Nanoscopy On-a-Chip

Ultra Large Field-of-View Super-Resolution Microscopy

The German Physicist Ernst Abbe has discovered the fundamental diffraction limit of optical resolution in 1873. Even being depicted on his grave, this relation was carved in stone for about 100 years until scientists started to reveal how to trick physics and build microscopes that enable much better resolutions than fixed by Abbe’s famous formula. The importance of these developments is underlined by 2014’s Nobel Prize in Chemistry being awarded to Eric Betzig, William E. Moerner, and Stefan W. Hell. Such approaches to super-resolution fluorescence microscopy [1], also termed “nanoscopy”, exploit dynamics of either the illumination of the sample [2-4], the fluorescent probes [5-10], or both [11-13] to increase the resolution.

Several of today’s most widespread techniques rely on the temporal separation of individual fluorophores by making them blink, i.e. by switching the fluorophores between a fluorescent “on”-state and a non-fluorescent “off”-state. The coordinate of each emitter can then be localized with precisions up to a few nanometers. In this manner, millions of localizations from several hundred to thousand raw images can be used to reconstruct the super-resolved image at a resolution down to 20 nm. The key to these techniques is the ability of making the probes blink, for instance by the use of switchable fluorescent proteins as in (F)PALM [5, 6], organic dyes as in dSTORM [7, 8] or transient binding as in (DNA)PAINT [14, 15]. The extremely high resolution of these realizations comes with the cost of long acquisition times because up to tens of thousands of frames need to be recorded to reconstruct one image. Accordingly, low throughput is one of the main drawbacks of localization microscopy. However, the use of modern scientific CMOS cameras in principle allows for simultaneous imaging of more than 2000 x 2000 pixel. This enables fields-of-view (FOV) of 200 µm x 200 µm and more that can fit many samples, e.g. cells or bacteria, at once. Now the sample illumination is becoming a new bottleneck as this large area has to be illuminated simultaneously. The use of sufficiently high powered lasers in combination with either free-space [16] or fiber-based [17] beamshaping has enabled dSTORM imaging in epi-illumination on FOV with up to 221 µm width and height.
However, many researchers tend to use total internal reflection fluorescence (TIRF) illumination because of TIRF illumination because of the excellent optical sectioning capability of the evanescent field and significantly reduced background signal that favors higher single molecule localization precisions.

**Results**

We present an approach to localization microscopy that further increases the FOV and, hence, throughput by more than a factor of four to 500 µm x 500 µm. Also, it provides TIRF excitation over almost arbitrary large areas in a power-efficient way. Together with the Arctic University of Norway in Tromsø, we have developed nanoscopy based on waveguide chips [18]. The laser is coupled from the side into a planar waveguide made out of a high refractive index material (fig. 1). While most of the light is guided inside the waveguide, a part of the light field leaks out generating an evanescent field that can excite fluorescence in a TIRF-like manner. The fluorescent signal is then collected by an orthogonal beam path. In contrast to other implementations of TIRF microscopy, which use high magnification/high NA lenses that restrict both, the FOV and the illuminated region of the sample, almost arbitrary objective lenses can be used in the waveguide-based implementation. Using a water immersion lens with 60x magnification, uncompromized resolution can be achieved with dSTORM imaging, here demonstrated for the cytoskeleton of liver cells (fig. 2, right). The FOV size of 70 µm x 70 µm already exceeds that of many commercial localization microscopes.

The planar waveguides are highly multi-moded. This results in an inhomogeneous illumination of the sample that could obscure the observed structure. We compensate for this effect by scanning the input facet of the waveguide, which changes the mode distribution and virtually averages out the inhomogeneity during the dSTORM measurement. But it is also possible to harness this fluctuating illumination of the sample: Recording about 200 frames while constantly changing the illumination pattern enables the use of a statistic analysis in entropy-based
super-resolution imaging (ESI) [10]. The computation is performed on a pixel-basis and does not require molecule blinking in contrast to the fitting of separate emitters as in dSTORM. Hence, image acquisition is significantly sped up at the cost of a lower resolution. Figure 2 shows the incremental resolution enhancement when using diffraction limited imaging (left), ESI (center), and dSTORM (left) on the same sample.

If a larger FOV is desired, it is possible to switch the objective lens for the detection. The sample illumination is not affected by this, so the waveguide also allows for imaging of the same sample at different magnifications. For instance, a low magnification image can be used to gain an overview and identify cells of interest, which can then be imaged at higher magnification and best possible resolution. However, some scenarios do not require the ultimate resolution, but instead looking at many cells at once. In this case, the chip-based approach offers to choose the tradeoff between resolution and FOV size. Recording raw data with a 20x/NA 0.45 objective lens results in a dSTORM image with a resolution on the order of 140 nm, which is still well below the diffraction limit. However, the biggest advantage in this case is the imaging of more than 50 cells at once (fig. 3). Realizing a similar sample size on conventional systems would require the recording of up to 50 single images, thus, the throughput is tremendously increased by our waveguide chip-based platform.

Furthermore, the setup complexity is dramatically reduced as illumination and detection beam paths are decoupled completely. This also allows for retrofitting of existing microscopes for chip-based imaging and making nanoscopy available for a wider range of users. Complementing the current trend of integrating more and more photonic features on-chip, future developments towards on-chip laser generation and steering of entire illumination systems will potentially further extend the capabilities. Combinations with different lab-on-a-chip methods, e.g. microfluidics, optical trapping, and other detection techniques, become also straightforward to implement.

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