Microscopy at a glance

New poster article series exploring the intersection of art, science and imaging

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EDITORIAL

Microscopy at a glance: New poster article series exploring the intersection of art, science and imaging

The COVID-19 pandemic in early 2020 caused widespread disruption, particularly impacting the scientific community involved in microscopy. Laboratories were shut down, and traditional lecture methods, collaboration, and conferences became untenable. In response, the 'Imaging ONEWORLD' (IOW) online microscopy lecture series was launched in May 2020 to provide a virtual platform for education, discussion and community engagement.[1] This series covers a wide range of topics, from the history of microscopy to advanced techniques in fields, continuing to connect the global microscopy community through recorded sessions and social media. As a follow-up step, in collaboration with the *Journal of Microscopy*, we invited speakers to summarise their talks in poster articles. With these visual articles, we aimed to communicate complex scientific ideas intuitively emphasising the importance of creativity and innovation in scientific discourse (Figure 1).

Curd et al.[3] addressed the challenge of detecting and analysing molecular structures in dense biological samples using single-molecule localisation microscopy (SMLM). The authors introduce a software package called PERPL designed to model and infer the 3D supramolecular structures from sparse SMLM data, even when detection efficiency is extremely low.[4,5] PERPL aggregates the relative positions of fluorescently labelled molecules across multiple instances of a complex, allowing for identifying characteristic features in 3D structures. The study demonstrates the application of PERPL in analysing the 3D lattice of Z-disk proteins in mammalian cardiomyocytes. By employing PERPL, the authors successfully identified both known and novel structural features with nanometre precision. PERPL thus offers a significant advantage by not requiring the segmentation of individual complexes, which is often challenging in biological SMLM data.[6] Instead, it fits structural models to the aggregated relative position data, providing a quantitative measure of the likelihood of various structural models. This capability allows for exploring complex molecular arrangements and could be a powerful tool in elucidating the architecture of other supramolecular complexes.

Cremer et al.[7] explored the application of Modulated Illumination Microscopy (MIM), particularly its combination with single-molecule localisation microscopy (SMLM), for analysing the intricate nanostructures within the cell nucleus, that is, complex chromatin structures and small macromolecular assemblies. The authors discuss various MIM techniques and the integration of SMI with SMLM, potentially achieving a localisation precision of less than 1 nm. They highlight how SMI has been successfully used to estimate the size of nuclear chromatin domains and other small nuclear structures, offering insights into their spatial organisation and density. The authors propose that the combination of MIM with MINFLUX and cryoelectron microscopy could further enhance the analysis of nuclear nanostructures. This approach could also facilitate high-resolution studies of drug interactions with chromatin and other macromolecular complexes, potentially advancing the field of medical therapy by providing a deeper understanding of chromatin remodelling and gene regulation at the nanoscale.

Smith et al.[8] explored the precision limits in single-molecule localisation microscopy (SMLM), specifically focusing on iterative techniques such as MINFLUX. The article demonstrates how incorporating prior information can enhance precision and reduce the number of photons needed for accurate localisation.[9] The authors propose using the Van Trees inequality (VTI) as a Bayesian alternative to the Cramér-Rao lower bound (CRLB) for assessing optimal precision in iterative localisation methods. Their findings reveal that while ideal conditions can lead to exponential improvements in precision with each iteration, practical limitations like background noise and imperfect modulation significantly reduce these gains. The study challenges several existing assumptions in the field, such as the belief that smaller step sizes always yield better precision and the expectation of exponential precision improvement. The results underscore the importance of optimising parameters like modulation contrast and photon budget distribution to achieve the best possible outcomes in iterative localisation microscopy. The framework developed in this study offers new insights and is expected to influence future research and applications

