

Bruker Super-Resolution Publications

All Authors	Title	Journal	Year	Abstract	Top Four Applications
Zung N, Aravindan N, Boshnakovska A, Valenti R, Preminger N, Jonas F, Yaakov G, Willoughby MM, Homberg B, Keller J, Kupervaser M, Dezorella N, Dadosh T, Wolf SG, Itkin M, Malitsky S, Brandis A, Barkai N, Fernandez-Busnadiego R, Reddi AR, Rehling P, Rapaport D, and Schuldiner M	The molecular mechanism of on-demand sterol biosynthesis at organelle contact sites.	bioRxiv	2024	Contact-sites are specialized zones of proximity between two organelles, essential for organelle communication and coordination. The formation of contacts between the Endoplasmic Reticulum (ER), and other organelles, relies on a unique membrane environment enriched in sterols. However, how these sterol-rich domains are formed and maintained had not been understood. We found that the yeast membrane protein Yet3, the homolog of human BAP31, is localized to multiple ER contact sites. We show that Yet3 interacts with all the enzymes of the post-squalene ergosterol biosynthesis pathway and recruits them to create sterol-rich domains. Increasing sterol levels at ER contacts causes its depletion from the plasma membrane leading to a compensatory reaction and altered cell metabolism. Our data shows that Yet3 provides on-demand sterols at contacts thus shaping organelle structure and function. A molecular understanding of this protein's functions gives new insights into the role of BAP31 in development and pathology.	
Brian D. Mueller, Sean A. Merrill, Lexy von Diezmann, and Erik M. Jorgensen	Using Localization Microscopy to Quantify Calcium Channels at Presynaptic Boutons.	Bioprotocol	2024	Calcium channels at synaptic boutons are critical for synaptic function, but their number and distribution are poorly understood. This gap in knowledge is primarily due to the resolution limits of fluorescent microscopy. In the last decade, the diffraction limit of light was surpassed, and fluorescent molecules can now be localized with nanometer precision. Concurrently, new gene editing strategies allowed direct tagging of the endogenous calcium channel genes-expressed in the correct cells and at physiological levels. Finally, the repurposing of self-labeling enzymes to attach fluorescent dyes to proteins improved photon yields enabling efficient localization of single molecules. Here, we describe tagging strategies, localization microscopy, and data analysis for calcium channel localization. In this case, we are imaging calcium channels fused with SNAP or HALO tags in live anesthetized <i>C. elegans</i> nematodes, but the analysis is relevant for any super-resolution preparations. We describe how to process images into localizations and protein clusters into confined nanodomains. Finally, we discuss strategies for estimating the number of calcium channels present at synaptic boutons.	Direct Imaging of Synaptic Structure
Natalie J. Guzikowski and Ege T. Kavalali	Functional specificity of liquid-liquid phase separation at the synapse.	Nature Communications	2024	The mechanisms that enable synapses to achieve temporally and spatially precise signaling at nano-scale while being fluid with the cytosol are poorly understood. Liquid-liquid phase separation (LLPS) is emerging as a key principle governing subcellular organization; however, the impact of synaptic LLPS on neurotransmission is unclear. Here, using rat primary hippocampal cultures, we show that robust disruption of neuronal LLPS with aliphatic alcohols severely dysregulates action potential-dependent neurotransmission, while spontaneous neurotransmission persists. Synaptic LLPS maintains synaptic vesicle pool clustering and recycling as well as the precise organization of active zone RIM1/2 and Munc13 nanoclusters, thus supporting fast evoked Ca ²⁺ -dependent release. These results indicate although LLPS is necessary within the nanodomain of the synapse, the disruption of this nanoorganization largely spares spontaneous neurotransmission. Therefore, properties of in vitro micron sized liquid condensates translate to the nanostructure of the synapse in a functionally specific manner regulating action potential-evoked release.	Synaptic Organization, Control of Signalling
