OPEN ACCESS

Molecular & Cell COMMENTARY

The Innovation Life

Interferometric image scanning microscopy: A new route to label-free super-resolution imaging in live cells

Xin Xu,[1] Jixiang Wang,[1] Jing Lu,[1] and Guohua Shi[1,*]

[1]Hangzhou Institute of Medicine, Chinese Academy of Science, Hangzhou 310018, China

*Correspondence: shiguohua@him.cas.cn

Received: April 16, 2026; Accepted: June 22, 2026; Published Online: June 24, 2026; https://doi.org/10.59717/j.xinn-life.2026.100231

Citation: Xu X., Wang J., Lu J., et al. (2026). Interferometric image scanning microscopy: A new route to label-free super-resolution imaging in live cells. *The Innovation Life* **4**:100231.

Throughout the development of optical microscopy, live-cell imaging has faced a central challenge: how to achieve spatiotemporally resolved observation beyond the diffraction limit under label-free, low-phototoxicity conditions. Over the past two decades, super-resolution fluorescence microscopy has achieved nanoscale resolution by leveraging the molecular specificity of fluorescent probes, with representative examples including stimulated emission depletion microscopy (STED), structured illumination microscopy (SIM), and stochastic optical reconstruction microscopy (STORM).[1] However, fluorescence imaging remains limited by phototoxicity, labeling efficiency, and the possibility that exogenous probes may perturb native cellular physiology. As a

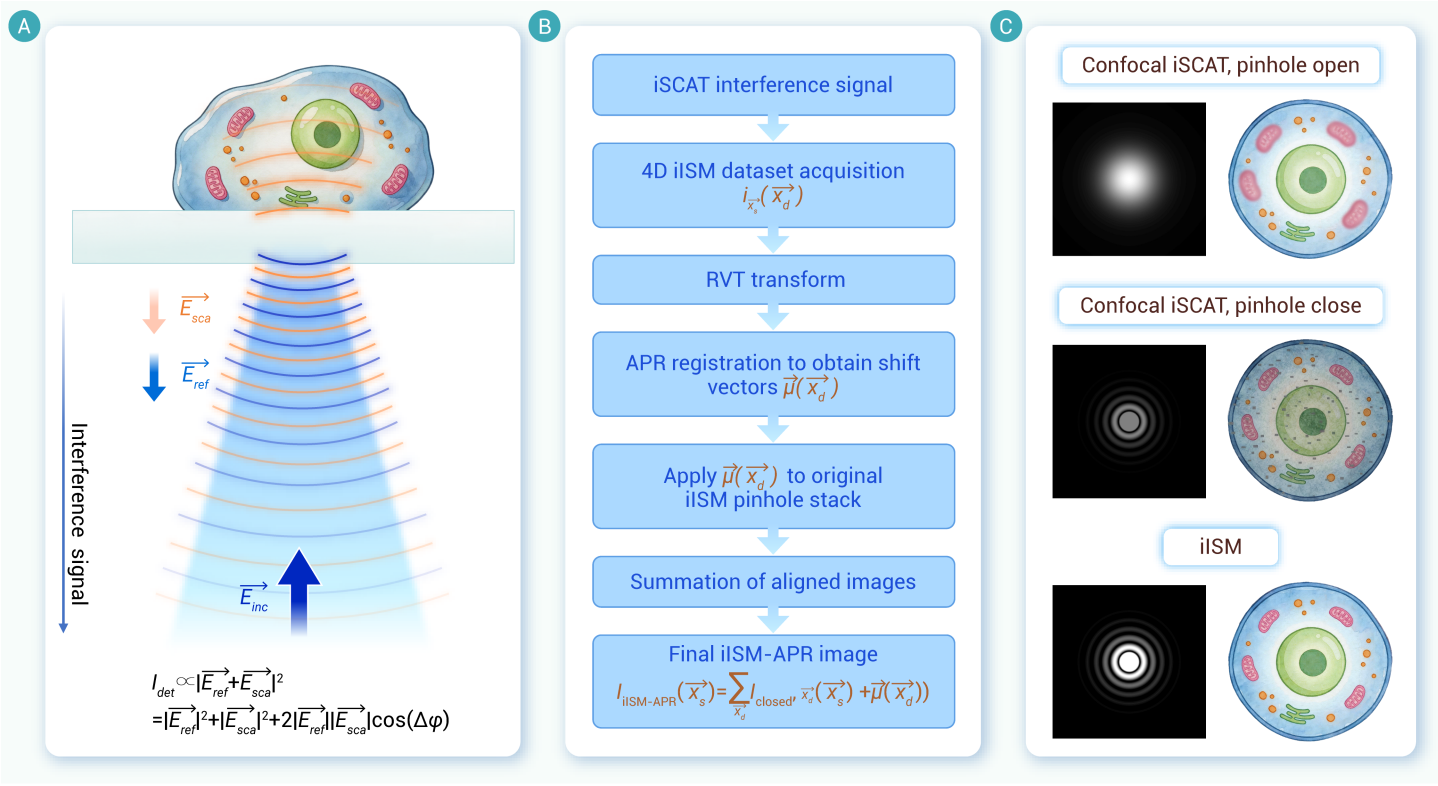

Figure 1. Schematic of the imaging principle and reconstruction workflow of iISM (A) Basic physical principle of interferometric scattering imaging. Upon illumination by the incident field , the sample-scattered field interferes with the reference reflected field , generating the interference signal at the detector. (B) Core algorithmic workflow of iISM. (C) Schematic illustration of the PSF and signal-processing outcome, showing the progression from the initial detected pattern to the final iISM-APR image with improved contrast and resolution.

