A New Twist for 3D-SIM

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We have developed a novel concept for superresolution microscopy based on structured illumination with a hexagonal illumination pattern (for details see the presentation by Schropp and Uhl). Rather than using a laminar pattern, which illuminates the whole field of view simultaneously, we create the pattern dynamically on the sample and make it vanish again while a pattern bar is continuously scanned over the sample. This way only a small fraction of the sample is illuminated at a given time, and by synchronizing the rolling shutter of an sCMOS camera in such a way that only light from the transiently illuminated area contributes to the continuously building up image, most light scattered from other areas of the sample is removed optically and does not need to be removed mathematically. This results in a greatly increased signal modulation depth and, through a greatly improved signal-to-noise ratio in a significantly improved image quality. The approach also enhances the penetration depth, which usually limits the effectiveness of SIM in deeper layers of a cell or tissue.

Our approach permits the acquisition of a 3D cellular map of 100,000 µm³ with superresolution in less than 10 s.