Zhu S, Bradfield CJ, Maminska A, Park ES, Kim BH, Kumar P, Huang S, Kim M, Zhang Y, Bewersdorf J, and MacMicking JD	Native architecture of a human GBP1 defense complex for cell-autonomous immunity to infection.	Science	2024	All living organisms deploy cell-autonomous defenses to combat infection. In plants and animals, large supramolecular complexes often activate immune proteins for protection. In this work, we resolved the native structure of a massive host-defense complex that polymerizes 30,000 guanylate-binding proteins (GBPs) over the surface of gram-negative bacteria inside human cells. Construction of this giant nanomachine took several minutes and remained stable for hours, required guanosine triphosphate hydrolysis, and recruited four GBPs plus caspase-4 and Gasdermin D as a cytokine and cell death immune signaling platform. Cryo-electron tomography suggests that GBP1 can adopt an extended conformation for bacterial membrane insertion to establish this platform, triggering lipopolysaccharide release that activated coassembled caspase-4. Our "open conformer" model provides a dynamic view into how the human GBP1 defense complex mobilizes innate immunity to infection.	Cells; Membrane; Bacteria; Cellular Structures
Yuan Z, Pavel MA, and Hansen SB	GABA and astrocytic cholesterol determine the lipid environment of GABA(A)R in cultured cortical neurons.	bioRxiv	2024	The gamma-aminobutyric acid (GABA) type A receptor (GABA(A)R), a GABA activated pentameric chloride channel, mediates fast inhibitory neurotransmission in the brain. The lipid environment is critical for GABA(A)R function. How lipids regulate the channel in the cell membrane is not fully understood. Here we employed super resolution imaging of lipids to demonstrate that the agonist GABA induces a rapid and reversible membrane translocation of GABA(A)R to phosphatidylinositol 4,5- bisphosphate (PIP(2)) clusters in mouse primary cortical neurons. This translocation relies on nanoscopic separation of PIP(2) clusters and lipid rafts (cholesterol-dependent ganglioside clusters). In a resting state, the GABA(A)R associates with lipid rafts and this colocalization is enhanced by uptake of astrocytic secretions. These astrocytic secretions enhance endocytosis and delay desensitization. Our findings suggest intercellular signaling from astrocytes regulates GABA(A)R location based on lipid uptake in neurons. The findings have implications for treating mood disorders associated with altered neural excitability.	

Bruker Super-Resolution Publications

Wang Z, Zhou Y, Hao Y, Zhao Z, Gao A, Ma H, Zhang P, Shen Q, Xu R, Xu Y, Dang D, and Meng L	One Stone, Two Birds: High-Brightness Aggregation-Induced Emission Photosensitizers for Super-Resolution Imaging and Photodynamic Therapy.	Nano Lett	2024	Most aggregation-induced emission (AIE) luminogens exhibit high brightness, excellent photostability, and good biocompatibility, but these AIE-active agents, which kill two birds with one stone to result in applications in both stimulated emission depletion (STED) super-resolution imaging and photodynamic therapy (PDT), have not been reported yet but are urgently needed. To meet the requirements of STED nanoscopy and PDT, D-A-pi-A-D type DTPABT-HP is designed by tuning conjugated pi spacers. It exhibits red-shifted emission, high PLQY of 32.04%, and impressive (1)O(2) generation (9.24 fold compared to RB) in nanoparticles (NPs). Then, DTPABT-HP NPs are applied in cell imaging via STED nanoscopy, especially visualizing the dynamic changes of lysosomes in the PDT process at ultrahigh resolution. After that, in vivo PDT was also conducted by DTPABT-HP NPs, resulting in significantly inhibited tumor growth, with an inhibition rate of 86%. The work here is beneficial to the design of multifunctional agents and the deep understanding of their phototheranostic mechanism in biological research.	Diseases; Eukaryota; Chemicals and Drugs
Struckman HL, Moise N, Vanslebrouck B, Rothacker N, Chen Z, van Hengel J, Weinberg SH, and Veeraraghavan R	Indirect Correlative Light and Electron Microscopy (iCLEM): A Novel Pipeline for Multiscale Quantification of Structure From Molecules to Organs.	Microsc Microanal	2024	Correlative light and electron microscopy (CLEM) methods are powerful methods that combine molecular organization (from light microscopy) with ultrastructure (from electron microscopy). However, CLEM methods pose high cost/difficulty barriers to entry and have very low experimental throughput. Therefore, we have developed an indirect correlative light and electron microscopy (iCLEM) pipeline to sidestep the rate-limiting steps of CLEM (i.e., preparing and imaging the same samples on multiple microscopes) and correlate multiscale structural data gleaned from separate samples imaged using different modalities by exploiting biological structures identifiable by both light and electron microscopy as intrinsic fiducials. We demonstrate here an application of