Symposium “Tracking Life”
Interdisciplinary meeting bringing together experts from imaging single molecules to whole organisms.
Monday | 8th October 2018 | 9.00 am
Generali-Saal | RWTH Aachen University
Free of charge!

Program and Abstracts

Keynote Speakers
Fritjof Helmchen, Brain Research Institute, University of Zürich
Daniel Razansky, Helmholtz Zentrum München
Steven Lee, Cambridge University

Organizers
Ulrike Endesfelder, Max Planck Institute for Terrestrial Microbiology & LOEWE Center for Synthetic Microbiology
Mangala Srinivas, Radboud Institute for Molecular Life Sciences (RIMLS)
Markus Rothermel, RWTH Aachen University
Program “Tracking Life”

9:00 - 9:15  Welcome note Prof. Dr. Doris Klee
Vice-Rector for Human Resources Management and Development, RWTH Aachen

Introduction by local host Markus Rothermel

9:15 - 12:00  SESSION I: Nanoscale imaging
chair Ulrike Endesfelder, Max-Planck-Institute for Terrestrial Microbiology, Marburg

9:15 - 10:00  Next-Generation Multidimensional Super-resolution Imaging
Steven Lee, Cambridge University, UK

10:00 - 10:30  COFFEE and POSTERS

10:30 - 10:50  Imaging intracellular and extracellular receptor dynamics in neurons at the nanoscale
Barbara Biermann, Heinrich-Heine-Universität Düsseldorf

10:50 - 11:10  Cellular division of Schizosaccharomyces pombe studied by single molecule localization microscopy
David Virant, Max-Planck-Institute for Terrestrial Microbiology, Marburg

11:10 - 11:30  Systematic superresolution analysis of endocytosis reveals an actin nucleation nano-template that drives efficient vesicle formation
Markus Mund, University of Geneva, Switzerland

11:30 - 11:50  Exploring the Escherichia coli nucleoid under various conditions using single-molecule localization microscopy
Christoph Spahn, Goethe-University, Frankfurt am Main

12:00 - 13:00  LUNCH SNACKS with POSTERS

13:00 - 14:45  SESSION II: Cellular imaging
chair Markus Rothermel, RWTH Aachen University

13:00 - 13:45  Neocortical dynamics during tactile discrimination behavior
Fritjof Helmchen, Brain Research Institute, University of Zürich

13:45 - 14:05  Dual-color imaging for isolating different olfactory bulb output streams in mice
Kim Chi Le, RWTH Aachen University

14:05 - 14:25  High-resolution 3D multi-site photostimulation of olfactory bulb granule cells using a holographic projector module
Max Müller, Universität Regensburg

14:25 - 14:45  High turnover of dendritic spines in the mouse hippocampus in vivo
Stefanie Poll, German Center for Neurodegenerative Diseases, Bonn

14:45 - 15:15  COFFEE and POSTERS

15:15 - 17:45  SESSION III: Imaging of Multi-cellular Organisms
chair Mangala Srinivas, Radboud University Medical Center, Netherlands

15:15 - 16:00  Advanced optoacoustic methods for imaging of fast biological dynamics
Daniel Razansky, Technical University of Munich & Helmholtz Center Munich

16:00 - 16:20  Interactive Analysis of Large-Scale Cell Tracking Data with EmbryoMiner
Johannes Stegmaier, Institute of Imaging and Computer Vision

16:20 - 16:40  Determinants of Intermediate Filament Dynamics in vivo
Nicole Schwarz, Universitätsklinikum Aachen

16:40 - 17:00  Analyzing mitotic chromatin decondensation through live-cell imaging
Daniel Moreno-Andrés, Universitätsklinikum Aachen

17:00 - 17:20  S100A11 is involved in plasma membrane repair in human endothelial cells
Arsila Ashraf, University of Muenster

17:20 - 17:40  DSper - The depolarizing channel of human sperm
Nadine Mundt, RWTH Aachen University

17:45 - 18:00  Meeting closure
informal pub night
P1: Single Molecule Localization Microscopy (SMLM) of autophagy-related proteins in mammalian cells
Iman Abdollahzadeh, Heinrich-Heine-Universität Düsseldorf

P2: Simplified multi-colour super-resolution imaging in E. coli
Mathilda Glaesmann, Goethe University, Frankfurt

P3: Towards kinetic studies of the RNA Helicase Dbp5
Vidura Liyanage, Rheinische Friedrich-Wilhelms-Universität Bonn

P4: Intranuclear dynamics of the 40S and 60S ribosomal subunits
Jan Ruland, Rheinische Friedrich-Wilhelms-Universität Bonn

P5: A rat monoclonal antibody which specifically detects mammalian GABARAP in confocal immunofluorescence imaging
Indra Maria Simons, Heinrich-Heine-Universität Düsseldorf

P6: Digital Tracking and Motion Analysis of Keratin Intermediate Filaments in Living Cells
Reinhard Windoffer, RWTH University Aachen

