18F-fluorinated deoxyribose (18F-FDG) tracer. The findings from experiments involving phantoms and live subjects present a case for the potential of PRLI. PRLI achieves signal intensities that are approximately 100 times higher than those obtained through CLI and surpasses flexible radioluminescence imaging (FRLI), a flexible scintillation based RLI method, in terms of resolution. This approach translates into substantial improvements in signal-to-background ratios within pseudotumor and tumor models. Specifically, in the pseudotumor model, PRLI increases the signal-to-background ratio by 81.8% compared to CLI and by 73.9% compared to FRLI. In the tumor model, PRLI exhibits increases of 60.0% compared to CLI and 33.3% compared to FRLI.

Light-sheet fluorescence microscopy (LSFM) is renowned for its ability to provide detailed optically sectioned imaging while minimizing issues like photobleaching and phototoxicity. To achieve high-speed 3D LSFM imaging without disturbing the sample too much, it is crucial to incorporate remote refocusing into the detection setup. Various methods, such as electrically tunable lenses and tunable acoustic gradient index of refraction lenses, have been attempted in the past. However, these approaches mostly address basic defocusing issues and are not well suited for high-NA objectives that require more advanced defocus correction. The remote-refocusing technique introduced by Botcherby *et al.* (2007) elegantly addresses this but can result in reduced optical efficiency. Hong *et al.* (2022) proposed a solution to these challenges by integrating a deformable mirror into the detection path. This deformable mirror possesses the capability to correct higher-order defocus and aberrations in optical systems with numerical apertures (NA) ranging from 0.72 to 0.75. The study achieved high-speed 3D imaging at a remarkable rate of 26.3 volumes per second, encompassing 35 frames in each volume. Notably, this achievement covers a substantial defocus range from -50 to 50 μm.

Navigating the intricate tradeoffs between field of view, optical sectioning, resolution, and detection efficiency presents challenges for light-sheet microscopes. High-numerical-aperture (NA) detection objective lenses, while offering improved resolution, struggle to efficiently capture fluorescence signals generated throughout the thickness of the illumination light sheet, particularly during the imaging of large volumes. Keomanee-Dizon *et al.* (2022) introduced a light-sheet microscopy approach known as ExD-SPIM (extended depth-of-field selective-plane illumination microscopy) to address this issue by aligning the depth of field (DOF) of high-NA detection objectives with the thickness of the illumination light sheet. The extended DOF achieved through ExD-SPIM involves the use of a phase mask to elongate the point-spread function of the objective lens along the axial direction while preserving lateral resolution. This alignment between the detection DOF and the thickness of the illumination sheet offers significant advantages. Notably, it enhances the overall collection of fluorescence, reduces background interference, and greatly improves the signal-to-noise ratio (SNR).

Structured illumination microscopy (SIM) is highly regarded in the field of fluorescence super-resolution microscopy due to its ability to provide comprehensive imaging and minimize photodamage, making it a cornerstone of interest for the biological sciences community (Heintzmann and Huser, 2017; Prakash *et al.*, 2021). However, traditional SIM techniques have their limitations, typically requiring a minimum of nine images for precise image reconstruction. Additionally, achieving high-quality super-resolution heavily depends on accurately estimating illumination parameters, a process often demanding significant computational effort and time. In response to these challenges, Qian *et al.* (2022) introduced a seven-frame SIM reconstruction algorithm that incorporates accelerated correlation-based parameter estimation. The proposed algorithm introduces two key innovations to enhance efficiency while maintaining accuracy. First, it employs a modulation-assigned spatial filter to effectively eliminate unreliable backgrounds associated with low signal-to-noise ratios, thus improving the quality of reconstruction. Second, it introduces a novel coarse-to-fine accelerated correlation approach that streamlines the iterative correlation-based process. Notably, this reduction in computational iterations is achieved through a carefully designed phase-shifting strategy and pixel-wise fluorescence pre-calibration.

