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25th Anniversary of STED Microscopy and the 20th Anniversary of SIM: feature introduction

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Abstract: This feature issue commemorating 25 years of STED microscopy and 20 years of SIM is intended to highlight the incredible progress and growth in the field of superresolution microscopy since Stefan Hell and Jan Wichmann published the article Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy in Optics Letters in 1994.

This feature issue commemorating 25 years of STED microscopy and 20 years of SIM is intended to highlight the incredible progress and growth in the field of superresolution microscopy since Stefan Hell and Jan Wichmann published the article *Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy* in Optics Letters in 1994 [1]. Only a few years after that, Tony Wilson's group published the first paper to use structured illumination for optical sectioning [2]. And in 1998, Rainer Heintzmann and Christoph Cremer presented their idea for using laterally modulated excitation to improve resolution in fluorescence microscopy. The next year, the conference proceeding was published [3], and, the year after that Mats Gustafsson demonstrated a factor of two resolution improvement, referring to "structured illumination microscopy" (SIM) for the first time [4].

Since the first paper, STED has been improved and refined. The first STED paper has now been cited 2,849 times, and last year 314 papers were published on STED. STED microscopy has now been commercialized and is used not only for live cell superresolution imaging [5], but also for novel applications such as laser lithography [6]. Rather than use stimulated emission to turn off fluorescence, RESOLFT takes advantage of reversibly photoswitchable fluorescent proteins, extending the STED concept to lower light levels more amenable to live cell imaging [7]. STED has also been massively parallelized to make live imaging over large fields of view possible [8]. STED has been combined with adaptive optics [9,10] and light sheet imaging [11]. STED microscopy has enabled video rate imaging of the synapse [12] and live imaging in mouse brains at better than 70 nm resolution [13]. In this issue, we have an invited paper on the modelling and characterization of RESOLFT imaging and resolution [14], and Hernandez *et al.* discuss an improved approach to combining STED with light-sheet microscopy [15].

Mats Gustafsson's original SIM paper has been cited 1,541 times. Last year, 303 papers were published in OSA journals referring to structured illumination microscopy techniques. There are now several successful commercial SIM instruments. After the initial work on 2D SIM, the technique was extended to three-dimensions [16], live imaging [17–19], and even higher resolution [20,21]. Mats Gustafsson developed a multifocal widefield microscope, and this technique has been combined with SIM [22]. The Lattice Light Sheet Microscope is a highly successful combination of SIM with light sheet imaging [23]. SIM has been combined with Adaptive Optics [24–26], and SIM concepts have been applied to phase imaging and to

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Biomedical Optics EXPRESS

fluorescence imaging with speckle illumination instead of conventional structured illumination patterns [27–29]. SIM has been used to image the nuclear pore complex [30], the synaptonemal complex [31], and live cytoskeletal dynamics [19,32]. In this issue, James Manton *et al.* present a novel concept for increasing axial resolution in SIM [33].

In 2006, PALM [34], FPALM [35], and STORM [36] were introduced. These papers have now been cited collectively over 10,000 times. These techniques spawned a plethora of acronyms and are now frequently but not always collectively referred to as single molecule localization microscopy (SMLM). SMLM has been used to study the cytoskeletal structure of the axon [37], the spatial organization of chromosomes [38], the structure of bacteria [39]. SMLM techniques have become increasingly sophisticated. Multi-emitter fitting now enables faster imaging with higher labeling densities [40], and single-particle reconstruction allows the structure of molecular machinery to be determined from 1000s of SMLM images [41,42]. SMLM has been optimized and extended in a multitude of ways including extending the axial range with PSF engineering [43,44], PSF engineering for multicolor imaging [45,46], optimizing blinking rates [47], light sheet illumination to reduce background [48,49], doughnut spot based triangulation for increased precision with minimal photon flux [50], and increasing the resolution with structured illumination [51,52]. Deep learning has become an important tool in superresolution microscopy, and there are several papers on the use of deep learning in SMLM [53,54]. In an invited article in this issue, deep learning is reviewed by Möckl *et al.* [33]. Along with these new developments in superresolution microscopy have come important and necessary developments in image evaluation as well. With the rise of superresolution microscopy, it has become increasingly important to quantify the resolution of an image in an objective manner, and Fourier Ring Correlation has become the accepted standard for this [55].

In this collection there are 4 articles on SMLM. Philipp Zelger *et al.* describe a new method for axial localization [56]. Sha An *et al.* discuss a method for SMLM along axial cross-sections [57]. Arne Bechensteen *et al.* write on a new algorithm for single molecule localization [58], and Marijn Siemons *et al.* look at astigmatism based localization [59].

Other forms of superresolution microscopy have been developed as well including SOFI [60] and MUSICAL [61]. We have papers on MUSICAL by Sebastian Acuna *et al.* [62] and papers on SOFI by Benjamien Moeyaert *et al.* [63] and Xiyu Yi and Shimon Weiss [64]. A paper on resolution beyond the diffraction limit using deep learning in infrared spectroscopy by Kianoush Falahkheirkhah *et al.* [65], and on a cost-efficient laser source for superresolution microscopy by Daniel Schröder *et al.* [66] round out the collection.

Disclosures

The authors declare that there are no conflicts of interest related to this article.

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