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Editorial: Optical microscopy: advances and applications

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Editorial on the Research Topic
Optical microscopy: advances and applications

Optical microscopy is widely used in biomedical and life science studies nowadays. It allows researchers to observe biological processes at the cellular and molecular levels and offers several advantages over other imaging modalities, such as minimal invasiveness and compatibility with live samples. The last decade has witnessed several dramatic improvements in optical microscopy such as super-resolution, observation of 4D (*xyz* + time) dynamic biological events, and label-free quantitative phase imaging. Single-molecule localization microscopy and stimulated emission depletion microscopy were recognized by the 2014 Nobel Prize for Chemistry [1, 2]. Super-resolution microscopy has been applied to visualize subcellular structures and intracellular events like microtube growth, shrinkage dynamics, and endoplasmic reticulum interaction with microtubes and mitochondria [3]. Light sheet fluorescence microscopy (LSFM) alias selective plane illumination microscopy (SPIM) reveals the zebrafish embryo growth developing events, which were hard to observe previously [4]. Vector beams like Bessel, Airy, and optical vortex (OV) are gradually adopted in optical microscopy to extend the microscopy functionality [5, 6]. Quantitative phase microscopy (QPM) has become a powerful label-free and quantitative imaging tool for transparent live cells [7].

However, optical microscopy still faces challenges, such as the trade-off between resolution, speed, and field of view (FOV), photobleaching and phototoxicity, and time-consuming image reconstruction algorithms. Therefore, researchers are constantly developing new methods and technologies to overcome these limitations and enhance the capabilities of optical microscopy. The articles on this Research Topic aim to address the above challenges and report the latest technological advances, specifically in LSFM, nitrogen fluorescence emissions, OV beam evaluation method, and QPM.

LSFM uses a thin sheet of laser light to illuminate and excite only a thin slice of the fluorescence sample. The fluorescence light is then imaged by an objective lens placed perpendicular to the light sheet. LSFM confines the illumination to the in-focus region of the specimen, minimizing the harmful phototoxicity and photobleaching. Thus, LSFM is a desired microscopy for long-term volumetric imaging of living specimens with high temporal-spatial resolution. Traditional LSFM uses a Gaussian beam with a cylindrical lens for light-sheet illumination, but the light sheet is not always flat and thin in the FOV [4]. Recently, Bessel and Airy beams were introduced in LSFM due to their non-

diffraction characters and capability of FOV extension. However, the side lobes of Bessel and Airy beams significantly deteriorate the image signal-to-noise ratio. To address this shortage, Gu et al. proposed multiple Airy beams (MAB) based LSFM in this Research Topic. The MAB light sheet energy is more concentrated on the focal plane of the detection objective than that of a regular single Airy light sheet, obviously suppressing the side lobes and increasing the image contrast. In addition, the axial resolution can be further improved with a complementary beam subtraction (CBS) method. The CBS-MAB study provides an LSFM without a trade-off between high resolution and wide FOV.

Nitrogen fluorescence emissions are a phenomenon where nitrogen molecules emit light after being excited by an external source such as cosmic rays or laser beams. The predominate emission wavelengths at 337 nm and 391 nm provide valuable clues to identify the formation mechanism of the molecular excited states, which can be used to produce high-intensity lasers and study the properties of the atmosphere and cosmic rays. Recent studies found that the OV beam used as the external source can affect the population of vibrational levels of electronic states, the emission transition probabilities, and the interaction between electrons, molecules, and ions through the adjustable topological charges (TCs). Thus, the OV beam offers a unique alternative to studying nitrogen fluorescence emissions and revisiting the mechanism behind it. Chen et al. showed an experimental investigation on nitrogen fluorescence emissions pumped by a single 800 nm femtosecond OV beam with different TCs. They found that the 391 nm emission decreased sharply while the 337 nm emission decreased slightly as the TCs increased. Moreover, the 337 nm emission exhibits a nearly linear variation with the pulse energy for different TCs. In contrast, the dependences of the 391 nm intensity on pulse energy have an abrupt change when changing the TCs. The two emissions also exhibit different dependences on the nitrogen gas pressure in different TCs. These observations suggest that the OV beam is a flexible toolkit to manipulate nitrogen fluorescence emissions.

Including the nitrogen fluorescence emissions study, OV beams are widely used in many microscopy research and applications due to the singular properties of spiral phase and polarization distributions. Providing real-time and accurate measurements for the OV beam is vital. However, the traditional scanning-based measurement approach is time-consuming, and the measurement efficiency is too low to realize high-speed and large-area measurements. To solve this problem, Qiu et al. proposed a real-time phase distribution and dynamic evolution method of OV via digital holography. The experiment was conducted on a modified Mach–Zehnder interferometer and the OV light field was generated using an acoustically induced fiber grating. They continuously recorded the holograms of the optical vortex using a CCD camera and numerically reconstructed the phase maps. More importantly, although the CCD frame rate limits the real-time acquisition, digital holographic wavefront reconstruction can recover the missing frames from the wavefront evolution. By serializing these phase maps over time, the propagation and evolution of the spiral phase structure of the OV beam can be demonstrated in real-time.

QPM can visualize transparent samples quantitatively with high contrast as a label-free and noninvasive imaging technique. Today, diffraction-based QPM features a compact setup and low cost compared to the traditional wavefront- and interferometer-based QPM. Diffraction-based QPM is non-interferometric and immune to environmental disturbances. However, the phase reconstruction usually requires several raw images as input, and the reconstruction time generally takes seconds to minutes. Wang et al. proposed a deep learning-based QPM approach (DLQPM), which can reconstruct a phase image from a single defocused bright-field intensity image by using a U-net. Sufficient training data pairs are collected in an environment reproducing the practice experimental conditions by inserting a spatial light modulator into an off-axis digital holography microscope. The authors quantitatively analyzed the effect of defocusing distance on the phase recovery performance of DLQPM. The results show that the phase reconstruction quality increases with the defocusing distance until it becomes saturated after a defocusing distance of 3 cm. Moreover, DLQPM is also suitable for bright-field microscopes equipped with partially coherent or incoherent illumination; in that case, the training data pairs can be obtained with single-beam phase retrieval approaches.

In short, this Research Topic demonstrates the start-of-art developments in LSFM, nitrogen fluorescence emissions, OV beam evaluation method, and QPM. I hope this Research Topic attracts readers in multiple disciplines, and I believe that future efforts from optics, chemistry, computer science, and biomedical demands will promote optical microscopy to become more functional, less costly, and easier to use.

Author contributions

DD: Writing–original draft.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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