

Automated Exchange DNA-PAINT for multi color imaging with Abbelight Smart Flow

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Abstract

Over the last decades, fluorescence microscopy has rapidly evolved with the development of techniques such as single molecule localization microscopy (SMLM). Stochastic optical reconstruction microscopy (STORM) and photo-activated localization microscopy (PALM) have been widely exploited to solve many biological questions, previously impossible to decipher due to the light diffraction limit. In recent years, Point Accumulation for Imaging in Nanoscale Topography (PAINT) has emerged as a new approach of SMLM overpassing some of the limits of STORM and PALM. DNA-PAINT consists of the transient binding of short dye-labeled DNA strands to the complementary strand conjugated to the target protein. Binding and unbinding kinetics generates single fluorescent molecules in a diffraction-limited area. The camera captures this blinking, and the position is determined with extreme precision, like in techniques such as STORM and PALM.

DNA-PAINT enables to couple regular dyes to the DNA strand, removing the limitation of photo-switchable dyes. One of the major advantages is that photo-bleaching is almost neglectable due to the vast availability of dyes in the buffer, enabling long hours of acquisition. Besides, DNA-PAINT has enormous potential for multiplexing by just modifying the DNA strand coupled to the dye, allowing to target multiple proteins using a single laser source.

Abbelight Smart Flow is the perfect solution for scientists using DNA-PAINT, as it greatly simplifies washing the sample and changing imagers without taking the risk of losing the region of interest or altering the acquisition conditions. Abbelight Smart Flow can perfuse up to 10 different solutions to the sample with high precision controlling the volume, flow rate and timing of injection. You can automate any sequence or simply manually select the solution you want to inject to your sample. With its flexibility you can not only change in between imagers, but also adjust in real time the concentration of the imager, and even to change in between techniques like STORM.

With this workshop, we want to demonstrate the ease and optimization that the Abbelight Smart Flow and SAFe360 combination brings to DNA-PAINT imaging: From automated sample preparation to consecutive imaging of multiple targets with a single dye on Cos7 cells.

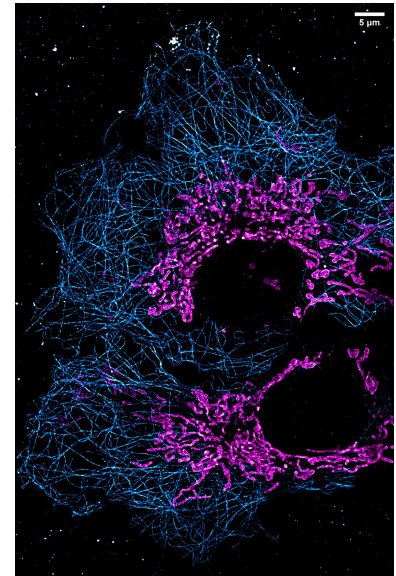
Schedule

10 min Introduction to DNA PAINT imaging

10 min Introduction to Abbelight Smart Flow and Safe 360

25 min Exchange DNA-PAINT for multi-color imaging

15 min Summary of the workshop & Questions



α -tubulin (magenta) and TOM20 (cyan-cool) both in Cy3B in Cos7 cells.