iCLEM, where we utilized gap junctions and mechanical junctions between muscle cells in the heart as intrinsic fiducials to correlate ultrastructural measurements from transmission electron microscopy (TEM), and focused ion beam scanning electron microscopy (FIB-SEM) with molecular organization from confocal microscopy and single molecule localization microscopy (SMLM). We further demonstrate how iCLEM can be integrated with computational modeling to discover structure-function relationships. Thus, we present iCLEM as a novel approach that complements existing CLEM methods and provides a generalizable framework that can be applied to any set of imaging modalities, provided suitable intrinsic fiducials can be identified.	Cells; Membrane; Cellular Structures; Microscopy
Smyth JW, Guo S, O'Rourke L, Deaver S, Dahlka J, Nurmamedov E, Sheng Z, Gourdie RG, and Lamouille S	Increased interaction between connexin43 and microtubules is critical for glioblastoma stem-like cell maintenance and tumorigenicity.	bioRxiv	2024	Glioblastoma (GBM) is the most common primary tumor of the central nervous system. One major challenge in GBM treatment is the resistance to chemotherapy and radiotherapy observed in subpopulations of cancer cells, including GBM stem-like cells (GSCs). These cells hold the ability to self-renew or differentiate following treatment, participating in tumor recurrence. The gap junction protein connexin43 (Cx43) has complex roles in oncogenesis and we have previously demonstrated an association between Cx43 and GBM chemotherapy resistance. Here, we report, for the first time, increased direct interaction between non-junctional Cx43 with microtubules in the cytoplasm of GSCs. We hypothesize that non-junctional Cx43/microtubule complexing is critical for GSC maintenance and survival and sought to specifically disrupt this interaction while maintaining other Cx43 functions, such as gap junction formation. Using a Cx43 mimetic peptide of the carboxyl terminal tubulin-binding domain of Cx43 (JM2), we successfully ablated Cx43 interaction with microtubules in GSCs. Importantly, administration of JM2 significantly decreased GSC survival in vitro, and limited GSC-derived tumor growth in vivo. Together, these results identify JM2 as a novel peptide drug to ablate GSCs in GBM treatment.	

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Saladin L, Breton V, Le Berruyer V, Nazac P, Lequeu T, Didier P, Danglot L, and Collet M	Targeted Photoconvertible BODIPYs Based on Directed Photooxidation-Induced Conversion for Applications in Photoconversion and Live Super-Resolution Imaging.	J Am Chem Soc	2024	Photomodulable fluorescent probes are drawing increasing attention due to their applications in advanced bioimaging. Whereas photoconvertible probes can be advantageously used in tracking, photoswitchable probes constitute key tools for single-molecule localization microscopy to perform super-resolution imaging. Herein, we shed light on a red and far-red BODIPY, namely, BDP-576 and BDP-650, which possess both properties of conversion and switching. Our study demonstrates that these pyrrolyl-BODIPYs convert into typical green- and red-emitting BODIPYs that are perfectly adapted to microscopy. We also showed that this pyrrolyl-BODIPYs undergo Directed Photooxidation Induced Conversion, a photoconversion mechanism that we recently introduced, where the pyrrole moiety plays a central role. These unique features were used to develop targeted photoconvertible probes toward different organelles or subcellular units (plasma membrane, mitochondria, nucleus, actin, Golgi apparatus, etc.) using chemical targeting moieties and a Halo tag. We notably showed that BDP-650 could be used to track intracellular vesicles over more than 20 min in two-color imaging with laser scanning confocal microscopy, demonstrating its robustness. The switching properties of these photoconverters were studied at the single-molecule level and were then successfully used in live single-molecule localization microscopy in epithelial cells and neurons. Both membrane- and mitochondria-targeted probes could be used to decipher membrane 3D architecture and mitochondrial dynamics at the nanoscale. This study builds a bridge between the photoconversion and photoswitching properties of probes undergoing directed photooxidation and shows the versatility and efficacy of this mechanism in advanced live imaging.	Cells; Nervous System; Eukaryota; Chemicals and Drugs