Comparative analysis against traditional iterative correlation-based techniques reveals that the proposed algorithm significantly enhances computational efficiency by a factor of 4.5 $\times$, all while preserving the precision of illumination parameter estimation. Remarkably, this method achieves high-fidelity super-resolution reconstruction even with a reduction to just two images.

In the realm of clinical applications, where noninvasive and high-speed optical coherence tomography (OCT) systems are widely employed, there has been remarkable progress in achieving higher-resolution OCT images. However, a persistent challenge for pathologists is the interpretability of OCT images in shades of gray, which lack the inherent diagnostic specificity found in hematoxylin and eosin (H&E)-stained images. To address this issue, Tsai *et al.* (2022) introduced a model that translates OCT images into H&E-like stained counterparts, making use of unpaired OCT and H&E image datasets. The primary objective of this translation is to attain a quality resembling that of H&E staining, ensuring alignment in features like the stratum corneum (SC) boundary and the dermal-epidermal junction (DEJ) between the original OCT images and the translated ones. A pivotal aspect of this research involves employing pre-trained segmentation models for the DEJ and SC, significantly improving the accuracy of anatomical image translation. This strategic integration effectively minimizes errors in determining the lower boundaries of DEJ and SC, reducing deviations to an impressive range of ± 2.3 and ± 1.7 μm, respectively. Additionally, the study harnesses a pre-trained VGG16 network to extract nuclear features. Notably, the consistency of nuclei location and size is quantified using Pearson's correlation coefficient, yielding a commendable score of 84% $\pm$ 1%.

The articles presented in this collection showcase progress in various fields of bioimaging and microscopy. From exploring novel imaging techniques for detecting nanoparticles within organisms to