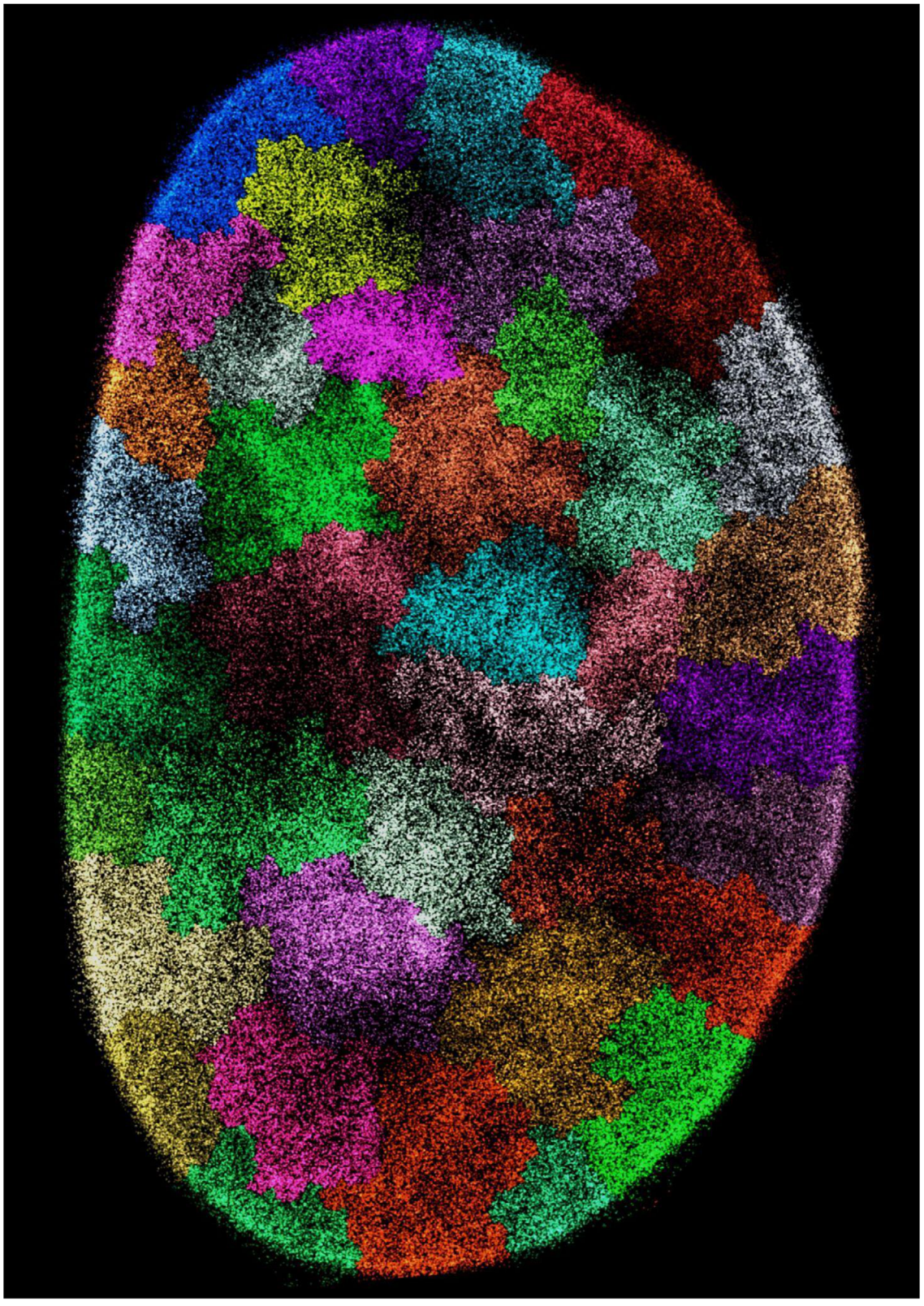

FIGURE 1 Single-molecule superresolution image of cell nucleus segmented to depict ONEWORLD.[2]

of MINFLUX and similar methodologies, particularly in scenarios where background noise and modulation imperfections are significant.