P7: Exploring cell-biology on a molecular level: Live-cell and quantitative localization microscopy
Ulrike Endesfelder, Max-Planck-Institute for Terrestrial Microbiology, Marburg

P8: Differential modulation of olfactory bulb activity by the basal forebrain
Erik Böhm, RWTH Aachen University

P9: AON top-down projections modulate olfactory bulb output activity
Renata Medinaceli Quintela, RWTH Aachen University

P10: Cellular division of Schizosaccharomyces pombe studied by single molecule localization microscopy
David Virant, Max-Planck-Institute for Terrestrial Microbiology, Marburg

P11: Pupillary dilations of mice performing a vibrotactile discrimination task reflect task engagement and response confidence.
Dan Ganea, RWTH Aachen University

P12: Mechanisms of plasma membrane repair in endothelial cells
Nikita Raj, Münster University
Super-resolution microscopy allows biological systems to be studied at the nanoscale, but has been restricted to providing only positional information. Here, we have developed a multi-dimensional super-resolution (md-SR) imaging technique to determine both the localization position and the environmental properties of single-molecule fluorescent emitters. The method, termed sPAINT, presented here exploits the solvatochromic and fluorogenic properties of Nile red to extract both the emission spectrum and the position of each dye molecule simultaneously enabling the mapping of the hydrophobicity with nanoscale resolution of biological structures. We first validated this sPAINT method by studying synthetic lipid vesicles of known composition. We then applied it to both, measure super-resolve the hydrophobicity of amyloid fibrils and oligomers implicated in neurodegenerative diseases, and to visualise the hydrophobic changes in a living mammalian cell. I will also present our latest unpublished 3D super-resolution imaging using a double-helix point spread function to study receptors clustering on complex cellular topology of human T cells.
The subcellular dynamics of receptor proteins is of pivotal interest to understand neuronal signaling. Using single-particle tracking with quantum dots (QDs) or the uPAINT technique, it was recently shown that neurotransmitter receptors move in and out of the postsynaptic density to regulate receptor numbers and to exchange desensitized receptors for native ones. It became feasible to track receptor dynamics not only in isolated neurons but also in native brain tissue. Yet, tracking intracellular receptor dynamics at the nanoscale is still a challenging task as diverse obstacles in the cytosol hinder diffusion of tags such as QDs. Here, we present a super-resolution approach for tracking neuronal receptors at the surface membrane and in the cytosol. We equipped the receptor of interest with a SNAP-tag in the extracellular protein domains and monitored the receptor dynamics with a 100x/NA1.46 oil objective and a microscope that allows for oblique laser illumination together with a fast EMCCD camera to image at 30 Hz. We estimated the localization accuracy by imaging immobilized QDs. To track the proteins we added SNAP-Cell 647-SiR to the culture medium, far-red fluorescent substrates that specifically label the SNAP-tag.

We used SNAP-tagged glycosyrophosphatidyinositol (GPI) - a lipid anchor - to analyze the basal membrane fluidity in axons, dendrites and synapses and compared them with GFP-GPI tracked QDs. We cloned the SNAP-tag to the C-terminal intracellular tail of dendritic metabotropic glutamate receptor 1a (mGluR1a) and monitored its postsynaptic dynamics. By fusing so-called sushi domains responsible for axonal targeting to the N-terminus of mGluR1a, we could redirect mGluR1a to axons and track its mobility. Currently, we are studying the efficiency of the SNAP substrates in penetrating the synaptic cleft by stimulated emission-depletion microscopy. Moreover, we aim to monitor altering receptor dynamics upon chemical long-term potentiation.
After DNA replication, each chromosome in the cell consists of two sister chromatids, both of which contain identical genetic information. In order for cell division to result in two fully functional new cells, these chromatids must be sequestered into the two cells with great precision. Microtubules that move the chromatids cannot bind directly to DNA. The kinetochore is a multi-protein complex that acts as the sole linker between DNA and microtubules. Since incorrect distribution of genetic material during division usually results in non-viable or heavily deficient cells, the kinetochore plays an absolutely vital role in the cell cycle. Despite its importance, relatively little is known about the structure and stoichiometry of the kinetochore complex.