Pourmorady AD, Bashkirova EV, Chiariello AM, Belagzhal H, Kodra A, Duffie R, Kahiapo J, Monahan K, Pulupa J, Schieren I, Osterhoudt A, Dekker J, Nicodemi M, and Lomvardas S	RNA-mediated symmetry breaking enables singular olfactory receptor choice.	Nature	2024	Olfactory receptor (OR) choice provides an extreme example of allelic competition for transcriptional dominance, where every olfactory neuron stably transcribes one of approximately 2,000 or more OR alleles (1,2). OR gene choice is mediated by a multichromosomal enhancer hub that activates transcription at a single OR (3,4), followed by OR-translation-dependent feedback that stabilizes this choice (5,6). Here, using single-cell genomics, we show formation of many competing hubs with variable enhancer composition, only one of which retains euchromatic features and transcriptional competence. Furthermore, we provide evidence that OR transcription recruits enhancers and reinforces enhancer hub activity locally, whereas OR RNA inhibits transcription of competing ORs over distance, promoting transition to transcriptional singularity. Whereas OR transcription is sufficient to break the symmetry between equipotent enhancer hubs, OR translation stabilizes transcription at the prevailing hub, indicating that there may be sequential non-coding and coding mechanisms that are implemented by OR alleles for transcriptional prevalence. We propose that coding OR mRNAs possess non-coding functions that influence nuclear architecture, enhance their own transcription and inhibit transcription from their competitors, with generalizable implications for probabilistic cell fate decisions.	Cells; Nervous System; Genetic Phenomena; Genetic Structures
Petersen EN, Pavel MA, Hansen SS, Gudheti M, Wang H, Yuan Z, Murphy KR, Ja W, Ferris HA, Jorgensen E, and Hansen SB	Mechanical activation of TWIK-related potassium channel by nanoscopic movement and rapid second messenger signaling.	Elife	2024	Rapid conversion of force into a biological signal enables living cells to respond to mechanical forces in their environment. The force is believed to initially affect the plasma membrane and then alter the behavior of membrane proteins. Phospholipase D2 (PLD2) is a mechanosensitive enzyme that is regulated by a structured membrane-lipid site comprised of cholesterol and saturated ganglioside (GM1). Here we show stretch activation of TWIK-related K(+) channel (TREK-1) is mechanically evoked by PLD2 and spatial patterning involving ordered GM1 and 4,5-bisphosphate (PIP(2)) clusters in mammalian cells. First, mechanical force deforms the ordered lipids, which disrupts the interaction of PLD2 with the GM1 lipids and allows a complex of TREK-1 and PLD2 to associate with PIP(2) clusters. The association with PIP(2) activates the enzyme, which produces the second messenger phosphatidic acid (PA) that gates the channel. Co-expression of catalytically inactive PLD2 inhibits TREK-1 stretch currents in a biological membrane. Cellular uptake of cholesterol inhibits TREK-1 currents in culture and depletion of cholesterol from astrocytes releases TREK-1 from GM1 lipids in mouse brain. Depletion of the PLD2 ortholog in flies results in hypersensitivity to mechanical force. We conclude PLD2 mechanosensitivity combines with TREK-1 ion permeability to elicit a mechanically evoked response.	Cells; Membrane; Signal Transduction; Cellular Structures
Pearlman BS, Burget N, Zhou Y, Schwartz GW, Petrovic J, Modrusan Z, and Faryabi RB	Enhancer-promoter hubs organize transcriptional networks promoting oncogenesis and drug resistance.	bioRxiv	2024	Recent advances in high-resolution mapping of spatial interactions among regulatory elements support the existence of complex topological assemblies of enhancers and promoters known as enhancer-promoter hubs or cliques. Yet, organization principles of these multi-interacting enhancer-promoter hubs and their potential role in regulating gene expression in cancer remains unclear. Here, we systematically identified enhancer-promoter hubs in breast cancer, lymphoma, and leukemia. We found that highly interacting enhancer-promoter hubs form at key oncogenes and lineage-associated transcription factors potentially promoting oncogenesis of these diverse cancer types. Genomic and optical mapping of interactions among enhancer and promoter elements further showed that topological alterations in hubs coincide with transcriptional changes underlying acquired resistance to targeted therapy in T cell leukemia and B cell lymphoma. Together, our findings suggest that enhancer-promoter hubs are dynamic and heterogeneous topological assemblies with the potential to control gene expression circuits promoting oncogenesis and drug resistance.	

