MICROSCOPY & IMAGING

EXPLORING DNA-PAINT

Savana Lipps discusses an advanced approach to single-molecule localisation microscopy

Super-resolution microscopy enables imaging of fluorescently labelled molecules at a resolution beyond the optical diffraction limit, down to 20nm laterally, and has propelled research forward in many disciplines, including genomics, cell biology, neuroscience and virology. One method of super-resolution microscopy – single-molecule localisation microscopy (SMLM) – provides the highest resolution compared to other available methods. SMLM achieves the separation of molecules by stochastically exciting individual molecules within the diffraction-limited volume at different time points. The data collected from the separation of molecules over time allows for the construction of an image of molecules within a diffraction-limited space, effectively surpassing the optical diffraction limit.

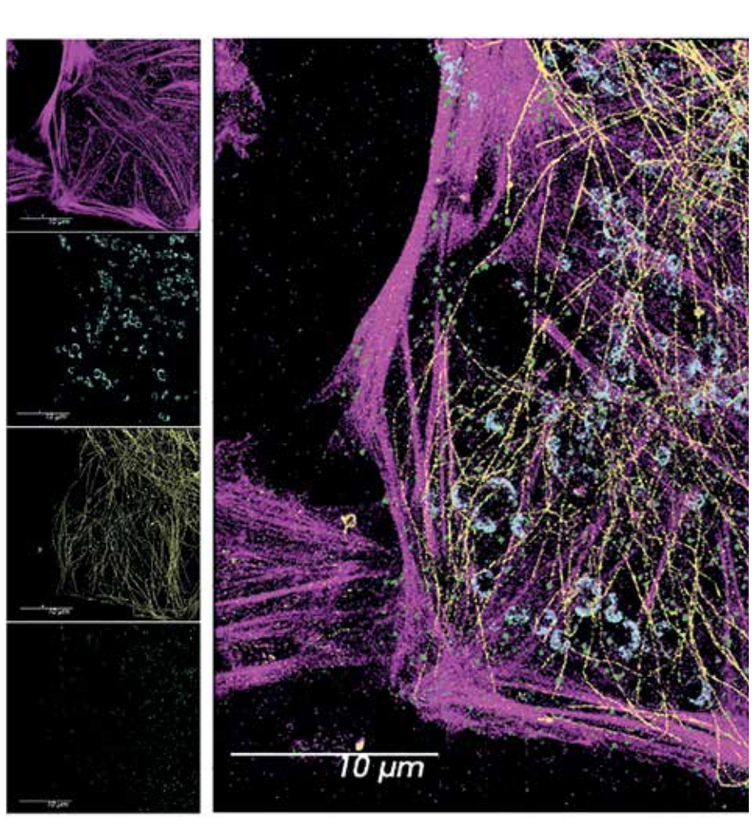

Fig. 1. DNA-PAINT multiplexed image. The images from each of the four targets (left) were acquired separately and combined automatically to create final image (right) in SRX software from Bruker. The image was coloured by molecule, with F-actin in magenta, mitochondria in cyan, microtubules in yellow, and clathrin in green

OVERVIEW OF TECHNIQUES

There are several techniques to achieving SMLM. Stochastic excitation of molecules can be achieved by either the use of fluorescent-labelled proteins or the use of organic fluorescent dyes. When using fluorescent protein labelling, proteins can be photoactivatable or photoconvertible. Photoactivated localisation microscopy (PALM) is an example of SMLM with photoactivatable protein. When using fluorescent dyes, fluorescence is controlled by either light-induced chemical reactions or the binding of dye to a specific cellular target. Stochastic optical reconstruction microscopy (STORM) and direct-STORM (dSTORM) are examples of light-induced chemical reactions. Points accumulation for imaging in nanoscale topography (PAINT) is an example of transient dye binding to a specific target.

With PAINT, transient binding and immobilisation of labels (e.g. Nile red) out of the buffer and onto the targeted structure provide the photoswitching on-off mechanism. This allows imaging of an unlimited number of structures of interest within the sample and makes it easier and more efficient to implement than other SMLM techniques. The PAINT technique does not result in photobleaching, and it provides the highest spatial resolution compared to other SMLM techniques.

PRINCIPLES OF DNA-PAINT

DNA-PAINT works through the transient binding of a short imaging oligonucleotide containing a fluorophore to a complementary oligonucleotide – called the docking strand – on the target of interest, such as an antibody, nanobody, aptamer or suicide enzyme ligand.

Super-resolution imaging with DNA-PAINT involves several steps. First, the sample is labelled with the "docking strand" through conventional techniques and prepared for imaging. For imaging, the sample is bathed in imaging buffer (typically PBS but can include oxygen scavengers) and a low (typically 0.1nM) concentration of imaging oligo complementary to the docking strand. The imaging oligo is typically 9-10 nucleotides in length and contains a fluorophore.

Once in the imaging buffer, the sample can be imaged. The transient binding of the imaging strand to the docking strand stops the diffusion of the fluorophore allowing it to be imaged on the camera. Since the sample is bathed in a large excess of constantly exchanging imaging strand, the target is essentially unbleachable, making it possible to batch-process many frames and extended Z-stacks. The nature of DNA-PAINT enables sequential labelling of multiple targets making multiplexed imaging easy, particularly when combined with an automated fluidic device.

DNA-PAINT CASE STUDY

DNA-PAINT has enormous potential for multiplexed imaging. Using orthogonal docking strands on different probes, imaging an unlimited number of targets is possible. In this case study, a multiplexed DNA-PAINT experiment was performed using a Vutara VXL microscope and integrated microfluidics unit from Bruker. The multicoloured images shown in Fig. 1 are a result of specific labelling and imaging four cellular targets including F-actin, mitochondria, microtubules, and clathrin. Four-colour imaging of these specifically labelled cellular proteins informs about their organisation and spatial relationships within the cell.

SUMMARY

DNA-PAINT is an emerging SMLM technique that supports the highest resolution, multiplexed imaging of fluorescently tagged molecules to seeing biology in nanoscale detail. This technique has transcending value across life sciences disciplines and will push the current boundaries to research that requires imaging multiple targets that are specifically labelled at super-resolution. ●

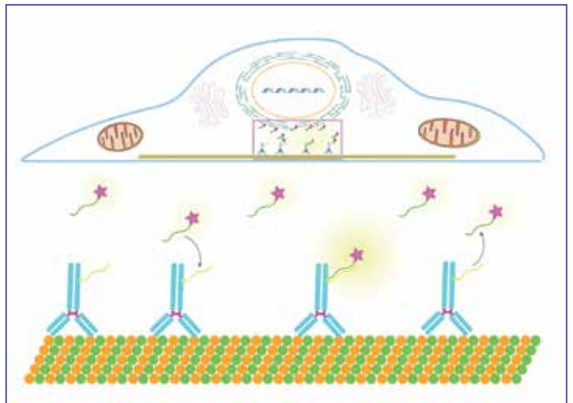

Fig. 2. Schematic of how DNA-PAINT works. The target protein (tubulin) is labelled with an antibody labelled with the docking strand oligo. The sample is then bathed in imaging strand oligos. The transient binding of the fluorescently labelled imaging strand to the docking strand causes the sample to appear to fluoresce, which can then be localised with advanced software. This process is repeated until the super-resolution image is formed

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