Subject: 3D Super-resolution Imaging of DNA-repair in live cells

Project type: MEP

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

Contact: Arti Tyagi, a.tyagi@tudelft.nl

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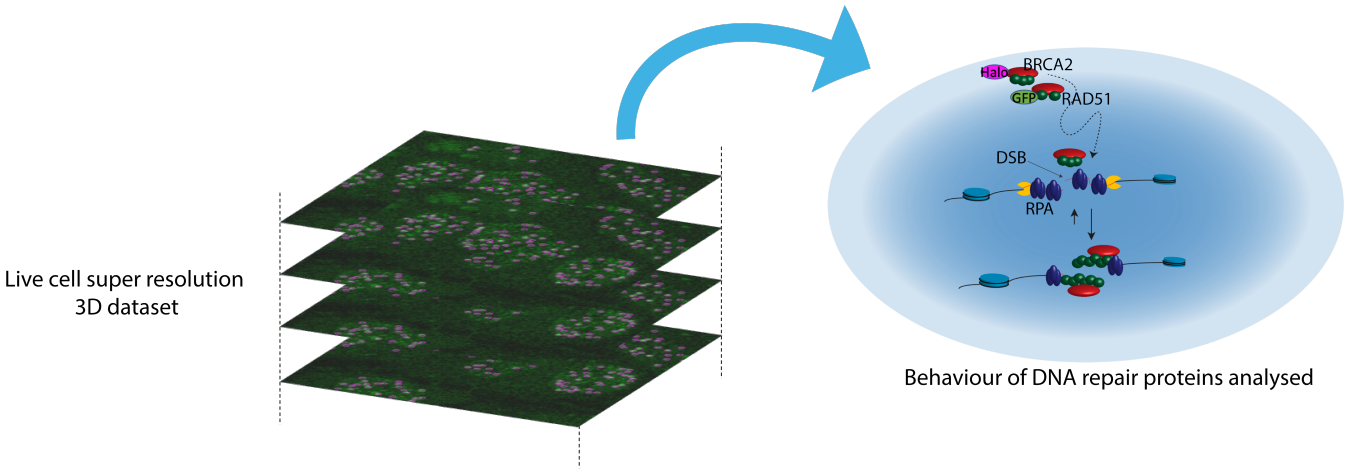
Project background

DNA damage repair is a central molecular process to ensure both genetic integrity as well as diversity. Cells must repair DNA damage to ensure their survival and prevent mutations that may lead to cancer. When DNA double strand breaks occur, triggered by factors such as ionizing radiation or drugs, repair proteins gather at the damaged sites, forming structures known as DNA repair foci. These foci can be observed using fluorescence microscopy. Various proteins engaged in DNA repair accumulate at distinct rates and quantities within these repair foci. Super-resolution (SR) microscopy studies have uncovered that repair factors exhibit distinct organization on the nanoscale at these sites. However, most of these are limited to fixed cell and 2D environments, thereby restricting any dynamic information gathered. Questions remain unanswered about how these proteins interact with each other, the timescales involved, and possible downstream effects on their individual functions in the repair pathways. We aim at observing these phenomenon via 3D multiplane SR framework in living cells to address these questions. This is a joint project with Maarten Paul and Roland Kanaar at Erasmus MC.

Project goals and activities

You will develop a pipeline for live cell 3D SR microscopy on our in-house microscope. This microscope uses a specialized prism to simultaneously acquire data from multiple sample planes. The idea is to optimize a SR method such as Halo-tag-PAINT or sptPALM to operate with live samples with sufficiently low phototoxicity and high SNR as well as temporal resolution . In addition, a computational analysis flow has to be set up. You will, then, apply this method to studying two major damage repair proteins, BRCA2 and RAD51, in cells which have endogenous tags on the proteins.



Your Profile

This project, as most other work in the Grussmayer Lab, is highly interdisciplinary. You work would combine techniques from cell/molecular biology, advanced super resolution microscopy and computational analysis. This implies learning tasks such as mammalian cell culture, fluorescent labelling, SR + 3D

imaging and data analysis. Students with a background in nanobiology or (bio-) physics who are interested in working on the interface of cutting-edge microscopy tools and real-world biological applications would be a great fit! So, if you are an TU Nanobiology or Applied Physics master student, this could be the project for you!