label-free imaging modality, interferometric scattering microscopy (iSCAT) has attracted broad attention because it can detect nanoscale structures without any additional labeling. Although iSCAT has already enabled the detection of proteins and viruses on clean substrates, in live cells, the coherent superposition of scattered light in complex media generates pronounced speckle, which degrades image quality. To suppress out-of-focus background and improve intracellular imaging contrast, recent studies have introduced confocal illumination and detection into iSCAT.[2] However, this method introduces a classic limitation of confocal microscopy: reducing the pinhole can improve lateral resolution, but at the cost of a substantial loss in signal-to-noise ratio. To address these challenges, the Moerner group introduced interferometric image scanning microscopy (iISM),[3] which for the first time combines image scanning microscopy (ISM) with interferometric scattering detection, thereby providing a new route for label-free super-resolution imaging in live cells.

Conventional fluorescence ISM replaces the point detector with an array detector and collects off-axis signals for pixel reassignment,[4] combining the high resolution of a closed pinhole with the high signal-to-noise ratio of an open pinhole. However, fluorescence emission is an incoherent process, and image formation is governed primarily by the intensity distribution of the point spread function (PSF). In contrast, the signal detected in iISM is coherent and contains both amplitude and phase information. Therefore, the adaptive pixel reassignment (APR) strategies used in conventional ISM,[5] whether based on simple geometric shifts or fluorescence PSFs, cannot be directly applied to interferometric data processing.

To address this problem, the authors optimized both the hardware and the algorithm (Figure 1). On the hardware side, they analyzed how polarization affects the interferometric point-spread function (iPSF). Under a high-numerical-aperture objective, a tightly focused Gaussian beam at a dielectric interface exhibits strong polarization dependence. When linearly polarized light is used for excitation, the illumination PSF becomes elongated along the polarization axis, leading to anisotropy in both the illumination PSF and the detected iPSF, and the phase reversals at off-axis positions. These effects compromise the robustness of the subsequent pixel reassignment procedure. Therefore, the

system incorporates a polarizing beam splitter cube and a quarter-wave plate to generate circularly polarized light at the sample plane, producing an iPSF with greater rotational symmetry and improved phase stability. On the algorithmic side, the authors first acquire a four-dimensional dataset containing both scan coordinates and detector coordinates, and then introduce the Radial Variance Transform (RVT), which converts interferometric images with fringe patterns into intensity maps that reflect local radial symmetry. APR is subsequently performed on the transformed data, and the resulting displacement vectors are finally mapped back onto the original iISM dataset to enable super-resolution reconstruction while retaining phase-related information. This strategy preserves the spatial information of interferometric imaging while avoiding the difficulties associated with direct phase-based registration.

Regarding imaging performance, this method achieves a lateral resolution of approximately 120 nm, close to the theoretical limit. The authors further present a quantitative comparison among three configurations: open-pinhole iSCAT, closed-pinhole iSCAT, and iISM. Nanoparticle measurements further confirmed that iISM preserves the resolution advantage of closed-pinhole confocal iSCAT while substantially improving image quality. It achieved a lateral resolution of approximately 120 nm and increased the CNR to about 38, compared with about 10 for closed-pinhole and 14 for open-pinhole confocal iSCAT. Thus, iISM recovers useful photons that would otherwise be rejected by a conventional pinhole, enabling lower-power imaging without sacrificing resolution.

In live COS-7 cell experiments, the authors demonstrated the imaging capability of iISM in biological samples. The system required only about 0.5 μW of incident power to generate clear images of intracellular structures. This power level is approximately one tenth of that used in their previous live-cell confocal iSCAT experiments. The field of view for a single optical section is approximately 40 × 80 μm. Within this relatively large imaging area, the nucleus, mitochondria, endoplasmic reticulum, vesicles, actin cytoskeleton, and lamellipodia can all be clearly identified. Under such low illumination, cells can be monitored almost continuously for essentially unlimited periods while maintaining a low risk of photodamage. More importantly, because the interferometric signal is highly sensitive to axial phase, the same type of structure can exhibit different contrast at different axial positions. This means that iISM provides not only lateral super-resolution, but also intrinsic sensitivity to three-dimensional information. After flat-field background correction, the authors further tracked dynamic processes such as vesicle motion and endoplasmic reticulum remodeling, highlighting the method's potential for time-resolved imaging under near-physiological conditions.

