This project is part of the Nanoscopy-consortium financed by STW

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PALM and STORM are very successful in obtaining images with a resolution ten times better than conventional microscopy. Both make use of repetitive stochastic photo-activation of individual fluorescent probes, followed by localization of the probes with an accuracy down to ~20nm.

A disadvantage is that thousands of images are required to reconstitute a high-resolution image. This results in long measurement times and restricts appl ications to static or even fixed samples. In our cells, essential processes such as signaling, transport and DNA damage repair are driven by proteins interacting intermittently with other biomolecules, diffusing or moving directionally.

In order to fully understand biological processes it is not enough to obtain a static high-resolution picture of the processes, also the dynamics of the key players need to be quantified. Current PALM/STORM methods excel in obtaining static structural insight, but are not well suited to capture dynamics. The aim of the project is develop and apply Dyna-Storm.

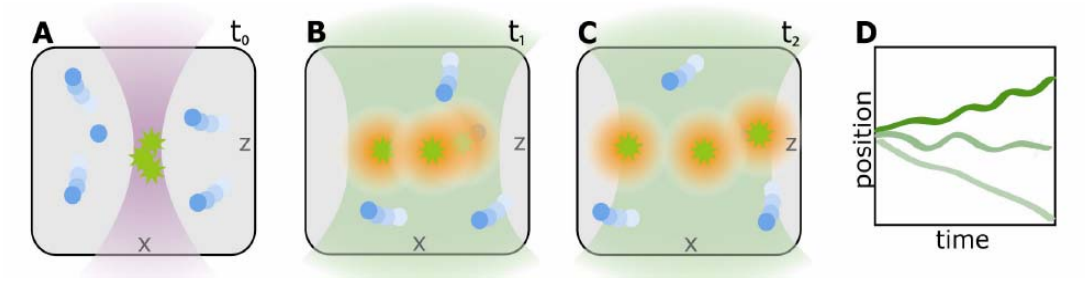

Figure. Schematic representation of the principle of dyna-STORM

dyna-STORM is based on stochastic photo-activation of individual fluorophores, but in contrast to conventional STORM/PALM with a high level of spatial and temporal control. Spatial light patterns (localized dots, stripes or more complex) are generated for photoactivation using a computer-controlled digital mirror device (DMD). Subsequently, with controlled time delay, the location of the activated fluorophores can be determined with ~20 nm accuracy using traditional widefield epi-laser fluorescence microscopy .

Multiple trajectories of individual proteins are generated and automatically analyzed to quantify the nature of the underlying dynamics: binding/unbinding, diffusion, directional motion, with high spatial and temporal control. In addition we develop methods allowing 3-D-localization of the probes (astigmatic or biplane imaging) and with other, conventional fluorescence microscopy modalities, such as Structured Illumination (SIM) and conventional confocal microscopy.

This allows to study the dynamics of a specific protein at high spatial and temporal resolution (with dyna-PALM), while a 'roadmap', reflecting global subcellular structure, is provided by the conventional images. In addition the conventional images are important to identify structures or compartments where we wish to activate and fol low specific tagged molecules.