To access this information at nanometer resolutions, we utilize state-of-the-art quantitative single molecule localization microscopy (SMLM). SMLM techniques, supported by localization-based data analysis algorithms enable us to obtain quantitative data on the individual proteins of the kinetochore complex. Through the use of sophisticated multi-color 3-D imaging schemes, we can also access structural information of the complex. We have developed a novel technique for 2-color SMLM imaging and applied it to the kinetochore, allowing us to measure distances between several of the proteins that make up the kinetochore. Our aim is to combine this structural data with quantitative data into a full model of the kinetochore complex.
Clathrin-mediated endocytosis is an essential cellular function of all eukaryotes, which relies on a self-assembled macromolecular machine of over 50 different proteins that mediate vesicle formation. Although most endocytic proteins have been studied extensively, it is not understood how all these proteins are organized to produce endocytic vesicles with high precision and efficiency. To address this fundamental gap in our knowledge, we developed a high-throughput superresolution microscopy and image analysis pipeline that allowed us to image more than 100,000 endocytic sites in yeast. From these superresolution images, we reconstructed where 23 proteins are localized within the endocytic machinery, and how their structural organization changes throughout endocytosis. By integrating our superresolution images with data from live-cell and conventional fluorescence microscopy, we derived a dynamic, time-resolved architectural model of the endocytic machinery throughout the entire process. We found that proteins assemble by radially-ordered recruitment according to function. During assembly, WASP family protein Las17 forms a circular nano-scale template on the flat membrane to spatially control actin nucleation during vesicle formation. We then used Brownian dynamics simulations to model actin polymerization at endocytic sites. We showed that the actin network nucleated by this WASP nano-template creates sufficient force for membrane invagination, and that the circular geometry substantially increases the efficiency of endocytosis. Such nanoscale pre-patterning of actin nucleation may represent a general design principle for localized, directional force generation in membrane remodeling processes such as during cell migration and division.
Bacteria essentially contribute to the balance of ecosystems and have significant influence on human life. This influence can either be beneficial (e.g. symbiotic bacteria in our gut such as *Escherichia coli*) or harmful (pathogenic bacteria such as *Salmonella* or *Listeria*). In both cases, bacteria need to adopt to their environment in order to guarantee cell survival and propagation. The essential processes can be traced back to the bacterial nucleoid, which comprises the blueprint for all proteins required and which needs to be robustly replicated during the cell cycle. Concomitant with chromosome replication, plenty of other processes such as transcription, translation or signal transduction have to be orchestrated in the same, highly confined space. In our work, we apply super-resolution microscopy, more precisely single molecule localization microscopy (SMLM), to visualize the bacterial nucleoid in unperturbed and drug-treated cells at a spatial resolution of about 20-30 nm. In order to shut down the fast cellular dynamics, *E. coli* cultures were chemically fixed at various time points of antibiotic treatment and screened using confocal microscopy. Time points exhibiting potentially interesting changes were further investigated using multicolor SMLM. By combining different SMLM approaches, we could visualize the bacterial nucleoid within the highly resolved cell envelope, revealing a high nucleoid complexity with partially strongly condensed chromatin filaments. We observe that the nucleoid is positioned close to the inner membrane, which might be a result of coupled transcription, translation and insertion of membrane proteins. This so-called transertion hypothesis was tested by specifically perturbing the contributing processes mentioned before. Other processes such as replication itself and cell growth were also perturbed in order to draw a more global picture of nucleoid organization in *E. coli* cells.
Neocortical dynamics during tactile discrimination behavior
Fritjof Helmchen, Brain Research Institute, University of Zürich

Through the combination of in vivo optical imaging and chronic expression of genetically encoded calcium indicators it is now feasible to directly 'watch' neuronal population dynamics in the neocortex of awake, head-restrained mice during specific behaviors. Here, I will present results from calcium imaging experiments in mouse neocortex while the animal performs a whisker-based texture discrimination task. Using retrograde tracing we identified distinct types of neurons in primary somatosensory area (S1), projecting to either S2 or M1, and characterized their dynamic features and sensory discrimination power during task execution and during learning. In addition, we used wide-field calcium imaging and multi-fiber photometry to resolve activation patterns across large parts of the neocortex and in subsets of subcortical regions, respectively. These experiments revealed wide-spread, coordinated activation of multiple brain regions, which correlated with various behavioral aspects such as whisking, body movements, licking. In particular, we identified highly distinct patterns of persistent cortical activity during a short-term memory phase, which were contingent of the animal’s behavior (active versus passive). Our results indicate that larger networks of neurons distributed across multiple cortical and subcortical regions are engaged in order to solve the task. Our data may help in understanding the principles of these large-scale activation patterns and the specific role of cross-regional interactions in sensory discrimination and the generation of the behavioral response.
Mitral and tufted cells (MTCs) are the main output neurons of the olfactory bulb (OB) project to different areas within the olfactory cortex (OC). Different brain areas can process segregated aspects of the sensory space as, e.g., the “what & where” pathway in vision. Whether different regions in the OC receive different functional input from MTCs however, remains unknown.

Here, we established a dual-color imaging approach to visualize activity from anatomically defined MTC subpopulations. We performed AAV-mediated retrograde tracing from two regions of the OC (anterior olfactory nucleus (AON) & anterior piriform cortex (aPC)) in order to isolate MTC output streams based on their axonal targets. We used green or red fluorescent calcium indicators; GCaMP6 and JRCaMP1a. Retrograde tracing from the AON or aPC yielded a strong expression in OB output neurons: labeled somata were mainly localized in the mitral cell and external plexiform layer (MCL, EPL).

Using widefield imaging we visualized AON or aPC traced MTC activity at the population level and compared their responses with PCD-GCaMP animals expressing GCaMP6 in olfactory sensory and OB output neurons. Odor presentation in PCD-GCaMP mice exhibited a defined activity map whereas those from AON or aPC traced MTCs were rather diffuse confirming the specificity of our tracing approach.

