Scientists advance important microscopic technique for biomedical research

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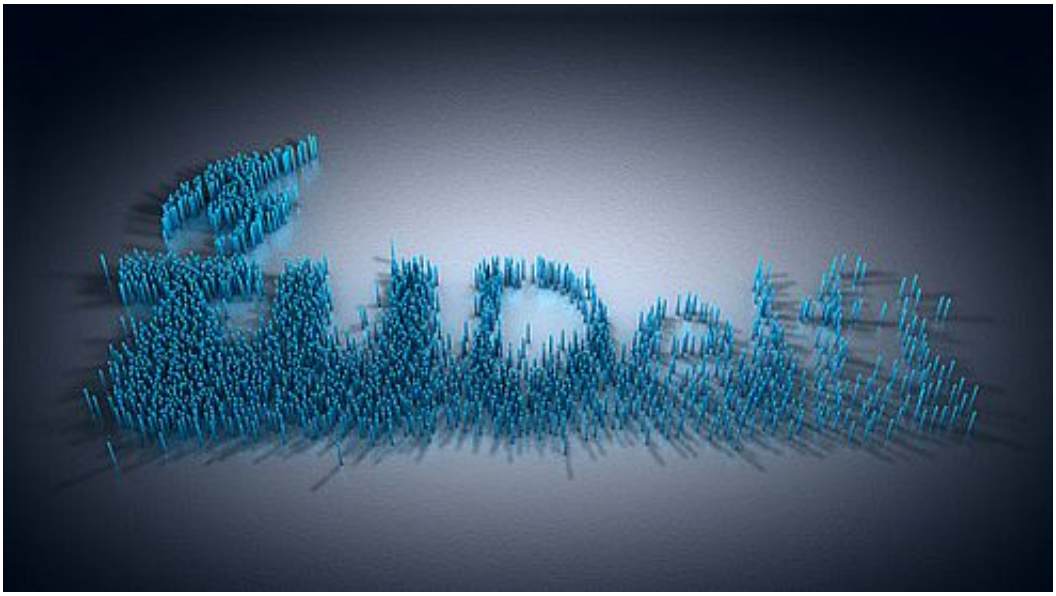

The logo becomes sharper as the pilars are positioned more precisely and the density increases. Credit: Tremani

Scientists at TU Delft have made an important advancement in a new microscopic technique that is widely used in medical research. They demonstrate what the resolution of this localisation microscopy is and how the best resolution can be achieved as quickly as possible. This week their findings are being published online in the scientific journal *Nature Methods*.

Fluorescence microscopy is an important technique in biomedical

research. This method makes it possible to deduce information, for example about the functioning of cells, from the light emitted by certain fluorescent molecules in cells. Fluorescence microscopy used to produce images with resolution ranging from 200 to 300 nanometres. In recent years, however, scientists have employed a trick that allows you to view images around ten times sharper: localisation microscopy. This technique makes it possible to obtain much better and much more informative images of the interior of the cell.

Localisation microscopy involves analysing the light of single molecules in several places. This is repeated for many molecules in succession. When the data from these individual molecules is combined, a much clearer picture emerges.

One number for the resolution

"Everyone started using the new technique. In practice, however, these great localisation-microscopy resolutions of 20 to 30 nanometres could not be obtained quickly", notes researcher Dr Bernd Rieger of TU Delft. "The emission of fluorescent light by the molecules is a statistical process that is thus partly determined by chance. One consequence is the need for highly complicated calculations. Fellow researcher Dr Sjoerd Stallinga, PhD candidate Robert Nieuwenhuizen and I wondered what would be really a feasible with this technique.

The resolution achieved depends upon the uncertainty in the location of labelled fluorescent molecules, the density of the applied labels and the shape of the sample under investigation. Nieuwenhuizen explains, "Until recently, there was no practical, integrated method for considering all of these factors. Now we are able to do that. We can derive a single number directly from the images, which indicates the resolution achieved. We do this using a statistical-mathematical analysis known as Fourier Ring Correlation".

Recipe

"We also provide a kind of recipe for localisation microscopy", Stallinga adds. "Our approach makes it possible to compare the resolution of images taken with different nanoscopic methods. It also makes it possible to optimise and rank several methods, in addition to determining when sufficient data have been obtained to produce a good image. In this way, we show how to achieve the best resolution as quickly as possible".

For this publication, the TU Delft scientists collaborated with the NKI, the German Max Planck Institute, the University of New Mexico and the University of Massachusetts. The study received financial support from STW.

The researchers published their findings in the online edition of *Nature Methods* on 28 April.

More information: Measuring resolution in optical nanoscopy, www.nature.com/nmeth/journal/v … full/nmeth.2448.html

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