How to get started with 3D-DNA-PAINT Superresolution Microscopy

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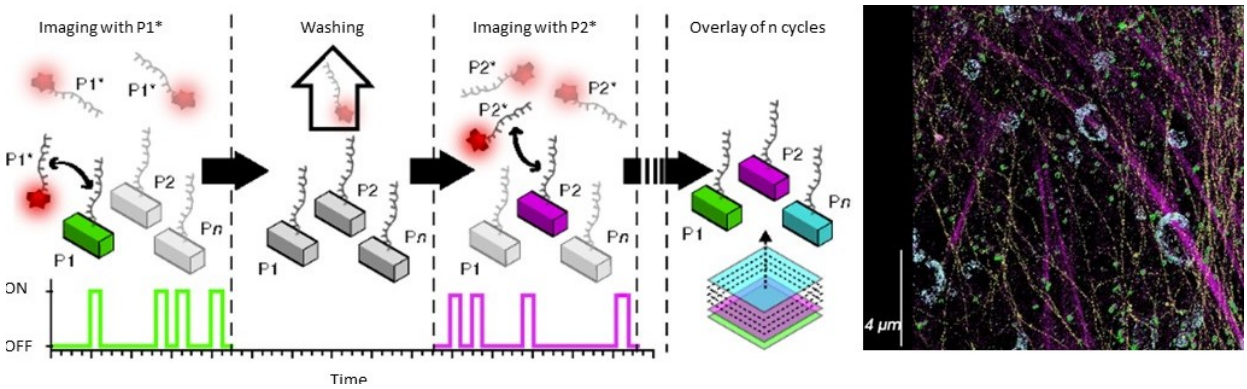
Abstract

Superresolution Microscopy based on **Single Molecule Localization (SML)** can provide **resolutions better than 10 nm laterally and 20 nm axially**. To achieve this, many critical parameters in sample preparation and experimental design need to be optimized, because resolution heavily depends on the photons collected per localization. One elegant way to increase the photon count of individual localizations and thus, increase resolution, is to use the **DNA-PAINT** approach.

Regular SML-microscopes are limited in their ability to acquire 3D-data and image more than 3-4 µm away from the coverslip. This can limit the scientific insights than can be obtained from such systems. The **Bruker Vutara VXL** system used in this workshop is different in this respect, because **widefield illumination** instead of TIRF / HILO illumination and the patented **biplane detection** instead of PSF-engineering are used. The optical concept of widefield illumination and biplane detection is ideally suited for **deep 3D-SMLM** acquisitions.

Combining the advantages of DNA-PAINT with the possibility to image deep inside the sample, paves the way for extending **SMLM to the third dimension**, which will be demonstrated and discussed in this workshop. DNA-PAINT samples will be prepared and provided by **Massive Photonics**.

In summary, this workshop will **cover the whole workflow of DNA-PAINT** from **sample preparation** to **data acquisition** and finally **data processing and analysis**.



Principle of multiplexed DNA-PAINT imaging. Image on the right: 4-plex DNA-PAINT experiment with markers for Actin, Mitochondria, Tubulin and Clathrin.