Prakash[10] discusses the challenges and potential solutions for correlating two super-resolution microscopy techniques: Stimulated Emission Depletion (STED) and Single-Molecule Localisation Microscopy (SMLM), which is complicated by issues such as photobleaching of fluorophores during STED and the difficulty of reactivating these fluorophores for subsequent SMLM imaging. This could be mitigated by the use of deep ultraviolet (DBUE) wavelengths for continuous SMLM imaging.[11] The author demonstrates that Alexa Fluor 594, in combination with the imaging buffer Prolong Diamond, is particularly effective for correlative STED and SMLM imaging. This approach enables high-resolution imaging of biological structures, such as the synaptonemal complex in meiotic chromosomes. The article also presents a framework for sequential imaging with confocal microscopy, STED and SMLM on the same sample, which allows for the validation and interpretation of nanoscale structures with enhanced confidence. The findings demonstrate that high-resolution, single-molecule imaging can be achieved using conventional microscopes and non-coherent light sources, making advanced imaging techniques more accessible to a broader range of laboratories.

Rooney et al.[12] discuss the innovative use of the Mesolens, a unique microscope, to study large, complex microbial specimens like biofilms and bacterial colonies. The Mesolens combines low magnification with a high numerical aperture, allowing detailed imaging of entire samples. The article highlights its applications in exploring biofilm architecture, nutrient transport channels, and interactions in dual-species biofilms. It also emphasises the potential of the Mesolens in other areas of microbiology, such as studying microbial communities, pathogens, and astrobiology.

Hartley et al.[13] emphasise the importance of open access to biological imaging data for scientific reproducibility and innovation. It discusses when and where to publish imaging data, highlighting the BioImage Archive as a key resource. The article also outlines the steps for data submission, the importance of proper metadata, and future directions for enhancing data reusability, including AI-ready data. The article advocates for standardised practices to facilitate data sharing and reuse in the scientific community.

Koerfer et al.[14] explore the impact of various glass cleaning methods on the calibration accuracy of STED-FCS. Calibration in these techniques is essential for measuring molecular mobility and diffusion dynamics in biological systems. The study compares three cleaning methods: the conventional piranha solution, an acetone-based method with sonication, and chloroform rinsing. The findings indicate that acetone cleaning is as effective as piranha cleaning but safer and easier to use. The research concludes that acetone cleaning combined with point STED-FCS provides accurate and reliable calibration, making it a suitable alternative to more complex methods.

Kalyviotis and Pantazis[15] explore the technique of primed conversion, a method for converting green-to-red photoconvertible fluorescent proteins using a combination of blue and far-red light. This technique offers precise, non-toxic imaging, particularly useful for single-cell labelling and lineage tracing in live organisms. Primed conversion is advantageous over traditional methods due to its reduced phototoxicity, axial confinement, and compatibility with existing confocal microscopes, making it a promising tool for developmental biology and disease research.

Kale et al.[16] present computational methods for analysing differential interference contrast (DIC) microscopy images, focusing on quantifying cellular dynamics. They enhance image quality through filtering to improve gradient visibility and develop a segmentation pipeline that leverages the apparent shadow of rod-shaped bacterial cells. The study includes edge detection via thresholding the global gradient in both X and Y directions to outline cell contours, which are then skeletonised to 1-pixel-wide representations for accurate length estimation. Additionally, single particle tracking (SPT) is utilised to monitor the movement of intracellular organelles and beads over time, allowing for quantitative analysis without the need for fluorescent labelling.

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This special issue is dedicated to Joseph Gall who passed away on September 12, 2024. The idea of this poster series was inspired by his book “Views of the Cell – A Pictorial History.” He exemplified the true spirit of science—pursuing truth through evidence and embracing new facts, even if they challenged his hypotheses. The best way to honor his memory is by following his commitment to scientific integrity and discovery.

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