# ABSTRACTS

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## "Extracting quantitative microbiological data from single-molecule microscopy: Concepts and applications"

Over the last few years, there has been extensive research into the development and application of novel spectroscopic and microscopic fluorescence methods with high spatial and temporal resolution. Stochastic methods, such as PALM or (d)STORM, are particular compared to other methods, as they build on registering individual fluorescent signals. In most applications so far, these coordinates have been used to reconstruct an intensity-like image with superior spatial resolution reaching 20 nm or less.

However, as single-molecule technique, more valuable information is available: single-molecule registration allows for molecular counting, as well as for discovering sub-populations and heterogeneities which are otherwise hidden through ensemble averaging. Thus, the important add-on these techniques can provide is, next to super-resolution images, quantitative, single-molecule resolved information which can then be analysed by new localisation-based algorithm routines for spatial analysis of protein distributions and colocalisation.

It has therefore now become possible to address a great deal of microbiological questions on the organisation, interaction and dynamics of individual proteins in a cellular context in a new quantitative manner.

Here, we introduce this methodological toolbox on exemplary microbiological questions:

(i) the organization of RNA polymerase in transcription foci of Escherichia coli and the question on whether there are transcription factories;

(ii) the organization of replication and chromosome segregation of E.coli to spot highly-defined cell-cycle dependent hetero-structures;

(iii) the change of the number of CENP-A(Cnp1) proteins in the yeast centromere of Schizosaccharomyces pombe with respect to the cell cycle, and the question at which stage before the next cell division the protein population is replenished.

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## Cristina FLORS

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### "Improving super-resolution microscopy with correlative imaging"

The expansion of super-resolution techniques has allowed a whole new range of biological problems to be explored. However, super-resolution techniques are still in continuous development, and new fluorescent labelling and analysis methods need to be tested. Both labelling and post-processing analysis are prone to imaging artifacts, therefore new tools that allow robust validation of super-resolution images are needed. For that purpose, we have implemented a novel correlative microscope that allows sequential in situ imaging of the same sample area by atomic force microscopy (AFM) and super-resolution localization microscopy [1]. The technical aspects of the correlative microscope, including image alignment and sample preparation requirements will be discussed, as well as its application in optimizing DNA super-resolution imaging using intercalating dyes. This novel tool is able to reveal typical artefacts in super-resolution imaging related to labelling and image reconstruction algorithms. The combination of super-resolution and AFM is not only a useful tool to improve current nanoscopy methods but also to answer new biological questions.

[1] A. Monserrate, S. Casado, C. Flors, ChemPhysChem 2014, 15, 647.

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## Jonas RIES

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### "Superresolution Microscopy of Protein Structures in Situ"

"Single-molecule localization based superresolution microscopy (localization microscopy) nowadays reaches a resolution sufficient to determine structures of protein assemblies in the cellular context. It is therefore a technique complementary to classical structural techniques such as x-ray crystallography or electron microscopy to investigate, how molecular machines are organized.

Here we present two alternative labeling schemes for localization microscopy and report on our progress towards resolving a fundamental multi-protein machinery on the nanometer scale, namely the endocytotic machinery in S. cerevisiae.

Localization microscopy requires a high degree of labeling with bright and switchable dyes. Until now however, this required special fluorescent proteins to be cloned or high-affinity antibodies to be generated for specific labeling. On the other hand, many laboratories will have most of their constructs in GFP form and entire genomes are available as functional GFP-fusion proteins. Here, we report a method that makes all these constructs available for superresolution microscopy by targeting GFP with tiny, high-affinity antibodies coupled to blinking dyes [1]. It thus combines the molecular specificity of genetic tagging with the high photon yield of organic dyes and minimal linkage error. We show that in combination with GFP-libraries, virtually any known protein can immediately be used in superresolution microscopy and that high-throughput superresolution imaging using simplified labeling schemes is possible.

As an alternative to using photo-switchable fluorophores, we introduce binding-activated localization microscopy (BALM), which employs fluorescence enhancement of fluorogenic dyes upon binding to target structures for superresolution microscopy. We used this approach to study DNA structures [2] and a-synuclein amyloids [3] and could demonstrate a superb labeling density combined with a very high resolution.

Endocytosis is a highly intricate cellular process, which in yeast involves the ordered recruitment and disassembly of around 60 proteins. Our current efforts focus on understanding the intermediate and late coat assembly preceding scission. Here, we were able to reveal subdiffraction features regarding shape and structure of endocytic coat proteins that were previously inaccessible. By visualizing many proteins pairs with dual-color superresolution microscopy, we are pursuing to obtain a comprehensive structural picture of the endocytic proteome.

References:

1. Ries, J., Kaplan, C., Platonova, E., Eghlidi, H. & Ewers, H. A simple, versatile method for GFP-based super-resolution microscopy via nanobodies. Nat Methods 9, 582–584 (2012).
2. Schoen, I., Ries, J., Klotzsch, E., Ewers, H. & Vogel, V. Binding-Activated Localization Microscopy of DNA Structures. Nano Letters 11, 4008–4011 (2011).
3. Ries, J. et al. Superresolution imaging of amyloid fibrils with binding-activated probes. ACS Chem Neurosci 4, 1057–1061 (2013).

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