Few studies specifically compared differences of mitral and tufted cells, based on depth, and found TCs being more sensitive and unselective for odorants. Comparing MTCs based on their axonal targets at a single-cell level using 2P microscopy, our study revealed MTCs projecting to the AON being more selective compared to MTCs innervating the aPC.

In dual-color tracing experiments we achieved labeling of AON and aPC output streams revealing green, red or yellow fluorescent MTCs in the OB. Future dual-color experiments from two traced MTC populations will directly compare the physiological properties of defined MTC output streams.
The inhibitory axonless olfactory bulb granule cells (GCs) form reciprocal dendrodendritic synapses with mitral and tufted cells, the main projection neurons of the olfactory bulb (OB), via large spines. These synapses are substantially involved in odor signal processing. GC dendrites are highly excitable in multiple ways: synaptic inputs to individual GC spines can generate local Na+ spine spikes, and stronger activation results in global low-threshold Ca2+ spikes (LTS) and Na+ spikes. To optimally investigate the transition from local to global signaling, we implemented a holographic two-photon uncaging system which allows simultaneous photostimulation of multiple spines in 3D in acute brain slices. A holographic volume of interest of 70x70x70 µm³ can be chosen at arbitrary positions within the microscope’s field of view. The function of the system was demonstrated via simultaneous photolysis (0.5 - 1.5 ms dwell time) of caged glutamate at 10 spines along basal dendrites of patch-clamped cortical pyramidal cells (PC) of juvenile rats, eventually resulting in APs. The system allows to simultaneously perform two-photon Ca2+ imaging in 2D and thus to monitor synaptic Ca2+ entry in selected spines and also local regenerative events such as dendritic spikes. Next, we investigated the conditions for Na+ spike generation in GCs. Although GC resting potentials are hyperpolarized compared to PCs by approx. -10 mV, their Na+ spike threshold potential requires similar numbers of simultaneously activated spines (9 ± 2, n = 27 in 26 GCs vs 10 ± 1, n = 7 in 4 PCs). Within the subthreshold regime GCs can display both sub- and supralinear integration of inputs. As to LTS, activation of a significant lower number of spines already suffices to elicit dendritic Ca2+ transients. (5 ± 2 spines, n = 24 in 23 GCs, P < 0.001 vs Na+ spike). We show that these Ca2+ signals are able to propagate far along the dendrite and thus might correspond to the previously observed LTS.
The adaption of neuronal networks upon experience is thought to underlie the formation and elimination of synaptic connections. Dendritic spines represent the postsynaptic part of excitatory synapses and have been extensively studied in different areas of the mouse cortex in vivo. By contrast, studies in the deeply embedded hippocampus, the archetypical memory center of the brain, are rare, due to limited access. To address this issue, we established chronic in vivo super-resolution microscopy in mouse hippocampus by combining a hippocampal window and two-photon stimulated emission depletion (2P-STED) microscopy. We measured two-fold higher spine density compared to previous studies using conventional two-photon microscopy. Moreover, we observed a spine turnover of 40% within four days, which primarily affected small spines, underscoring the high synaptic rewiring potential of the hippocampus. Our study demonstrates chronic super-resolution microscopy in the mouse hippocampus in vivo, enabling longitudinal analysis of nanoscale neuroanatomical structures. This technique will be highly beneficial for the investigation of behavior-dependent structure and function relationships of synaptic connections in the living brain.
Visualization of biological dynamics in an unperturbed in vivo environment is essential for understanding the complex biology of living organisms and disease progression. To this end, optoacoustic sensing and imaging have shown unmatched capacity for high-resolution visualization of structural, functional, metabolic and molecular information deep from optically opaque living tissues. The modality is uniquely endowed with rich and label-free hemodynamic contrast, excellent spatial and temporal resolution, centimeter scale penetration into living tissues, and versatile exogenous contrast approaches. The talk focuses on the recent advances in multi-spectral optoacoustic tomography (MSOT) methods assisted by smart molecular labeling and dynamic contrast enhancement approaches that enable new types of fast multi-scale observations not attainable with other bio-imaging modalities. To this end, the unprecedented imaging performance of MSOT has been successfully exploited in various pre-clinical applications, including large-scale recording of fast neural activity, imaging of inflammation, tissue metabolism, and angiogenesis, volumetric visualization of contrast agent kinetics, molecular imaging using targeted and genetically expressed labels, monitoring of therapeutic interventions and drug efficacy. State-of-the-art handheld solutions are further transforming medical diagnostics by offering new level of precision in non-invasive clinical observations of patients, demonstrating high diagnostic efficacy in a number of indications, including skin and breast lesions, cardiovascular and inflammatory diseases and metastatic lymph node detection.
State-of-the-art light-sheet and confocal microscopes allow recording of entire embryos in 3D and over time (3D+t) for many hours of their early existence. Fluorescently labeled nuclei and cellular membranes can be segmented and tracked automatically in these terabyte-scale 3D+t images, resulting in thousands of cell migration trajectories. Analyzing these movement trajectories can provide detailed insights in large-scale tissue reorganization and morphological changes in early developmental stages at the cellular level. We developed a new interactive framework called EmbryoMiner that allows in-depth analyses and comparisons of entire embryos in unprecedented detail, including an extensive set of trajectory features to quantitatively describe the characteristics of the investigated tracks for feature-based change detection. Starting at the whole-embryo level, the framework can be used to iteratively focus on a region of interest within the embryo, and to investigate and test specific trajectory-based hypotheses. After the selection process is done, quantitative features can be computed from the isolated trajectories, to analyze and compare their properties. Identified groups can be easily transferred to additional data sets, allowing the comparison of corresponding structures in different embryos. All steps can be interactively refined to cope with the inherent biological variation present in different data sets. As a proof-of-concept, we analyzed 3D+t light-sheet microscopy images of zebrafish embryos and interactively extracted involuting cells during zebrafish gastrulation in four wild-type embryos.
Epithelial functions are fundamentally determined by cytoskeletal keratin network organization. However, our understanding of keratin network plasticity is only based on analyses of cultured cells overexpressing fluorescently tagged keratins. In order to learn how keratin network organization is affected by various factors in functional epithelial tissues in vivo, we generated a knock-in mouse that produces fluorescence-tagged keratin 8. By imaging of living pre-implantation embryos, we observe for the first time de novo keratin network biogenesis, keratin turnover in interphase cells and keratin rearrangements in dividing cells at subcellular resolution during formation of the very first epithelial tissue. In order to investigate the contribution of different cytoskeletal components to keratin network formation and dynamics, we interfered with desmosomal components, actin, as well as myosin. This mouse model will help to further dissect keratin network dynamics in its native tissue context during physiological and also pathological events.