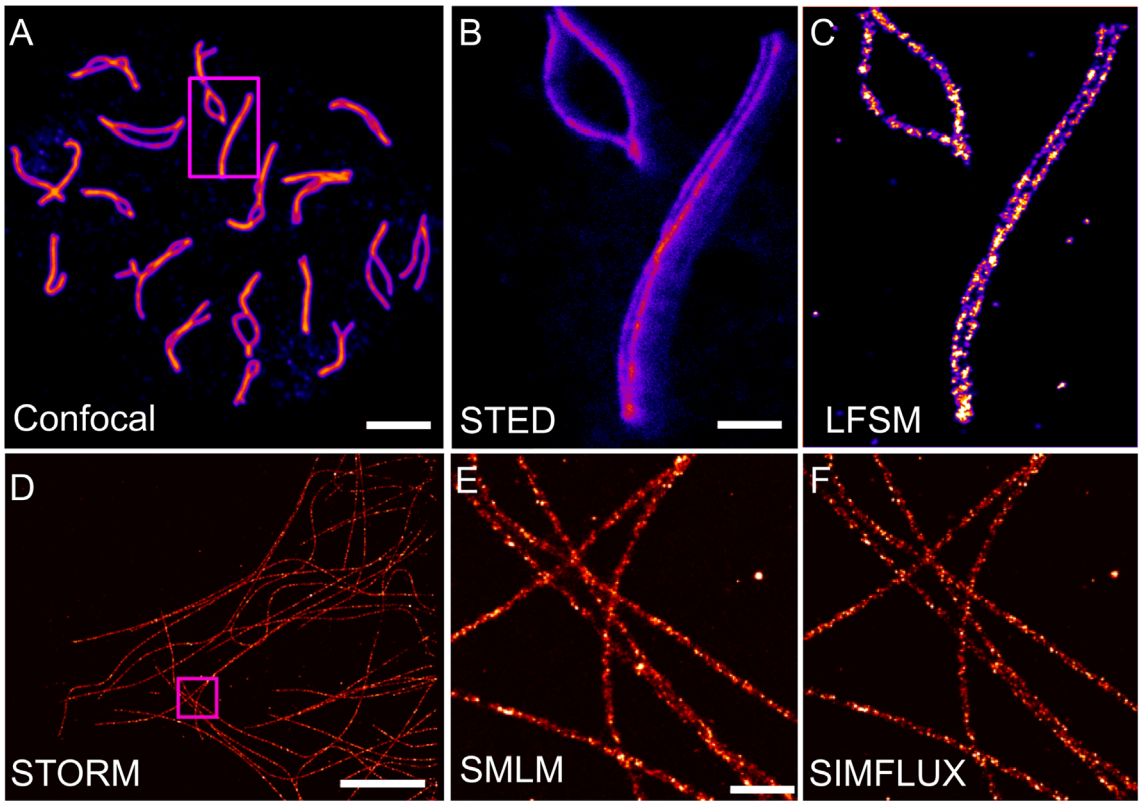

FIG. 1. The next frontier in bioimaging: correlative super-resolution microscopy. (a) The confocal image at a lower magnification displays all the chromosomes present. (b) The enclosed region from (a) as imaged by the STED microscope, and (c) the same chromosome was imaged by the LFSM microscope. It is noteworthy that the synaptonemal complex proteins remain indistinct in the confocal image. However, both STED and LFSM microscopies reveal their two distinct halves. Employing sequential correlative imaging can be instrumental in verifying this newly discovered structure beyond the limits of diffraction. (d) Demonstration of SIMFLUX on cellular tubulin with dSTORM. (e) and (f) Zoom-in on SMLM and SIMFLUX images of boxes in (d); all reconstructions are based on the same underlying data. Direct correlative methods like SIMFLUX can improve precision over standard localization with the same photon count. Scale bar in (b), (c), (e), and (f), 1 μm; in (a) and (d), 5 μm. Image modified from Prakash (2021) and Cnossen *et al.* (2020).

revolutionizing light-sheet microscopy for high-speed volumetric imaging, these studies push the boundaries of what is possible in scientific exploration.

The special issue did not encompass the latest developments in correlative super-resolution microscopy (CRM), which are extending the limits of what can be achieved in biological imaging. CRM has broadened the horizons, enabling scientists to merge various imaging techniques for improved resolution and a deeper understanding of intricate biological systems. Correlative super-resolution methodologies can be categorized into two primary groups: sequential combination and direct combination (Fig. 1).

The sequential combination involves using different super-resolution techniques in succession to obtain complementary information about a biological sample (Rossberger *et al.*, 2013; Prakash, 2021). For example, the combination of stimulated emission depletion (STED) microscopy and single-molecule localization microscopy (SMLM) has been proposed (Prakash, 2021). By sequentially applying these techniques, researchers can cross-validate the observed structures and enhance confidence in the obtained results. The two strands of synaptonemal complex proteins (Prakash *et al.*, 2015) were resolved with laser-free super-resolution microscopy (LFSM) and STED [Figs. 1(a)–1(c)].

Direct combination techniques, such as MINFLUX and SIMFLUX, offer the promise of significantly increased resolution. MINFLUX combines the principles of STED and SMLM, utilizing a minimal number of photons for precise localization (Balzarotti *et al.*, 2017; Cnossen *et al.*, 2020; Reymond *et al.*, 2020; and Gwosch *et al.*, 2020). Similarly, SIMFLUX merges SIM and SMLM, offering improved resolution through direct combination. However, the application of these to thick, complex biological structures presents challenges (Prakash and Curd, 2023; Prakash, 2022; and Helmerich *et al.*, 2022) to achieve the desired resolution.

The special issue introduces inventive approaches to address specific challenges, whether it is enhancing the precision of imaging, improving signal-to-noise ratios, or bridging the gap between different imaging modalities. These innovations not only expand our understanding of complex biological systems but also hold promise for practical applications in fields like medicine and materials science. We anticipate that they will inspire further discoveries and pave the way for new frontiers in scientific exploration.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Kirti Prakash: Conceptualization (equal); Data curation (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal). **Rainer Heintzmann:** Conceptualization (equal); Supervision (equal); Writing – review & editing (equal). **Uri Manor:** Writing – review & editing (equal). **Carlas Smith:** Data curation (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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