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PHOTONICS

WHITEPAPER

SINGLE-MOLECULE LOCALISATION MICROSCOPY (SLMS)

How to overcome the Abbe diffraction limit?

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The Challenge

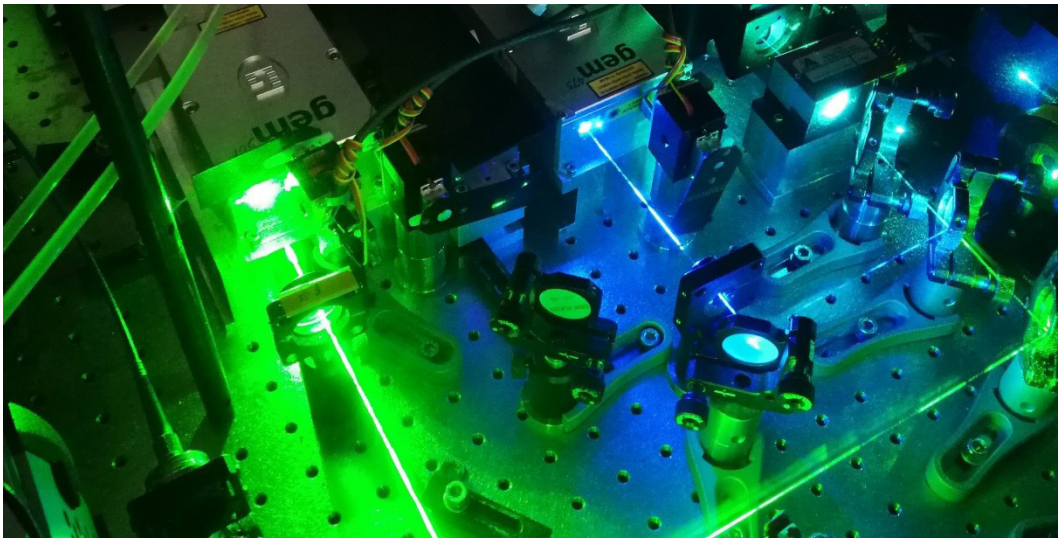
The Abbe diffraction barrier, which fundamentally restricted the lateral optical resolution to 200 – 300 nm until the early 1990s, can be overcome by super-resolution optical fluorescence imaging techniques. These techniques have been so groundbreaking that the Nobel Prize 2014 in Chemistry was awarded to Eric Betzig, Stefan Hell and W. E. Moerner “for the development of super-resolved fluorescence microscopy”.

Background

There are two different techniques commonly used so far; one is based on point spread function engineering, the other on single-molecule localization. Stefan Hell’s stimulated emission depletion (STED) microscopy belongs to the first class and is a so called deterministic super-resolution technique. In contrast, Eric Betzig’s *photoactivated localization microscopy* (PALM) is a stochastic technique.

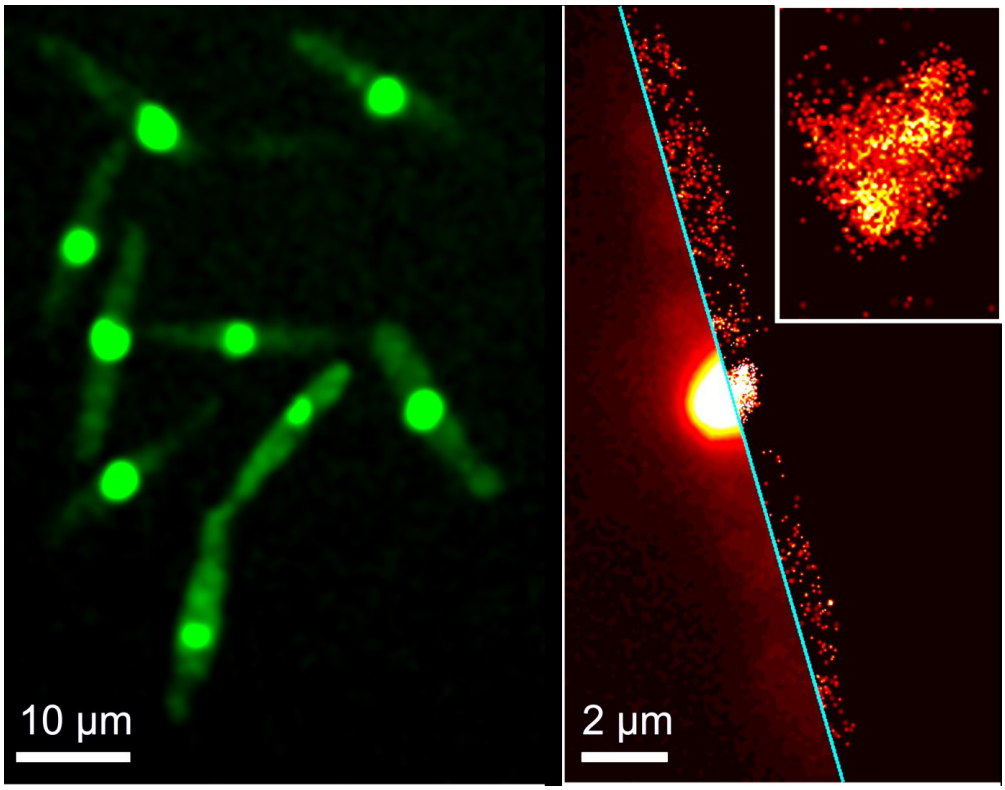
How does SMLM work?