During mitosis in higher eukaryotes, chromatin is highly compacted generating individualized and rod-shaped chromosome bodies that will reach their maximum packing density in anaphase. During mitotic exit, chromosomes return to their functional interphase state by a still poorly characterized decondensation process. We have shown that this process is energy dependent and that two ATPases, Pontin and Reptin, also known as RuvBL1 and RuvBL2, are required but not sufficient for chromatin decondensation at the end of mitosis. To shed light on the Pontin-Reptin mediated decondensation process we identified proteins that specifically interact with Pontin and Reptin during mitotic exit by SILAC based mass spectrometry. Then, those specific interaction partners were analyzed using live-cell imaging based phenotypic screening for extended telophase, which is characteristic of Pontin and/or Reptin downregulation by small interference RNA. The hits were considered potential mitotic chromatin decondensation factors, which we are currently studying in detail by means of biochemical, cell biological and other live-cell imaging approaches.
The plasma membrane of a cell demarcates its cytoplasm from the environment. This maintains the cellular homeostasis which is inevitable for cell viability. The integrity of the plasma membrane is often compromised since the cells are under constant mechanical stress. Plasma membrane repair in response to mechanical strain has been studied in detail in myocytes, however much less is known about other cell types and the underlying molecular mechanisms. We now show that human vascular endothelial cells which are physiologically subjected to mechanical stress, are able to repair the wounds induced by laser ablation or mechanical damage; a process dependent on the presence of Ca2+. In search for a factor involved in membrane resealing, we identified S100A11, a member of S100 protein family characterized by two helix-loop-helix ("EF-hand type") calcium-binding sites which is recruited immediately to the site of the wound in a wave like pattern in a Ca2+-dependent manner. Knockdown experiments reveal that S100A11 is a positive regulator of plasma membrane repair in human endothelial cells. Moreover, S100A11 and AnnexinA2, a Ca2+-regulated membrane binding protein known to be involved in membrane resealing (Koerdt and Gerke, 2017), are recruited to the wound site with similar kinetics. Pull down experiments show that S100A11 is a potential interaction partner of AnnexinA2. Furthermore, our results with employing ectopic expression of fluorescent microtubule binding proteins and pharmacological inhibition of microtubule polymerization suggests a prominent role of microtubules in S100A11 translocation to the wound site and wound repair. Thus, S100A11 and AnxA2 likely interact with each other to coordinate efficient plasma membrane repair in endothelial cells. In addition, our observations shed light on a novel link between cytoskeleton integrity and plasma membrane repair efficacy in human vascular endothelial cells.
Ion channels control the ability of human sperm to fertilize the egg by triggering hyperactivated motility, which is regulated by membrane potential, intracellular pH, and cytosolic calcium. Previous studies unraveled three essential ion channels that regulate these parameters: (1) the Ca²⁺ channel CatSper, (2) the K⁺ channel KSper, and (3) the H⁺ channel Hv1. However, the molecular identity of the sperm Na⁺ conductance that mediates initial membrane depolarization and, thus, triggers downstream signaling events is yet to be defined. Here, we functionally characterize DSper, the Depolarizing Channel of Sperm, as the temperature-activated channel TRPV4. It is functionally expressed at both mRNA and protein levels, while other temperature-sensitive TRPV channels are not functional in human sperm. DSper currents are activated by warm temperatures and mediate cation conductance, that shares a pharmacological profile reminiscent of TRPV4. Together, these results suggest that TRPV4 activation triggers initial membrane depolarization, facilitating both CatSper and Hv1 gating and, consequently, sperm hyperactivation.
Autophagy is a homeostatic process enabling cells to survive under stress and starvation. It plays an important role both for basal turnover of intracellular proteins and organelles and for maintenance of homeostasis under stress such as nutrient deprivation. The formation of cup-shaped double membrane phagophore is the unique hallmark of autophagy. Pioneering studies in yeast have led to the identification of a core machinery of autophagy-related (ATG) proteins, and most of these were found to be conserved in mammalian cells. In human cells, the ATG8 family consists of six members grouped into two subfamilies including GABARAP-type and LC3-type proteins. Given the size of the intracellular ATG8-positive structures (50 nm to 1.5 µm), which is on the order of the wavelength of visible light or well below, the distribution of proteins on these organelles cannot be resolved by conventional fluorescence microscopy. Using Single Molecule Localization Microscopy (SMLM), which relies on the precise localization (~ 40 nm accuracy) of single fluorescent molecules decorating the autophagy-relevant and other cellular structures, it is possible to retrieve the localization of all fluorophores that label a certain structure by imaging only a subset of the emitters at a time and repeat the imaging process many times with new subsets activated to the fluorescent state. The localization of the ATG8 proteins, their trafficking, their interplay and their interaction with other autophagy-relevant and lysosomal proteins is the central research topic of this project. HEK293 cells have been transiently transfected with expression plasmids encoding EYFP-GABARAP or LC3B-mCherry fusion proteins, and after fixation have been measured with SMLM. Based on categorizing the identified ATG8-containing structures in SMLM images of fixed cells, we investigated the different size and shape distributions of GABARAP and LC3B.
Life arises from an incredible number of highly coordinated interactions of innumerable individual parts. Therefore, it is necessary to study the interactions between different cellular compounds to get knowledge about highly synchronized biological processes. Especially in confined and crowded bacterial cells investigating the interplay between different parts of a process is challenging. With single molecule localization microscopy (SMLM) techniques it is possible to visualise individual molecules in bacteria, but often labelling is limiting the possibilities to investigate multiple cellular compounds. Point Accumulation for Imaging in Nanoscale Topography (PAINT) allows long-term single-molecule super-resolution imaging independent of photobleaching due to fluorophores that bind highly specific, but transiently, to their target structure. In addition to known techniques, like binding-activated localization microscopy (BALM) and integrating exchangeable single-molecule localization (IRIS), we show an easy and versatile application of innovative dyes that allow to super resolve several cellular structures in a PAINT-like approach. The different spectral properties allow to couple the PAINT-like imaging approach with other SMLM techniques such as dSTORM in a single cell to study interactions between various cellular constituents. This provides a wide ranging toolbox for an easy and widely applicable multi-color SMLM imaging of bacterial cell structure.
The nuclear pore complex (NPC) is the only route into and out of the nucleus, but it cannot define the directionality of transport itself. Transport cargo, which can enter the central pore due to its receptor-mediated interaction with the FG repeats, can theoretically leave the pore on both sides. For the nucleo-cytoplasmic transport of proteins, directionality is mediated by the small GTPase Ran, which either resolves import complexes in the nucleus or co-escorts export cargo in dependence on its GTP status. The RNA helicase-Dbp5 accomplishes the directionality of mRNA transport. The substrates and co-factors of Dbp5 are well known, however, the exact sequential arrangement of the biochemical reactions is still under debate.[1],[2] By means of confocal and total internal reflection microscopy, we studied the interactions of the Dbp5/mRNA reaction cycle using recombinant wild-type and mutant Dbp5, the co-factors Nup214, Gle1 and a mRNA substrate. In this manner, we already demonstrated the expected binding between Dbp5, -RNA and Nup214 both at the bulk and at the single molecule level.

