

## SUPER-RESOLUTION OPTICAL MICROSCOPY

## Seeing the smaller picture

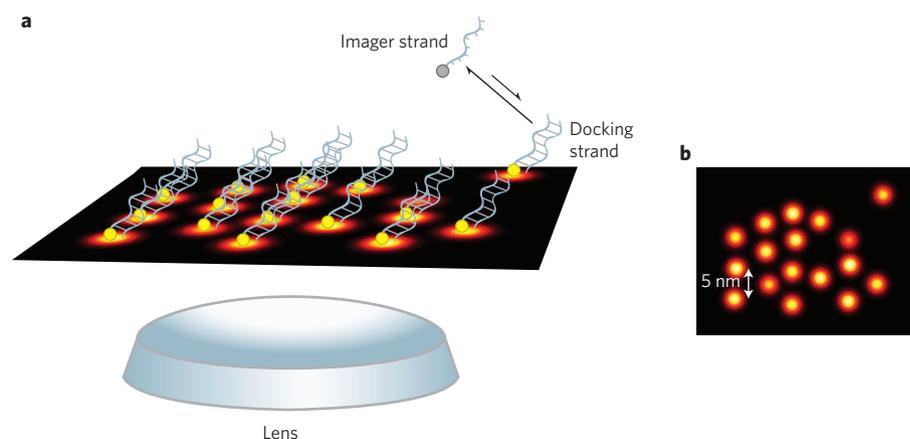
Optical microscopy goes subnanometre with DNA-PAINT.

Jörg Enderlein

Since the advent of super-resolution microscopy, it has become possible to directly detect and image individual molecules. Although the size of each molecule's image on the detector is limited by the resolution of the microscope, the centre position of the molecule can be determined with essentially unlimited accuracy. With the invention of stimulated emission depletion (STED) microscopy<sup>1</sup> and later with the development of single-molecule localization-based methods, such as photoactivated localization microscopy (PALM)<sup>2</sup> and stochastic optical reconstruction microscopy (STORM)<sup>3</sup>, there has been a race towards increasingly higher spatial resolution.

Due to photophysical limitations, in particular the limited photostability of all available fluorescent labels suitable for STED microscopy, PALM or STORM, the achievable resolution has so far stagnated around 10–20 nm. Although this resolution is much better than that of conventional optical microscopy and has produced a remarkable number of new and important results, it is still well above the length scale of small molecular complexes or aggregates. Writing in *Nature Nanotechnology*, Peng Yin and colleagues at the Wyss Institute at Harvard University and Harvard Medical School now report the use of a DNA-based discrete molecular imaging technique, termed DNA-PAINT (point accumulation for imaging in nanoscale topography)<sup>4</sup>, to resolve molecular structures down to 1 nm (ref. 5). This offers the fascinating prospect of using PAINT to resolve structural details in small molecular complexes or even within single bio- or macromolecules. In contrast to super-resolution microscopy techniques, which look to show a continuous morphology of a structure, PAINT aims to identify the position of each discrete molecular component in a complex synthetic or biological system.

PALM and STORM reconstruct an image by first sequentially localizing all individual molecules within a sample, and second by using all those localizations to back-calculate a final synthetic image with an effective spatial resolution that is determined



**Figure 1** | Working principle of DNA-PAINT. **a**, The recursive binding of fluorescently labelled (imager) strands to specific docking sites on a target structure produces an unlimited number of fluorescence photons for high-precision single-molecule localization. The reversible binding continuously replenishes fluorescent labels at a given binding site, addressing the main drawback imposed by the limited photostability of fluorescent molecules to the achievable localization accuracy in STORM or PALM. **b**, Using DNA-PAINT, Yin and colleagues demonstrate a localization accuracy (and thus optical resolution) of better than 1 nm. Figure adapted from ref. 5, Nature Publishing Group.

by the localization accuracy. The accuracy of these techniques depends only on the number of detected photons from the imaged molecule. The core trick is to employ photoswitchable fluorescent labels that, by various methods, can be switched from a non-fluorescent to a fluorescent state, so that in each recorded frame only a few labels fluoresce. By adjusting the photoswitching procedure, the chance that the images of two molecules overlap in one recorded frame can be made arbitrarily low, so that each molecule can be localized with high accuracy without interference from nearby molecules. The main limitation of PALM and STORM is the limited photostability of the employed fluorescent molecules, which ultimately limits the localization accuracy. An alternative to using photoswitching fluorescent molecules is to employ the recurrent binding–unbinding of fluorescent molecules to fixed binding sites of the sample, which is the principle of PAINT. Because the technique is no longer restricted by the finite number of detectable photons from one molecule before photobleaching

(even if a molecule is bleached, it will eventually unbind and be replaced by a new unbleached molecule), PAINT promises unlimited resolution power in resolving structural details of a given sample.