Bruker Super-Resolution Publications

Nakatani Y, Gaumer S, Shechtman Y, and Gustavsson AK	Long axial-range double-helix point spread functions for 3D volumetric super-resolution imaging.	bioRxiv	2024	Single-molecule localization microscopy (SMLM) is a powerful tool for observing structures beyond the diffraction limit of light. Combining SMLM with engineered point spread functions (PSFs) enables 3D imaging over an extended axial range, as has been demonstrated for super-resolution imaging of various cellular structures. However, super-resolving structures in 3D in thick samples, such as whole mammalian cells, remains challenging as it typically requires acquisition and post-processing stitching of multiple slices to cover the entire sample volume or more complex analysis of the data. Here, we demonstrate how the imaging and analysis workflows can be simplified by 3D single-molecule super-resolution imaging with long axial-range double-helix (DH)-PSFs. First, we experimentally benchmark the localization precisions of short- and long axial-range DH-PSFs at different signal-to-background ratios by imaging of fluorescent beads. The performance of the DH-PSFs in terms of achievable resolution and imaging speed was then quantified for 3D single-molecule super-resolution imaging of mammalian cells by DNA-PAINT imaging of the nuclear lamina protein lamin B1 in U-2 OS cells. Furthermore, we demonstrate how the use of a deep learning-based algorithm allows the localization of dense emitters, drastically improving the achievable imaging speed and resolution. Our data demonstrate that using long axial-range DH-PSFs offers stitching-free, 3D super-resolution imaging of whole mammalian cells, simplifying the experimental and analysis procedures for obtaining volumetric nanoscale structural information.	
Jain H, Kumar A, Almousa S, Mishra S, Langsten KL, Kim S, Sharma M, Su Y, Singh S, Kerr BA, and Deep G	Characterisation of LPS+ bacterial extracellular vesicles along the gut-hepatic portal vein-liver axis.	J Extracell Vesicles	2024	Gut microbiome dysbiosis is a major contributing factor to several pathological conditions. However, the mechanistic understanding of the communication between gut microbiota and extra-intestinal organs remains largely elusive. Extracellular vesicles (EVs), secreted by almost every form of life, including bacteria, could play a critical role in this inter-kingdom crosstalk and are the focus of present study. Here, we present a novel approach for isolating lipopolysaccharide (LPS)+ bacterial extracellular vesicles (bEV(LPS)) from complex biological samples, including faeces, plasma and the liver from lean and diet-induced obese (DIO) mice. bEV(LPS) were extensively characterised using nanoparticle tracking analyses, immunogold labelling coupled with transmission electron microscopy, flow cytometry, super-resolution microscopy and 16S sequencing. In liver tissues, the protein expressions of TLR4 and a few macrophage-specific biomarkers were assessed by immunohistochemistry, and the gene expressions of inflammation-related cytokines and their receptors (n = 89 genes) were measured using a PCR array. Faecal samples from DIO mice revealed a remarkably lower concentration of total EVs but a significantly higher percentage of LPS+ EVs. Interestingly, DIO faecal bEV(LPS) showed a higher abundance of Proteobacteria by 16S sequencing. Importantly, in DIO mice, a higher number of total EVs and bEV(LPS) consistently entered the hepatic portal vein and subsequently reached the liver, associated with increased expression of TLR4, macrophage markers (F4/80, CD86 and CD206), cytokines and receptors (Il1m, Ccr1, Cxcl10, Il2rg and Ccr2). Furthermore, a portion of bEV(LPS) escaped liver and entered the peripheral circulation. In conclusion, bEV could be the key mediator orchestrating various well-established biological effects induced by gut bacteria on distant organs.	Cells; EV; Bacteria; Cellular Structures
Chowdhury P, Wang X, Love JF, Vargas-Hernandez S, Nakatani Y, Grimm SL, Mezquita D, Mason FM, Martinez ED, Coarfa C, Walker CL, Gustavsson AK, and Dere R	Lysine Demethylase 4A is a Centrosome Associated Protein Required for Centrosome Integrity and Genomic Stability.	bioRxiv	2024	Centrosomes play a fundamental role in nucleating and organizing microtubules in the cell and are vital for faithful chromosome segregation and maintenance of genomic stability. Loss of structural or functional integrity of centrosomes causes genomic instability and is a driver of oncogenesis. The lysine demethylase 4A (KDM4A) is an epigenetic 'eraser' of chromatin methyl marks, which we show also localizes to the centrosome with single molecule resolution. We additionally discovered KDM4A demethylase enzymatic activity is required to maintain centrosome homeostasis, and is required for centrosome integrity, a new functionality unlinked to altered expression of genes regulating centrosome number. We find rather, that KDM4A interacts with both mother and daughter centriolar proteins to localize to the centrosome in all stages of mitosis. Loss of KDM4A results in supernumerary centrosomes and accrual of chromosome segregation errors including chromatin bridges and micronuclei, markers of genomic instability. In summary, these data highlight a novel role for an epigenetic 'eraser' regulating centrosome integrity, mitotic fidelity, and genomic stability at the centrosome.	