Protein biosynthesis is carried out by ribosomes, molecular machines, consisting of many different proteins and a ribosomal RNA (rRNA) backbone. The biogenesis of mammalian ribosomal subunits begins in the nucleolus with the 90S precursor particle, which is subsequently split into the pre-40S and pre-60S subunits. During further processing steps the subunits are loaded with export receptors, which allows for their passage through the nuclear pore complexes into the cytoplasm. Here the export factors are released and both subunits can form the final ribosome [1-4]. Ribosomal biogenesis has been studied in great detail by biochemical and genetic approaches; however, until now all views on the biogenesis were rather static. To gain insights in the dynamics of this process, we produced stable cell lines, which express one fluorescent assembly factor for each subunit, and NTF2-GFP. Combining state-of-the-art microscopy techniques, like highly-inclined-and-laminated-optical-sheet (HILO) and super resolution confocal microscopy, we studied the in vivo dynamics of both single ribosomal subunits in specific intranuclear regions of HeLa cells at the single particle level in a comparative manner [5]. Eventually, we aim at elucidating their interaction with nuclear pore complexes. [1]
Despite intense research, the precise cellular functions of GABARAP (gamma-aminobutyric acid receptor type A associated protein), a member of the conserved protein family of mammalian autophagy-related 8 proteins (mATG8s) are only partially understood. Especially the elucidation of unique roles of GABARAP within specific cellular processes including autophagy, receptor-trafficking or autosecretion remains a challenging task. Available anti-GABARAP antibodies often come along with a non-transparent and insufficient validation, e.g. regarding their performances within diverse microscopy-based applications. We developed a rat IgG2a subclass monoclonal antibody against human GABARAP (GABARAP-8H5). Following the latest recommendations, we characterized the performance of GABARAP-8H5 during confocal immunofluorescence (IF) imaging applications in-depth. We compared GABARAP-8H5-based staining with that obtained by ectopically expressed fluorescence protein (FP)-tagged GABARAP, FP-GABARAPL1 or FP-GABARAPL2 in HAP1 cells deficient for endogenous GABARAP. We stained HAP1 wildtype cells containing endogenous mATG8 levels, and checked GABARAP knockout cells for unspecific GABARAP-8H5 staining, both under fed and growth factor-depleted/BafA1-treated conditions. Finally, cells were simultaneously stained with GABARAP-8H5 and an antibody specifically recognizing LC3B, which is a common and independent marker for autophagosomes. In sum, we demonstrate that GABARAP-8H5 shows high specificity for GABARAP without cross-reactivity neither with GABARAPL1, -L2, nor with LC3B in IF. GABARAP-8H5 will be a valuable tool for future investigations to identify unique GABARAP functions.
Networks of keratin intermediate filaments have a dominant impact on the mechanical properties of epithelial cells. Remarkably the cellular keratin network topology is quite variable between different tissues and cell types. Within the keratin network of a single cell three major organization-types of filaments can be observed in many cell types. For a detailed analysis of keratin network structures a digital representation of the network containing its spatial and temporal network properties is needed. An accurate manual quantification of the network properties requires a lot of effort and is extremely time-demanding. We therefore developed an image analysis framework for automated localization and tracking of segments of keratin filaments. The framework consists of two main routines: one for detecting and one for tracking the filaments. The detection routine allows to segment filaments with prescribed properties. The tracking routine follows detected or manually identified filaments through the image sequence and determines how they deform. The algorithm was evaluated using 300 ground truth trajectories. Using this image analysis tool we now can automatically track segments of keratin filaments in fluorescently labelled living cells over time and determine their movements and deformations. First analyses show differences in curvature and virtual persistence length at different subcellular localizations. Further analysis are planned to show the impact of various factors on the keratin network organization and dynamics.

