New method gives microscope a boost in resolution

December 12 2018

Credit: CC0 Public Domain

Scientists at the University of Würzburg have been able to boost current super-resolution microscopy by a novel tweak. They coated the glass cover slip as part of the sample carrier with tailor-made biocompatible nanosheets that create a mirror effect. This method shows that localizing single emitters in front of a metal-dielectric coating leads to higher precision, brightness and contrast in Single Molecule Localization Microscopy (SMLM). The study was published in the *Nature* journal *Light: Science and Applications*.

The sharpness of a light microscope is limited by physical conditions—structures that are closer together than 0.2 thousandths of a millimeter blur, and can no longer be distinguished from each other. The cause of this blurring is diffraction. Each point-shaped object is therefore not shown as a point, but as a blurry spot.

With mathematical methods, the resolution can still be drastically improved. One method would calculate its exact center from the brightness distribution of the blurry spot. However, it only works if two closely adjacent points of the object are initially not simultaneously but subsequently visible, and are merged later in the image processing. This temporal decoupling prevents superimposition of the blurry spot. For years, researchers in life sciences have been using this tricky method for super high-resolution light microscopy of cells.

One such method was developed by the research group of Prof. Dr. Markus Sauer at the University of Würzburg: direct stochastic optical reconstruction microscopy (dSTORM). This powerful SMLM technique can provide a lateral resolution of ~ 20 nm. For this purpose, certain structures, for example, pores of a cell nucleus, are stained with fluorescent dyes. Each of the dye molecules blinks at irregular intervals and represents part of the pore. The image of the nuclear pores is therefore not initially visible, but arises after the image processing by the superposition of several thousand images. With the dSTORM technique, the resolution of a conventional light microscope can be increased by a factor of 10. "It allows us to visualise the architecture of a cell down to its molecular level, for example," explains Hannah Heil. The researcher is doing her doctorate at the Rudolf Virchow Center of the University of Würzburg in the group of Prof. Katrin Heinze.

However, the photon statistics define a virtual resolution limit in resolution. To address this issue, Katrin Heinze had the idea to use relatively simple biocompatible nanocoatings to boost the signal. In a joined effort with Markus Sauer and colleagues from the faculty of Physics, Hannah Heil designed and fabricated metal-dielectric nanocoatings that behave like a tunable mirror. It almost doubles the resolution.

Mirror, mirror on the wall: Which image is the sharpest of them all?

During the observation, they placed the cells on a vapor-deposited a coverslip with a thin reflective nano-coating consisting of silver and transparent silicon nitrite. The coating is biocompatible, so it does not damage the cell. With this method, the two groups achieved two effects: The mirror reflected the light emitted to the microscope, which increased the brightness of the fluorescence signal and thus also the effective image sharpness.

Second, the emitted and the reflected light waves are superimposed. This creates so-called interference. Depending on the distance to the mirror, the light is amplified or attenuated. "In this way, we primarily see structures in a certain image plane," says Heil. "Everything that is above or below and could possibly disturb the image is, on the other hand, hidden." To ensure that the exact parts of the image become visible, the thickness of the transparent layer applied to the mirror must be chosen appropriately. Among other things, Heinze and Heil use computer simulations to tailor the coating according to the object.

Overall, the method is surprisingly easy to use, says Hannah Heil. "That's what I really like about our approach." Prof. Heinze adds, "Except for the cheap metal-dielectric coated coverslip there is no need of any additional microscope hardware or software to boost the localization precision, and thus is a fantastic add-on in advanced microscopy."

More information: Hannah S. Heil et al, Sharpening emitter localization in front of a tuned mirror, *Light: Science & Applications* (2018). DOI: 10.1038/s41377-018-0104-z

Provided by University of Würzburg