The fundamental principle of all SMLM implementations is the random photoactivation and subsequent localization of individual fluorescence emitters by detecting their photon emission at a certain position and time in the complete absence of emission from neighboring emitters. The centroid of the diffraction-limited distribution of photons is a measure of the location of the emitter and is far better defined than the photon distribution itself. Thus, whereas a standard widefield image is an overlay of the photon distributions from a large number of emitters, a super-resolved SMLM image is a map of their individual locations that can yields lateral resolutions down to 5 nm.



Photograph of the laser compartment of a widefield microscope for SMLM with the beam paths of the gem 473 (blue) and gem 561 (lime). Courtesy of G. U. Nienhaus and A. Kobitski (KIT)

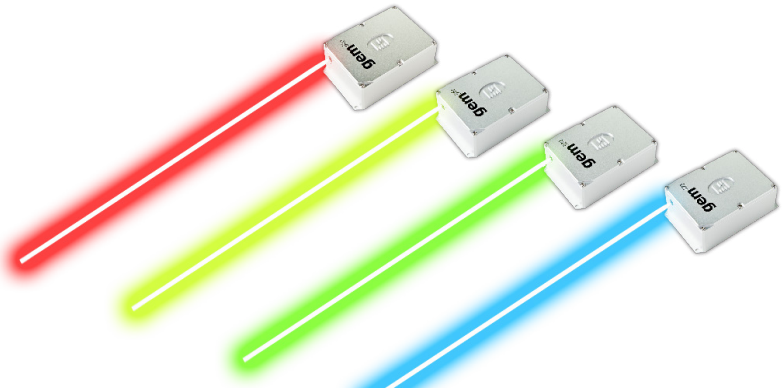
But how can one ensure that only a few fluorophores emit at a certain time making individual localization possible? Let us focus on the strategy used in PALM, which exploits specific properties of fluorophores that spontaneously form in photoactivatable fluorescent proteins (paFPs) of the green fluorescent protein (GFP) family. The key advantage of these GFP-like proteins is that they are genetically encodable, so that a cell endowed with this gene produces these markers itself with no further staining procedures required. Typically, weak laser light at 405 nm is chosen to photoconvert a few paPFs from green- to red-fluorescent emitters during acquisition of a single camera frame. Red fluorescence is typically excited with a 561 nm laser. The red emission appears then as a small number of isolated, diffraction-limited spots in the red color channel during each camera frame. Photobleaching quickly removes the red-converted paFPs, so new emitters can be activated and detected during acquisition of the next camera frame. Careful adjustment of the experimental parameters (laser powers, camera dwell times) ensures that the probability of detecting two fluorophores with spatially overlapping intensity distributions is almost zero within a single camera frame.



SMLM image of a selected cell with red-converted protein; the inset shows a close-up around the cell nucleus. SMLM images were rendered from 1000 successive camera frames taken with 561 nm illumination for fluorescence excitation and an additional weak 405 nm illumination for green-to-red photoconversion of the protein. Images taken from: Lu Zhou et al.: Cytoplasmic Transport Machinery of the SPF27 Homologue Num1 in Ustilago maydis, Sci. Rep. 8 (2018) 3611, http://creativecommons.org/licenses/by/4.0/

One supplier for all wavelengths

Unlike confocal microscopy, widefield imaging techniques require illumination of a larger area with constant high intensity. A key aspect in SMLM are lasers emitting at suitable wavelengths (typically 405, 473, 561 and 640 nm) in order to excite (and possibly photoactivate) the fluorescent molecules employed for visualization.



The Laser Quantum gem laser series with its 4 most popular wavelength (473, 532, 561 and 640 nm).

For fluorescence excitation of paFPs, high laser powers in the range of 500 mW are beneficial. The gem lasers from Laser Quantum offer these or even higher powers at various wavelengths (473, 532, 561, 640, 660 and 671 nm) with near perfect beam quality in a compact, cost-efficient platform. The high powers also allow advanced beam shaping techniques since power is not the limiting factor as with diode lasers.

Novanta Benefits

Novanta is uniquely positioned to solve even the most complex challenges for OEMs, system integrators, and end-use customers seeking to advance their manufacturing processes with high precision laser systems. With some of the most well-known brands in the industry and in-country application and service support, Novanta delivers reliable, precise, and durable components and sub-systems.

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