SMLM data is built on single-molecule localizations, and thus allows determining the stoichiometry and molecular architecture of subcellular structures. Here, not only individual proteins can be precisely localized, but the large molecular architecture of multiprotein complexes or the organization of the genome can be targeted in the native cellular environment. This yields detailed quantitative molecular maps that capture these assemblies. In our vision, SMLM imaging thus has the potential to place hundreds of different molecules into assembled three-dimensional structures while maintaining the high spatiotemporal resolution of the present methods, ideally in correlative approaches.

Uniquely, SMLM can be combined with single-particle tracking (SPT) to measure a large batch of statistics on single-molecule dynamics inside living cells. It is thus possible to obtain spatially and temporally highly resolved diffusion maps that combine the multitude of single-molecule trajectories and accordingly unravel possible dynamic heterogeneities and subpopulations. Behind todays attractive super-resolved images and analyses hides a rather high complexity of in large detail tailored experimental designs for specific organisms and environments. We cannot answer our research questions about the in situ behavior of molecular processes at a single molecules’ spatiotemporal resolution without highly optimized and robust tools - which are still largely missing for most biological research fields, e.g. for microbiology (as most proof-of-principle SMLM studies, are mostly conducted in mammalian cell culture).

In this poster, I will introduce some of our recently developed experimental and analytical tools alongside with our specific biological questions.
The olfactory bulb (OB) receives neuromodulatory input from diverse areas whose role in shaping early olfactory processing remain unclear. One of the major neuromodulatory centers implicated in attentional modulation of sensory processing, the diagonal band of Broca (HDB), is composed of cholinergic, GABAergic as well as glutamatergic projection neurons. Here we used optogenetic tools to selectively activate or inhibit cholinergic or GABAergic HDB terminals at the level of the OB while recording OB mitral/tufted output neuron activity using multichannel electrophysiological recordings. An optogenetic activation of cholinergic fibers was shown to add an excitatory bias to OB output activity, that is independent of the strength of sensory input. A selective activation of GABAergic neuromodulatory fibers at the level of the OB significantly decreases the activity of mitral/tufted cells. Similar to the cholinergic data, no correlation between modulation strength and sensory input could be observed. Experiments performed using two inhibitory opsins expressed either in cholinergic or GABAergic HDB fibers so far revealed only weak modulation effects, potentially arguing for a weak baseline activity of HDB neurons in the anesthetized condition. These experiments demonstrate that: 1) A differential modulation of neuronal responses can not only be achieved by activating different top-downs system but can also be mediated via different neuronal populations of the same top-down area. 2) Despite recent reports pointing to a potential coexpression of cholinergic and GABAergic markers at the level of the OB, our data argue for a functionally distinct neuronal population. 3) The interplay between different neuronal HDB subpopulation might be important for the fine tuning of sensory information 4) These modulations are likely to represent local effects mediated by neuromodulatory release at the level of the OB. Future experiments will investigate HDB stimulation effects also in the awake and behaving animal.
The olfactory bulb (OB) is the target of massive cortical top-down projections from the anterior olfactory nucleus (AON) whose role in modulating early olfactory processing remains elusive.

