Nanoscopy: Shedding Light on Life

Where traditional optical microscopy fails, a new tool, the nanoscope, overcomes the last barrier: the diffraction limit. It can explore the interior of cells in 3D, non-invasively, and with nanometric resolution.

Nanoscopy! Remember this word: it is here to leave its mark. Optical microscopy at the nanoscale is no longer science fiction. Indeed, researchers at the Max Planck Institute for Biophysical Chemistry in Göttingen (Germany), led by Stefan W. Hell, have significatively pushed the 3D resolution of optical microscopy down to the nanoscale, and have been able to non-invasively investigate the interior of cells.

"The resolution of a microscope," Hell explains, "is given by the 3D size of the focal spot, which usually has an elliptical shape, similar to a rugby ball — around 250 nm in the plane parallel to the sample and around 500 nm in the perpendicular plane. We have shown that it is possible to squeeze this ellipsoid to a sphere, downsizing the focus from a rugby ball to a tennis ball — around 45 nm in diameter. The squeezed spot leads to a substantial improvement in resolution which we have used to map out the distribution of proteins in a cellular organelle without needing to chop the cells into slices, as other techniques, such as electron microscopy, would have required."

Since its dawn, mankind has always been fascinated by the unknown. Unluckily, only a tiny part of the much broader natural complexity can be experienced by our physiological perception, the five senses. Curiosity urged our ancestors to open the way to further information by developing dedicated tools, such as microscopes, to empower the sense of sight.

More than three hundred years ago, the Dutchman Antonie van Leeuwenhoek and the Englishman Robert Hooke separately observed the existence of tiny living organisms in drops of water using a rudimentary microscope. Since then giant steps have been taken in the field of optical microscopy.

Unfortunately, an optical microscope, even a perfect one, cannot be used to see objects that are smaller than

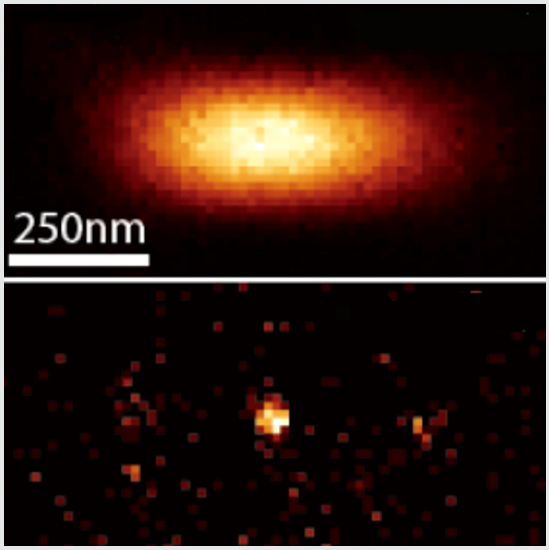

Figure 1: Microscope versus nanoscope. Diffraction-limited 3D focal spot in standard optical microscopy (top) compared to the 45 nm spherical one now achieved in optical nanoscopy (bottom).

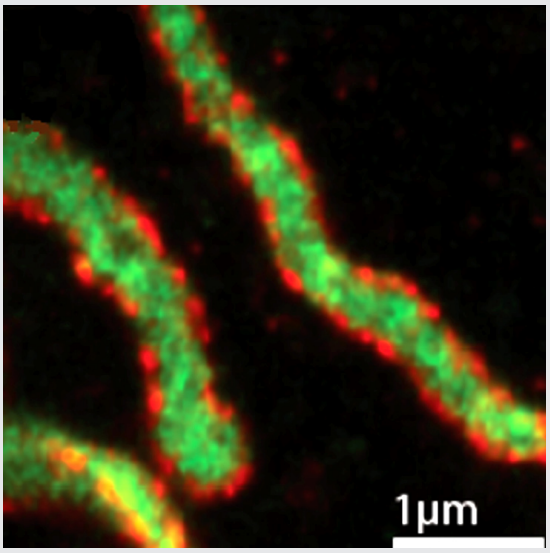

Figure 2: Cell nano-image. Distribution of membrane (red) and matrix (green) proteins in cellular mitochondria.

half the wavelength of the illumination light, a few hundred nanometres. It is a fundamental physical law: the diffraction limit. Have you ever tried to pick up a very thin needle using your fingers alone? Standard microscopy makes use of rather clumsy fingers (the wavelength of light) to discern tiny objects: any object smaller than the diffraction limit is invisible.

We need a more refined tool to break the diffraction barrier and eventually pick up the aforementioned needle. For example, electron microscopy has been widely employed in biology to gain resolution at the nanoscale. Indeed, the wavelength associated with fast electrons is much smaller than that of light. However, these unconventional microscopes have a major drawback: they are typically incompatible with life. As a result, the clumsy fingers of focused light continue to provide us with the most promising way to explore the 3D cell universe non-invasively. The challenge is to make them as little clumsy as possible.

One way to achieve this is by taking advantage of fluorescence, an optical property of some molecules. Fluorescent molecules absorb light at a certain wavelength to emit it again at a longer one. High resolutions have been achieved through several fluorescence-based approaches, but at most in 2D. Hell's group, for example, had previously studied the interaction between some fluorescent molecules and a focused laser beam in the shape of a doughnut. Even though the distribution of light is still diffraction limited, the interaction with the fluorescent molecule is not: instantly quenching the periphery, the excitation is effectively confined only to the hole of the doughnut, which is far below the diffraction limit. It is like placing ice on the rim of a hot plate to cool the edge down whilst leaving the centre hot.

Now Hell and coworkers have found the right recipe to place ice not only on a 2D plate, but in a whole 3D space. They have engineered the interference of laser beams in order to confine the fluorescence to a sphere of 45 nm in diameter. Hell affirms that "in theory this spot and hence the resolution can be squeezed down to the size of a molecule, a few nanometers. However, practical issues will probably limit it to around 10-20 nm."

Hell underlines that "the wavelength of light can be tuned so that it is not absorbed by the sample. Besides, the light intensity is almost three orders below that of multiphoton microscopy, which is nowadays broadly used for live cell imaging." Optical nanoscopy, therefore, would be a powerful tool to visualize and quantify molecular distributions in living cells without concern about biological damages.

At Harvard University (Massachusetts, USA), the group led by Xiaowei Zhuang had already achieved similar results with an alternative fluorescent-based method [1]. However, Lukas Novotny, from Rochester University (NY State, USA), explains that "Hell's technique has less stringent requirements and is faster in terms of video rate, since it can image many fluorescent molecules at once. This, I believe, will make it widely used in future." Bo Huang, from Zhuang's group, mostly agrees with Novotny's opinion, even though he adds that, regarding applications, "no approach will be more successful than others in full generality. Different problems will require different approaches."

[1] B. Huang *et al.*, *Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy*, Science **319**, 810-813 (2008).

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R. Schmidt, C. A. Wurm, S. Jakobs, J. Engelhardt, A. Egner, and S. W. Hell,
Spherical nanosized focal spot unravels the interior of cells,
Nature Methods (2008) **5**, 539-544.