MOLECULAR IMAGING

Seeing the target

> This discrete molecular imaging technique enables … the highest resolution optical imaging for molecular clusters to date

Individual molecules in densely packed clusters commonly found in biological environments are difficult to see using conventional and super-resolution imaging techniques. Super-resolution microscopy using fluorescent probes has enabled the imaging of structures as small as 10–20 nm; however, in biological and synthetic biomolecular systems, even smaller sizes need to be imaged to reveal individual molecules and interesting structural information.

Now, the optical imaging of synthetic DNA nanostructures with feature sizes of 5 nm (that is, the size of a small protein) is reported by Peng Yin and co-workers in *Nature Nanotechnology*. Using DNA-PAINT (points accumulation for imaging in nanoscale topography) — a technique that relies on transient binding events between DNA docking strands and dye-labelled imager strands — the slight offset of DNA strands could be observed with ångström-scale precision. "This discrete molecular imaging technique enables scientists to clearly visualize densely packed molecular targets, showing the highest resolution optical imaging for molecular clusters to date," says Yin. "We are able to visualize molecular targets that have been densely packed on a 5 nm by 5 nm grid." They also achieve multiplexed imaging by observing three distinct colours on the grid.

Yin and co-workers tackle the factors — which they call the 'three blinking requirements' — that, if addressed, could enhance the imaging capabilities of fluorescence super-resolution microscopy. First, a high photon count per molecular target is needed for precise localization of the DNA strands. Second, a large number of blinking events per molecule is required to achieve a high signal-to-noise ratio. Third, a low number of false localizations as a result of double blinking events is required. These three blinking requirements all benefit from longer imaging times, and thus there is a trade-off between these spatial and temporal constraints.

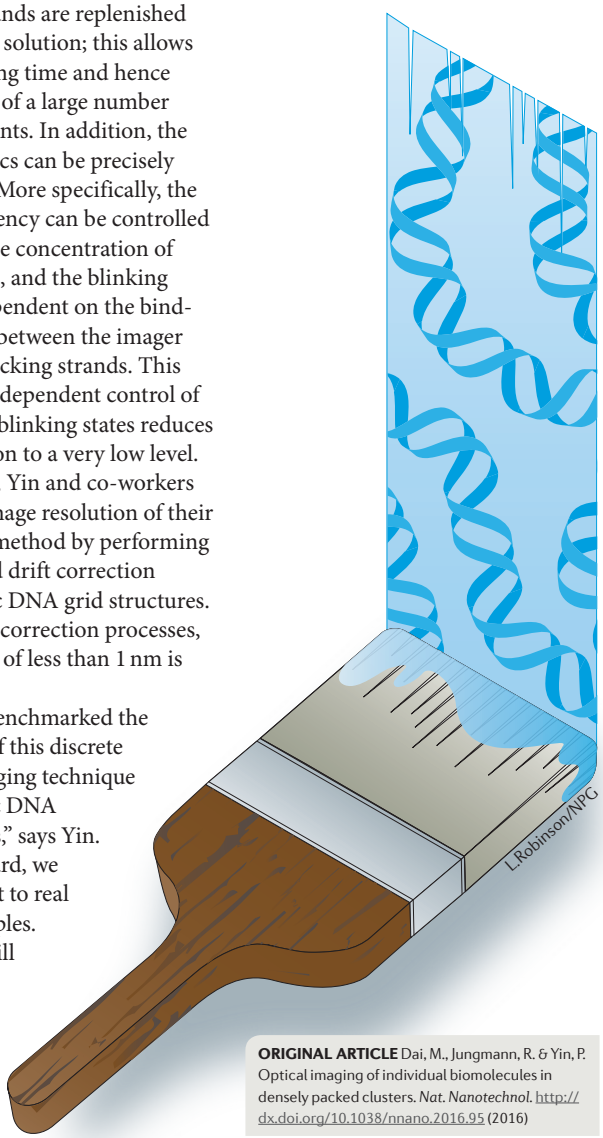
The DNA-PAINT technique is resistant to photobleaching because the imager strands are replenished by those in the solution; this allows a longer imaging time and hence the registering of a large number of blinking events. In addition, the blinking kinetics can be precisely programmed. More specifically, the blinking frequency can be controlled by adjusting the concentration of imager strands, and the blinking duration is dependent on the binding energetics between the imager strands and docking strands. This high level of independent control of the on and off blinking states reduces false localization to a very low level.

In addition, Yin and co-workers improve the image resolution of their DNA-PAINT method by performing software-based drift correction using synthetic DNA grid structures. Following two correction processes, a residual drift of less than 1 nm is observed.

"We have benchmarked the performance of this discrete molecular imaging technique using synthetic DNA nanostructures," says Yin. "Moving forward, we hope to apply it to real biological samples. To do so, we will need to label the biological targets with DNA docking strands."

If this technique is successfully translated into real biological environments, it may enable the visualization of the finer structures and molecular compositions of cellular species, or reveal the 3D architecture of chromosomes with such high resolution that important spatial and genomic information is obtained.

Alison Stoddart



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