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Summary

By directly visualizing biological processes at the level of individual cells down to single proteins, many fundamental biological questions can be answered. Fluorescence microscopy has emerged as the most widely used tool for this purpose as it has unique advantages. The technique is minimally perturbative allowing the study of living cells and proteins in their original biological context. State of the art fluorescent labels yield high specificity and achieve the ultimate contrast. Single fluorescent emitters can be imaged with high signal-to-noise ratios. By circumventing ensemble averaging, underlying heterogeneity in molecules can be resolved and dynamic events are observed without the need of synchronization.

This unique set of properties makes fluorescence microscopy the tool of choice for many biological questions and it has become a real workhorse technique. It has found application in the study of living eukaryotic cells and bacteria, and their interactions with each other and their environment. On the subcellular level, different cell organelles are imaged in unprecedented detail. In molecular biology, fluorescence microscopy provides insights in the mechanisms of protein synthesis and folding, DNA replication and transcription, and has enabled the tracking of single proteins in individual bacterial cells. The aim of this thesis is to address some of the current limitations in fluorescence microscopy. Specifically, the focus is on the development of new fluorescent probes used to acquire images, and the development of software for its analysis.

In Chapter 2, we characterize the performance of ‘self-healing’ dyes in optical super-resolution techniques. Fluorescent molecules were coupled to a chemical group which gives them ‘self-healing’ properties. Through this mechanism, the molecules are more resistant to light exposure, allowing them to be observed longer before being irreversibly damaged. Super-resolution techniques, which bypass the resolution limit imposed by the wave nature of light, are especially demanding with respect to the fluorescent dyes used. We characterized self-healing dyes in both the targeted-readout super-resolution technique stimulated emission depletion (STED) as well as the stochastical technique stochastic optical reconstruction microscopy (STORM). In STED, we found that our fluorescent derivatives allow not only the collection of more images in the same area, but we show that we can observe fluorescence emission from a single fluorophore in time.

In Chapter 3, we go further into the details of the mechanism of self-healing dyes.
When undergoing the process of fluorescence, the dyes transiently visit a reactive state called the ‘triplet state’. The photoprotecting chemical group can take the fluorescent dye back to its ground state via a process called ‘intramolecular triplet state quenching’. Here, we studied the kinetics and resulting photostability of self-healing dyes in the presence of other triplet-reactive reagents in solution. First, the photobleaching lifetime was measured in the presence of photostabilizing agents in the imaging buffer solution. To our surprise, when combining self-healing dyes with solution-based healing, the obtained photostability was limited to the self-healing result, despite more pathways for triplet-state quenching being present. Therefore, we hypothesized the presence of pathways in the intramolecular quenching mechanism which lead to photobleaching. Second, we found that commonly used photoswitching agents for STORM-type super-resolution microscopy are highly reactive toward the fluorophore’s triplet state. Using self-healing dyes reduces their efficacy and, on the other side of the same coin, the presence of these photoswitching agents can lead to apparent photobleaching.

In Chapter 4, we describe a software package called ‘ColiCoords’, developed to analyse fluorescence microscopy data of rod-shaped bacterial cells. Through the development of fully automated microscopes, the data generated has increased tremendously, moving the bottleneck more and more towards the data analysis. To address this, we have created a new analysis package, which is freely available and well documented, thereby making it straightforward to use or modify the code. The analysis pipelines and associated data can be easily shared with other researchers thereby promoting open and reproducible science. ColiCoords’s main feature is the transformation of Cartesian coordinates to cellular coordinates. Through this transformation, many cells of different shapes can be aligned and combined, increasing the statistical power of the dataset. Furthermore, ColiCoords features projections of any data type along the longitudinal, radial or angular axis.

In Chapter 5, novel probes to selectively image gram-negative bacterial infections are described. Multiresistant bacteria are on the rise, presenting a global health crisis. Rapid and accurate diagnosis of infections can make treatment more effective and reduce risk of further development of antimicrobial resistance. In this chapter, we present novel fluorescent probes which selectively stain gram-negative bacteria, enabling optical imaging of bacterial infections. Optical imaging is a promising technique for diagnostic purposes since it is non-invasive and allows for real-time detection of infections. Using mouse models, we show that the probe selectively targets E. coli over S. aureus when injected intravenously. Furthermore, we show that the probes are potent photodynamic
therapy (PDT) agents. PDT can be used to treat infections locally without inducing antimicrobial resistance.