Image scanning microscopy: Novel developments and applications

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The past 20 years have seen a revolution in optical microscopy, with the invention of a multitude of new superresolution methods and techniques, such as STED, PALM, STORM, or SOFI. They all provide resolution improvement beyond the diffraction classical limit, but require special labels and/or complex setups. However, within the realm of linear, diffraction-limited optics, a clever combination of structured illumination with wide-field imaging can already provide a lateral resolution improvement of nearly two times, without any need of special labeling or sample preparation. The first experimental realization of this concept was Structured Illumination Microscopy (SIM), which by now is a successfully commercialized and widely applied technique. In SIM, one illuminates the sample with a spatially modulated excitation intensity distribution, and the emerging fluorescence is imaged with a conventional wide-field imaging setup. By moving and rotating the excitation intensity distribution pattern in different positions and orientations, taking each time a wide-field image, a final fluorescence image is composed which has roughly double the resolution (laterally) of a conventional wide-field or a confocal laser scanning image alone. However, it requires complex image acquisition hardware and sophisticated data reconstruction procedures, which easily fail for non-prefect samples or imaging conditions. Moreover, a combination of SIM with non-linear microscopy techniques is rather challenging. As an alternative to SIM, we have recently developed Image Scanning Microscopy (ISM) which is based on a theoretical idea that was developed by Colin Sheppard already in 1988. In ISM, the focus of a conventional laser-scanning confocal microscope (LCSM) is scanned over the sample, but instead of recording only the total fluorescence intensity for each scan position, as done in conventional operation of an LCSM, one records a small image of the illuminated region. The result is a four-dimensional stack of data: two dimensions refer to the lateral scan position, and two dimensions to the pixel position on the chip of the image-recording camera. This set of data is then used to obtain a super-resolved image with doubled resolution, completely analogous to what is achieved with SIM. However, ISM is conceptually and technically much simpler, suffers less from sample imperfections like refractive index variations, and can easily be implemented into any existing LSCM. Meanwhile, many different variants of ISM have been developed and successfully implemented. In my presentation, I will report about the foundations and latest developments of ISM, from single focus to multi-focus schemes, from software-based to real-time all-optical implementations. Finally, because ISM uses the same excitation modality as a LSCM, one can use multiphoton excitation in ISM with the same ease as in a conventional LSCM. This makes ISM also ideally suited for non-linear optical microscopy including coherent microscopy such as SHG, THG, or even CARS.