Here, we examine how top-down projections from the AON modulate output neuron activity from the OB using imaging, electrophysiological and optogenetic approaches in vivo. Optogenetic activation of AON axon terminals in the OB led to a significant decrease in mitral/tufted (MT) cell spiking in the absence of inhalation-driven sensory input. The population time course showed a fast reduction of MT cell activity during light stimulation that was followed by a long lasting increase, reminiscent of OB offset responses. Since sensory input was shown to trigger AON feedback activity to the OB, we also tested for AON modulation effects during inhalation of clean air as well as during odor presentation. In both conditions, MT cells showed a decrease in activity similar to that observed in the absence of sensory input, arguing that our light stimulation protocol already strongly activated AON OB axons. Averaged normalized sniff-triggered spike histograms showed a decrease in both baseline and peak spike rate, consistent with an AON mediated effect on odor sensitivity rather than an influence on signal-to-noise ratio. Furthermore, AON simulation effects were independent of the strength and polarity of the odorant response.

Preliminary imaging experiments yielded similar results: AON stimulation caused a decrease in spontaneous as well as odor-evoked MT cell Ca2+ transients and so far, no odor specific effect could be observed.

Our results suggest that AON activation is capable of reducing OB output activity independent of sensory input strength. Future experiments will focus on translating our findings in anesthetized animals to the awake condition.
In this poster we would like to give more details about how we, by the use of single molecule localization microscopy (SMLM) access the kinetochore structure and function as presented in the talk. We introduce the experimental SMLM imaging routine, which is supported by molecular techniques for the design of fluorescently tagged strains and localization based algorithms for data analysis. Together, these tools make it possible to super-resolve and quantify the structure of multi-protein complexes and we established a sequential triple-color imaging routine to measure each structural protein of the kinetochore in relation to two reference proteins. This routine allows us to frame our previous single protein and single particle tracking work by detailed protein-protein distance measurements.
Pupillary dilations of mice performing a vibrotactile discrimination task reflect task engagement and response confidence.

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Pupillometry, the measure of pupil size and reactivity, has been widely used to assess cognitive processes. As such, changes in pupil size have been shown to correlate with arousal, locomotion, cortical state and decision-making processes. In addition, pupillary responses have been linked to the activity of neuromodulatory systems that modulate attention and perception as the noradrenergic and cholinergic systems. Due to the extent of processes reflected by the pupil, we aimed at resolving pupillary responses in context of behavioral state and task performance while recording pupillary transients of mice performing a vibrotactile two-alternative forced choice task (2-AFC). We show that pre-stimulus pupil size differentiates between states disengagement from task performance versus when actively engaged. In addition, when actively engaged, post-stimulus, pupillary dilations for correct responses are larger than for error responses with this difference reflecting response confidence. Importantly, in a delayed 2-AFC task we show that even though pupillary transients mainly reflect motor output following the response of the animal, they also reflect animal decision confidence prior to its response. Finally, in a condition of passive engagement, when stimulus has no task relevance with reward provided automatically, pupillary dilations rather reflect stimulation and reward and are reduced relative to a state of active engagement explained by shifts of attention from irrelevant task occurrences.

Our results provide further evidence of how pupillary dilations reflect cognitive processes in a task relevant context, showing that the pupil reflects response confidence and baseline pupil size encodes attentiveness rather than general arousal.
Eukaryotic cells are exposed to various environmental stresses which can cause cell membrane disruptions that need to be resealed rapidly to ensure cell survival. When the cell membrane is compromised, extracellular Ca^{2+} enters the cell and this Ca^{2+} entry acts as a signal to initiate membrane resealing processes. I am studying the poorly understood mechanisms of membrane resealing used by endothelial cells, which are subjected to high mechanical stresses. Using live cell laser wounding assays, I am examining the role of various internal membranes in the membrane resealing process. Among other things, I could show that various endosomal vesicles, in particular Rab5- and Rab7-positive early and late endosomes disappear near the wound site during resealing. Most likely, this disappearance represents exocytosis fusion events that contribute membrane for the resealing process. Further plans include studying these possible fusion events and the role of calcium in inducing resealing mechanisms.