Yin and co-workers present an in-depth analysis of the capabilities of DNA-PAINT for imaging and resolving molecular structures with greater than 1 nm accuracy. In DNA-PAINT, short, fluorophore-labelled imager DNA strands bind to complementary DNA strands on a target structure (Fig. 1). Due to the freedom in designing hybrid sequences of any given length and nucleotide composition, the binding–unbinding properties of imager strands to their target binding strands can be tuned with arbitrary precision. Previously, Yin and colleagues have published impressive results using DNA-PAINT, where the imaged structures are DNA origami and the fluorescently labelled ligands are DNA strands that hybridize to suitably designed complementary single strands on the origami<sup>6–8</sup>. In the current work, the authors now demonstrate subnanometre resolution,

and undertake a systematic and quantitative study of the accuracy and resolution capability of the technique<sup>5</sup>. In particular, they focus on three main topics: impact and optimization of the signal-to-noise ratio, suppression of false localizations due to unspecific or multiple-site binding, and sophisticated mechanical drift correction. For the latter, they introduce new concepts based on the imaging of complex *a priori* known DNA origami structures, which they term templated and geometry-templated drift correction. These corrections achieve <1 nm residual drift over the imaging timescale.

To demonstrate the imaging resolution of DNA-PAINT, a DNA-origami grid with a dense pattern of pixels, 5 nm apart, was prepared, mimicking the monomer arrangement in a microtubule. DNA-PAINT allows the visualization of each individual

target, and can also be used for multicolour grids. This work is an important step forward in the optical microscopy of molecular structures. The demonstrated resolution power of DNA-PAINT rivals that of electron microscopes, but with the added specificity of fluorescent labelling. The next important steps are (1) to extend the nanometre resolution to the third dimension, which would allow for obtaining 3D structural information of single biomolecules or biomolecular complexes that would come close to resolutions achieved with cryoelectron microscopy or X-ray scattering methods; and (2) to develop new toolboxes for applying the concept of PAINT to proteins, not just DNA, for example by introducing specific binding sequences into a protein of interest to which a suitable dye-labelled ligand can reversibly bind. The promise is that far-field

optical imaging can provide structural (and potentially dynamic) information on single molecules or molecular complexes with close to atomistic spatial resolution, which could open a new world to optical microscopy. □

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## TISSUE ENGINEERING

# Nanoelectronics for the heart

Real-time three-dimensional mapping and control of *in vitro* cardiomyocytes opens new paths for post-surgery heart monitoring and stimulation.

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**M**yocardial resection is an open-heart surgery removing damaged or diseased areas of the cardiac muscle to improve its rhythm and/or function. Biomedical engineering and synthetic biology have focused on the fabrication of cardiac patches that could be used to fill the resulting resection void. Various 3D scaffolds have been developed for the regeneration of heart muscle<sup>1</sup>, and postnatal dermal fibroblasts have been reprogrammed to become cardiomyocyte-like cells endowed with contractility<sup>2</sup>. Together, these results imply that a culture of the patient's own skin cells within a 3D scaffold could be used to populate the resected heart areas. However, this approach requires devices that are able to continuously monitor the functional status throughout the implanted patches and, simultaneously, control their electrical activity. Writing in *Nature Nanotechnology*, Charles Lieber and co-workers from Harvard University now report the development of a flexible scaffold that can house cardiomyocytes as well as map and modulate their electrical activity in three dimensions<sup>3</sup>.

In their bottom-up approach, the researchers first produced doped p-type silicon nanowires contact-printed onto an

SU-8 polymer film surface. They arranged the nanowires in a field-effect transistor (FET) configuration rather than as simple electrodes, minimizing the spurious increase of the impedance as a result of decreasing the device's physical dimensions<sup>4,5</sup>. Metal source-drain interconnects were inserted to address each FET individually, and the resulting rectangular pad (20 μm × 4 μm × 350 nm) corresponded to a single recording device. The electrical properties of individual sensor pads were characterized using phosphate-buffered saline. The quantified time resolution on the order of 0.01 ms, together with favourable sensitivity and high signal-to-noise ratio, made the pads suitable for the detection of action potentials.

The considered scaffold was made of four superimposed layers, each one containing a 4 × 4 array of pads; and four circular palladium–platinum microelectrodes were incorporated for stimulation. Passive poly(lactic-co-glycolic acid) electrospun fibre films were inserted between the four layers, and the resulting final 3D scaffold (5 mm × 5 mm × 200 μm) had a bending stiffness similar to that of conventional scaffolds used for cardiac tissue growth. The scaffold attached to a modified

Petri dish (Fig. 1) housed rat ventricular cardiomyocytes in culture. Their electrical activity was evident throughout the entire scaffold after 8 days *in vitro*, showing sarcomere length and conduction velocity similar to that found in *in vivo* rat heart tissue. Over the course of culture, there was an order-of-magnitude reduction in the beating frequency. This frequency could be acutely up- or down-modulated by the global application of norepinephrine or heptanol, respectively, the latter being a blocker of gap junctions, which connect cardiomyocytes. The focal application of norepinephrine caused arrhythmia, indicating that the original pacemaker activity could be modulated, as further explored by using the stimulation electrodes. The authors were able to lock the pacemaker activity to a given stimulation electrode and shift the directionality of the action potential propagation by changing the stimulus input between different electrodes, showing spatiotemporal control over the tissue excitability.

These proof-of-principle experiments indicate that the constructed 3D scaffold would make it feasible to monitor and stimulate the cardiac activity post-surgery.