This work is not intended to replace fluorescence microscopy, but rather to provide a valuable complementary modality. The authors fluorescently labeled actin in fixed COS-7 cells, and performed correlative imaging with iISM and fluorescence ISM.The results showed that the spatial localization of major filamentous structures is highly consistent between the two modalities, indicating that iISM can reliably reflect subcellular architecture such as the cytoskeleton. At the same time, iISM revealed additional structural information beyond the fluorescence channel, including locally unlabeled vesicles or adhesion-related structures, as well as finer variations in scattering contrast along the fibers. One particularly instructive result in the paper is that iISM and fluorescence imaging show strong agreement for thick actin bundles. In denser mesh-like regions, the fluorescence channel often reveals the fine network more clearly because of its molecular specificity. This difference highlights the complementary of the two imaging modalities. Fluorescence imaging provides molecular specificity, whereas iISM contributes label-free structural background, morphological context, and scattering-related physical information. In combination, the two approaches may help researchers interpret molecular events within a more complete cellular structural framework.

Despite these strengths, several practical boundaries should also be considered when evaluating the broader applicability of iISM. First, because the contrast originates from coherent interference between the reference and scattered fields, the recorded signal is sensitive not only to the scattering cross-section of intracellular structures, but also to their axial position and local refractive-index environment. This feature provides valuable three-dimensional sensitivity, but it may also complicate direct biological interpretation in samples with highly heterogeneous scattering backgrounds. In dense intracellular regions, overlapping scattering contributions may obscure weak or fine structures, as suggested by the comparison with fluorescence ISM in actin-rich regions. Second, the RVT-APR workflow relies on stable interferometric point-spread functions, appropriate polarization control, and four-dimensional data acquisition, which may increase computational and data-throughput demands for large field-of-view or high-speed imaging. Therefore, future applications of iISM will likely require not only optical optimization, but also faster reconstruction algorithms and more robust strategies for separating structure-specific scattering signals from complex cellular backgrounds.

In summary, this work represents an important advance in the system design, polarization control, and reconstruction algorithm of interferometric scattering imaging, and provides a new strategy for super-resolution imaging in live cells. Its significance lies not only in achieving label-free imaging with a resolution of approximately 120 nm, but also in demonstrating that super-resolution, reduced photodamage, and long-term dynamic observation can be achieved simultaneously. Importantly, the future development of iISM should be considered within its coherent-detection framework rather than simply as an extension of conventional fluorescence ISM. For example, single-photon avalanche diode (SPAD) array detectors and parallelized scanning architectures may improve temporal resolution, but their integration will need to preserve interferometric phase stability, detector-to-detector uniformity, and accurate pixel reassignment. Similarly, computational staining based on paired iISM and fluorescence datasets should account for the fact that iISM contrast reflects a mixture of scattering strength, refractive-index variation, and axial phase, rather than molecular identity alone. Therefore, deep-learning models for iISM may be most powerful when designed not merely as image-to-image translators, but as physics-informed tools that incorporate coherent contrast formation and correlative fluorescence ground truth. It is therefore reasonable to expect that this technique will find important applications in the study of host–pathogen interactions, intracellular transport, cytoskeletal remodeling, and cell dynamics under near-native conditions.

REFERENCES

1. Danial J.S.H. (2025). Super-resolution microscopy for structural biology. *Nat. Methods* **22**:1636–1652. DOI:10.1038/s41592-025-02731-1
2. Ginsberg N.S., Hsieh C.L., Kukura P., et al. (2025). Interferometric scattering microscopy. *Nat. Rev. Methods Primers* **5**:23. DOI:10.1038/s43586-025-00391-1
3. Küppers M. and Moerner W.E. (2026). Interferometric Image Scanning Microscopy for label-free imaging at 120 nm lateral resolution inside live cells. *Light: Sci. Appl.* **15**:129. DOI:10.1038/s41377-026-02210-y
4. Zunino A., Garrè G., Perego E., et al. (2025). Structured detection for simultaneous super-resolution and optical sectioning in laser scanning microscopy. *Nat. Photonics* **19**:888–897. DOI:10.1038/s41566-025-01695-0
5. Müller C.B. and Enderlein J. (2010). Image scanning microscopy. *Phys. Rev. Lett.* **104**:198101. DOI:10.1103/PhysRevLett.104.198101

FUNDING AND ACKNOWLEDGMENTS

This work was supported by the Chinese Academy of Sciences Project for Young Scientists in Basic Research (grant no. YSBR067), and the Leading Innovative and Entrepreneur Team Introduction Program of Hangzhou (grant no.TD2025014). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

All authors conceived the original idea. Xin Xu wrote the manuscript. Jixiang Wang, Jing Lu and Guohua Shi revised the manuscript. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.