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Article:
Curd, A. orcid.org/0000-0002-3949-7523 (2023) Cocktails of tags enhance resolution of microscopy technique. Nature, 617 (7962). pp. 681-682. ISSN 0028-0836

https://doi.org/10.1038/d41586-023-01632-7

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Getting to the points

Reinhardt and colleagues have developed a new light microscopy technique that can distinguish tiny objects that are within 1 nanometre (1 millionth of a millimetre) or several atoms apart from each other [Ref in Nature issue]. They have visualised adjacent points along the DNA double helix and proteins in cells found in pairs that other methods have struggled to see. This could lead to biologists probing the organisation of complex arrangements of the molecules of life in greater detail than before, within their native environment of the cell.

For over 100 years, light microscope builders understood that there was a limit to the detail we could see in an image, no matter how much we magnified it, or zoomed in. This happens because of the way light waves spread out after they are confined, for instance by the aperture of a lens. It prevented us from seeing the detailed arrangements of the living machinery within cells, which works at a scale well beyond this limit. However, over the last couple of decades, researchers have invented ways of working around these rules.

One important group of techniques revolves around labelling all of the copies of a molecule of interest with a fluorescent tag, and causing these tags to light up only a few at a time. The positions of their individual blurred image spots can be calculated and stored much more precisely than is possible when all of the molecules are visible at the same time. Over many repetitions of this process, the positions of many of the molecules of interest can be found, and their arrangement reconstructed from combining all of the precise positions in one dataset. This is known as localisation microscopy [SMLM REF].

Even in localisation microscopy, there is a precision limit, which is largely determined by the brightness of the 'blink', when one of the tags for a molecule lights up for a brief time. That means that it is still a significant achievement to be able to distinguish particular molecules, or rather their tags, that are within about 10 nanometres of one another. These researchers, however, found a way to advance such resolution down to below 1 nanometre. By labelling molecules this close to each other with different tags, they could determine which molecule and tag was the source of a particular blink. Next, they could collect multiple blinks from each one and find the average position of each set of multiple blinks with a precision much better than we can obtain for a single blink, sufficient to find the two separate molecular positions (or rather the positions of their tags) and the distance between them. In particular, their labelling method of choice, called DNA-PAINT [DNA-PAINT REF], gave them both completely clear differentiation of which blinks came from which molecular tag and many blinks from each tag, the two requirements for what they term "RESI" (Resolution Enhancement by Sequential Imaging) (**Figure**).

Figure: Resolution Enhancement by Sequential Imaging (RESI) allows the resolution of molecules at distances shorter than previous light microscopy techniques, including localisation microscopy. When the two molecules share a common labelling tag, conventional localisation microscopy cannot distinguish them. Labelling the molecules differently and detecting them separately reveals them and their different positions.

Demonstrations in their paper include seeing distinct pairs of tags a few atoms apart, less than 1 nanometre, along an engineered structure made of DNA. When labelling less controlled structures in real biological cells, they could use four different labelling tags to randomly label proteins about 10 nm apart in a pair, so that they would usually (in about 75% of the pairs) be distinguishable and precisely located in 3D. Finally, they labelled and imaged CD20, a molecule targeted by a cancer drug, Rituximab. They showed that RESI begins to provide new information about the way CD20 is organised within cells and how that arrangement is affected by treatment.

These experiments demonstrate improvements in imaging static arrangements of molecules compared with other impressive recent innovations [Masullo review]. However, another localisation technique called MINFLUX [MINFLUX Balzarotti] has come near to this ability to image detail but can also precisely track the movement of tagged single molecules, if they are very well separated from any other fluorescent molecules [MINFLUX Deguchi].

There is still room to improve light microscopy beyond these new capabilities. In common with localisation microscopy in general, the structures must stay still for long enough to get precise information across the field of view – over 100 minutes in this case to find molecules over a square smaller than 1/10 millimetre. This means that the cells must be “fixed”, which typically also introduces some level of structural distortion, and their dynamic processes cannot be visualised over time. The size of the tags that light up to provide the position information also becomes a limiting factor at these length scales, as it is the position of the fluorescent part of the tag that is found, not the interesting part of a molecule it is connected to. These current limitations are common across high-precision microscopy and work on them is ongoing.

More interesting for RESI: in samples where we need to label the molecules of interest by sending in a cocktail of different tags to distinguish them (say tags A, B and C), these tags will randomly land on the molecules of interest, which means the nearby molecules in cells will not always be resolvable. A pair of molecules labelled A, B could be resolvable, whereas a pair labelled A, A, which would often happen by chance, would not be resolvable. Reinhardt and colleagues do explore this issue and show how a greater number of distinguishable tags results in more frequent distinguishability of molecules.

It will be fascinating to see what tiny length scales light microscopy will go on to give access to, perhaps using more of these distinct labels, smaller labels and developing to image over a greater volume in a cell. On the hand, work will also press on to try to find these molecules and their arrangements, not only when they must be stopped in their tracks to image over minutes and hours, but when they are free to go about their business in cells and reveal ever more to us about the mechanisms of healthy life and disease at the smallest scale.

[Ref in Nature issue]

[SMLM REF] Lelek, M. et al. Single-molecule localization microscopy. Nat Rev Methods Primers 1, 39 (2021). https://doi.org/10.1038/s43586-021-00038-x

[DNA-PAINT REF] Schnitzbauer, J., Strauss, M., Schlichthaerle, T., Schueder, F. and Jungmann, R. Super-resolution microscopy with DNA-PAINT. Nat Protoc 12, 1198–1228 (2017). https://doi.org/10.1038/nprot.2017.024

[MASULLO Review] Masullo, L.A., Szalai, A.M., Lopez, L.F. and Stefani, F.D. Fluorescence nanoscopy at the sub-10 nm scale. Biophys Rev 13, 1101–1112 (2021). https://doi.org/10.1007/s12551-021-00864-z

[MINFLUX Balzarotti] Balzarotti, F. et al. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science 355, 606-612, doi:doi:10.1126/science.aak9913 (2017).

[MINFLUX Deguchi] Deguchi, T. et al. Direct observation of motor protein stepping in living cells using MINFLUX. Science 379, 1010-1015, doi:doi:10.1126/science.ade2676 (2023).