Subject: Multiplexing Super-resolution Optical fluctuation imaging (SOFI) with DNA-PAINT

Project type: BEP/MEP/Intern (3+ month)

Supervision: Dr. Kristin Grußmayer, k.s.grussmayer@tudelft.nl

Contact: Ran Huo, r.huo@tudelft.nl

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Project background

Super-resolution optical fluctuation imaging (SOFI) is a post-processing method that extracts high-resolution information from a series of fluctuating fluorescence imaging data. Such fluctuations are the result of ”blinking” fluorescent probes that are switching between ’on-’ and ’off-’ states. The blinking dynamics are characteristic of each fluorophore and can be used to identify and differentiate between different fluorophores. SOFI analysis is sensitive to the blinking kinetics (lag time and on-time ratio) amongst other molecular fluorophore parameters, opening the pathway to record multi-target images in one optical channel simultaneously by multiplexing fluorphores with different blinking characteristics.
DNA-PAINT is a technique that makes use of the transient and repetitive binding of two complementary single-stranded DNAs: the docking strand attaching to the molecule of interest, and the imaging strand coupled with a fluorophore diffusing freely in solution (see figure 1). It provides us with the flexibility to modulate the blinking dynamics for optimal SOFI multiplexing. DNA-PAINT and SOFI has proven to be a promising combination for a versatile super-resolution imaging method.

Project goals and activities

We aim to achieve fast multiplexed imaging in one cell without complicated optical setup by exploring the synergy of SOFI and DNA-PAINT. Previous work at the Grußmayer lab has verified their compatibility and established the experimental procedures to control their blinking kinetics for optimal resolution and imaging speed. You will establish blinking-based multiplexing and determine the limits using simulations and imaging experiments with different DNA-PAINT docking/imaging strands. How many ”color” channels can we achieve? You will also test the SOFI analysis workflow for a robust output of multiplexed imaging data.

For you?

You will learn about mammalian cell culture and fluorescent labeling techniques. You will get hands-on experience with our state-of-the-art super-resolution microscopes and advanced image analysis. You will work closely with the interdisciplinary team at Grußmayer lab. Depending on your background and interests, you could either focus more on optimization of data acquisition or data analysis. You will have have the chance to further extend the project to live-cell multi-color imaging of more significant biological significance. If you are an TU Nanobiology or Applied Physics master student, this could be a great fit for you!

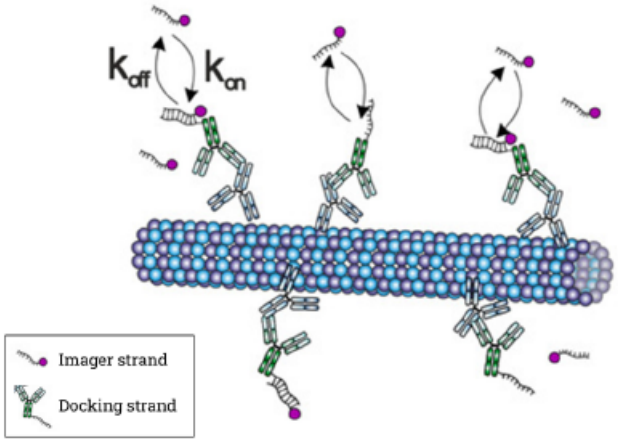

Figure 1: DNA-PAINT demonstration: docking strand labeling protein of interest and complementary imager strand with fluorescent dye floating in the solution. k_{on} and k_{off} represent the on- and off- time ratio respectively, which can be modified to be recognizable by SOFI. Adapted from 10.1002/anie.202013166

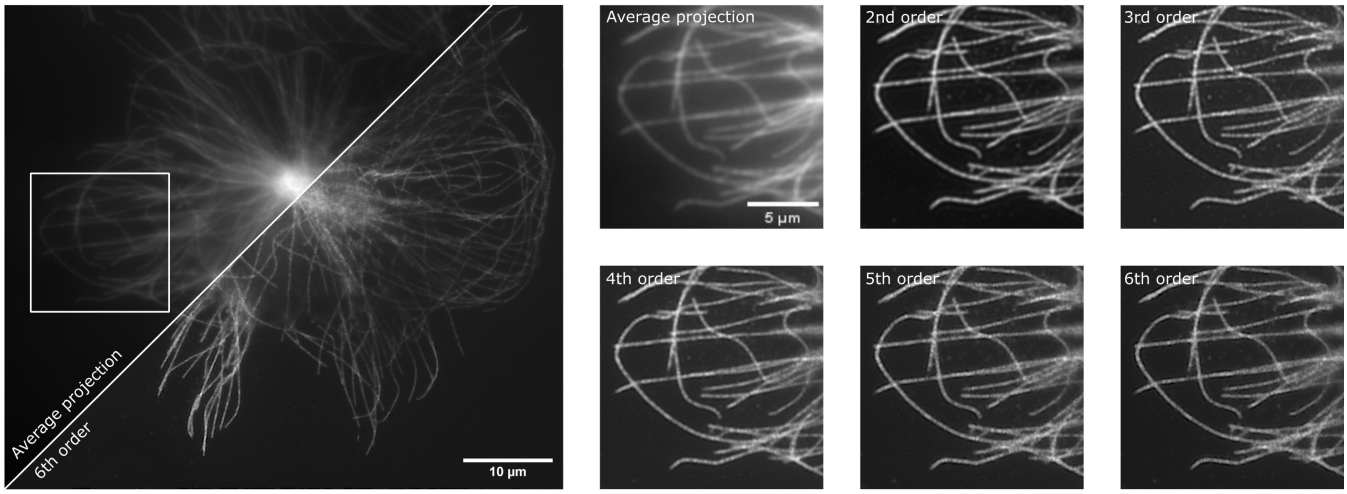

Figure 2: SOFI images of microtubules in COS-7 cell labelled with DNA-PAINT probes. Higher order SOFI indicates higher resolution enhancement compared to the diffraction-limited image (average intensity).