Comments on high-speed mesoscale light-field microscopy

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Since its invention in the 1600s, optical microscopy has significantly contributed to our understanding of biology, advancing fields such as cell biology and microbiology. Yet, with advancements in biotechnology and medicine, the demand for more comprehensive imaging has markedly increased. Understanding the dynamic complexities of biological systems requires high-resolution 3D imaging with both superior temporal and spatial resolution. Although various subcellular intravital microscopy (IVM) methods have been developed, they only partially meet these needs[1–4], and capturing fast and dynamic processes, such as cellular activities across large spatial ranges, remains a challenge[5].

Light-field microscopy (LFM) has emerged as an innovative single-shot 3D approach, offering rapid volumetric acquisition[6]. Instead of capturing images sequentially—point-by-point or plane-by-plane—LFM records four-dimensional (4D) light-field data—both spatial and angular—by incorporating a microlens array (MLA) at the image plane [Fig. 1(a)]. This enables computational reconstruction of three-dimensional (3D) biological structures from one exposure[7]. By boosting volumetric imaging speed and maximizing photon collection efficiency, LFM has become a powerful tool for applications including neural activity imaging in zebrafish, single-molecule tracking, and human organoid imaging[8–10]. However, LFM's inherent resolution limit propelled the development of scanning light-field microscopy (sLFM), which achieves near-diffraction-limited resolution and represents a significant leap over conventional LFM.

The development of sLFM has been pioneered by Prof. Qionghai Dai's group at Tsinghua University, successfully overcoming the spatial-angular resolution trade-off to achieve enable high-resolution volumetric imaging[2,11]. By integrating digital adaptive optics (DAO) with optimized imaging protocols, sLFM offers aberration-corrected mesoscale imaging at millisecond rates and low phototoxicity, enabling clear visualization of complex cellular environments[2,11]. Various sLFM hardware versions have been developed, each optimized for different needs, while computational methods have also evolved to enhance image quality and streamline data processing. Key sLFM platforms include digital adaptive optics scanning light-field mutual iterative tomography (DAOSLIMIT)[2], confocal line-scanning LFM[12], the meta-imaging sensor[13], and the RUSH3D real-time ultra-large-scale 3D mesoscope[14].

Advancements in sLFM arise from two core ideas: high-density light field imaging with periodic scanning and DAO. By employing dense acquisition plus scanning, sLFM attains near diffraction-limited resolution[2,15]. Integrating DAO into reconstruction enables multi-site 3D aberration correction for mesoscale IVM, ensuring high-fidelity imaging of complex structures.

Unlike conventional LFM using larger microlenses, sLFM adopts smaller microlenses—about 10 times the diffraction limit—introducing diffraction effects that preserve high-frequency details via frequency aliasing. Additionally, a scanning module applies small, periodic shifts of the image plane relative to the MLA [Fig. 1(b)][16], boosting spatial sampling density and enabling higher-resolution reconstruction. Incoherent synthetic aperture (ISA) merges angular and spatial data[2], greatly enhancing sLFM's fidelity for high-speed high-resolution biomedical imaging.

Like regular LFM, sLFM retains high photon collection efficiency by capturing the entire imaging volume each time. This lowers the required illumination intensity, minimizing the phototoxicity compared to methods like confocal or two-photon microscopy. These features make sLFM ideal for long-term studies of delicate biological processes, including embryonic development and *in vivo* cell migration[14,15].

sLFM uses DAO during reconstruction to estimate and correct optical aberrations, substantially boosting image quality. Biological specimens often exhibit heterogeneous composition, producing nonuniform aberrations that degrade resolution and reduce signal-to-noise ratio (SNR)[15,17]. Conventional adaptive optics (AO) systems rely on wavefront sensors and deformable mirrors or spatial light modulators, which are expensive and challenging to implement. By contrast, sLFM inherently records the light field, allowing aberration estimation and correction via computational post-processing [Fig. 1(b)].

DAO employs image-formation models and iterative updates of 4D spatial-angular data to refine the 3D volume. Large fields of view (FOVs) are processed in overlapping patches, combining wavefront correction with ISA. This approach excels at imaging uneven surfaces or densely labeled samples[11,12]. Unlike hardware-based AO, DAO in sLFM enables simultaneous multi-site aberration correction, making it highly effective for large-scale intravital imaging.

sLFM technologies have yielded major insights into complex biological processes. Its minimal excitation requirements enable hours-long imaging with reduced phototoxicity[2,11]. Unlike 2D methods with a shallow depth of focus (DOF), sLFM supports depths of tens to 100 μm, clarifying cellular views on uneven surfaces. Notable applications include migrasome dynamics in mammals, tumor cell migration in zebrafish and mice, and large-scale 3D calcium imaging at subcellular resolution [Fig. 1(c)][2,11,12]. For instance, sLFM reveals that neutrophils rapidly produce migrasomes via retraction fibers attached to blood vessels during migration in the mouse liver [Fig. 1(c)][2]. sLFM also enables extended 3D visualization of multiple germinal centers (GCs) in mouse inguinal lymph nodes at single-cell resolution. Moreover, it facilitates studies of reverse transendothelial neutrophil migration following traumatic brain injury,

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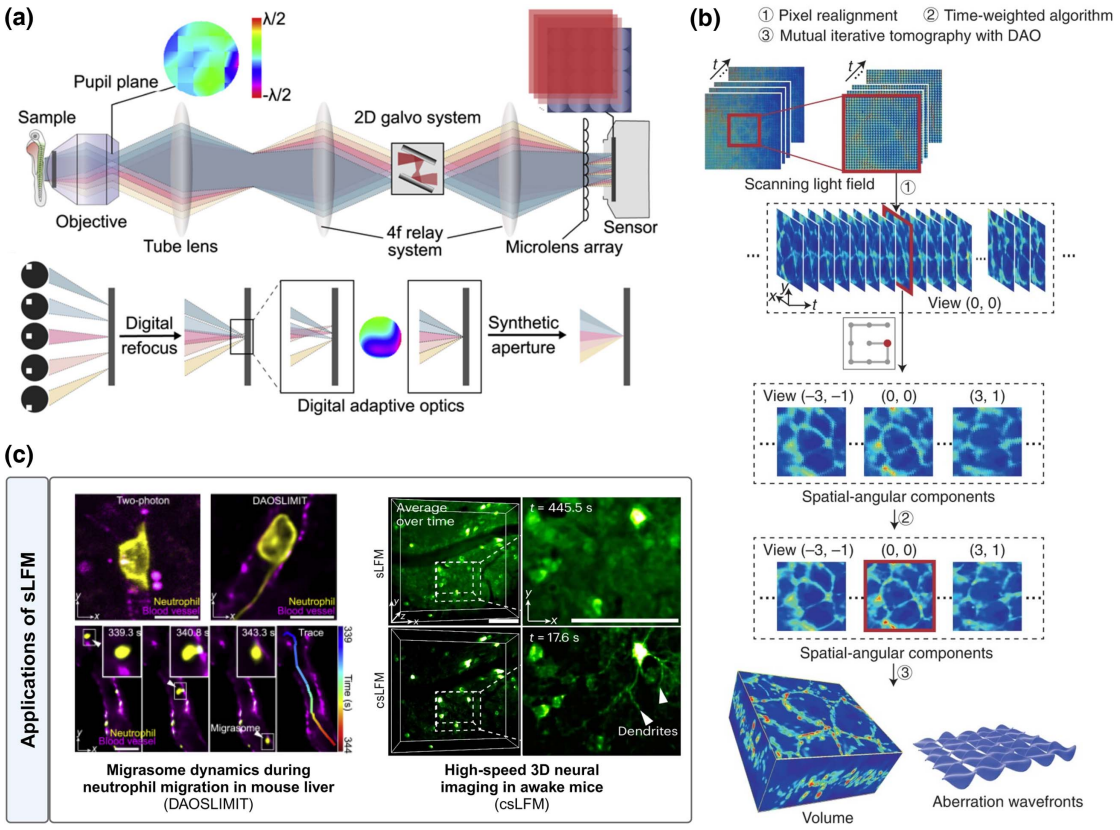

Fig. 1 Overview of scanning light-field microscopy (sLFM) and its applications. (a) In sLFM[1,2], a 2D scanning module and a microlens array capture high-resolution spatial–angular data at near-diffraction-limited resolution[2,9]. (b) After scanning, a time-weighted algorithm and mutual iterative tomography with digital adaptive optics (DAO)[2,13] enable pixel realignment and aberration correction, yielding a volumetric reconstruction. (c) These advancements support diverse biomedical applications, such as visualizing migrasome dynamics in mouse liver vasculature (DAOSLIMIT)[2], high-speed vascular imaging in zebrafish (MiSLFM)[11], and large-scale neural activity mapping in awake mice (csLFM)[12].

plus single-cell neural responses under multisensory stimulation and cross-day representation drift across brain regions[14].

Despite sLFM's advancements, imaging depth remains a bottleneck, crucial for investigating processes in intact organs. A promising solution is using long-wavelength or multi-photon excitation to enhance penetration. Additionally, machine learning (ML) methods, including virtual sLFM (VsLFM), can capture rapid dynamics without physical scanning[18]. Further ML progress should address motion artefacts and real-time aberration correction[13].

Commercial sLFM systems, integrating these features, are broadening the technology's reach[12]. Though primarily fluorescence-based, incorporating label-free approaches—such as scattering, phase contrast, or autofluorescence—would extend clinical utility. Moreover, new DAO algorithms accommodating varied contrast mechanisms can further widen sLFM's applications.

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