Bruker Super-Resolution Publications

Breton V, Nazac P, Boulet D, and Danglot L	Molecular mapping of neuronal architecture using STORM microscopy and new fluorescent probes for SMLM imaging.	Neurophotonics	2024	Imaging neuronal architecture has been a recurrent challenge over the years, and the localization of synaptic proteins is a frequent challenge in neuroscience. To quantitatively detect and analyze the structure of synapses, we recently developed free SODA software to detect the association of pre and postsynaptic proteins. To fully take advantage of spatial distribution analysis in complex cells, such as neurons, we also selected some new dyes for plasma membrane labeling. Using Icy SODA plugin, we could detect and analyze synaptic association in both conventional and single molecule localization microscopy, giving access to a molecular map at the nanoscale level. To replace those molecular distributions within the neuronal three-dimensional (3D) shape, we used MemBright probes and 3D STORM analysis to decipher the entire 3D shape of various dendritic spine types at the single-molecule resolution level. We report here the example of synaptic proteins within neuronal mask, but these tools have a broader spectrum of interest since they can be used whatever the proteins or the cellular type. Altogether with SODA plugin, MemBright probes thus provide the perfect toolkit to decipher a nanometricmolecular map of proteins within a 3D cellular context.	
Behrens L, Walter RM, Cai W, Ewers H, van Bommel B, and Zemella A	Fast In Vitro Synthesis and Direct Labeling of Nanobodies for Prototyping in Microscopy Applications.	ACS Omega	2024	Small antigen binders, such as nanobodies, have become widely used in biomedical research and pharmaceutical development. However, the pipeline for the generation of functional conjugated probes and drugs from identified binders remains a major time-consuming bottleneck. Here, we developed a method for fast nanobody production and conjugation based on an in vitro synthesis platform. Our system allows for small batch synthesis of nanobodies with the inclusion of a noncanonical amino acid (NCAA). This NCAA can then be used for direct conjugation of molecules to the synthesized nanobody using click-chemistry, reducing the time from binder-encoding DNA to a conjugated probe tremendously. In this study, we conjugated a fluorescent dye to an anti-Green fluorescent protein (GFP) nanobody and attained a fully functional probe suitable for advanced super-resolution microscopy within a short time frame of 2 days. Our work illustrates that an in vitro synthesis platform in combination with click-chemistry can be successfully employed to produce conjugated small antigen binding probes. The fast production and conjugation, combined with the possibility for parallelization as well as precise analysis by microscopy, forms an excellent platform for nanobody prototyping. The here-illustrated method can be used for quick selection and benchmarking of obtained nanobody sequences/clones, e. g., from a phage-display, for use as conjugated small-molecule carriers. This procedure can accelerate the bioengineering of nanobodies for research and pharmaceutical applications.	
Balakrishnan A, Glogger M, and Heilemann M	Quantitative Super-Resolution Imaging of ER-Phagy Initiation in Cells.	Methods Mol Biol	2024	Selective autophagy of the endoplasmic reticulum (ER-phagy) is a mechanism that is necessary for degrading damaged ER components and preventing cells from experiencing ER stress. Various ER-phagy receptors orchestrate this process by building protein assemblies with dedicated functions. In order to understand the molecular building principles of ER-phagy, it is important to reveal the assembly of ER-phagy receptors in a temporal and functional context. However, direct visualization is hampered by the diffraction limit in light microscopy. Super-resolution microscopy (SRM) can bypass this limitation and resolve single proteins and nanoscale protein clusters in cells. In particular, DNA points accumulation for imaging in nanoscale topography (DNA-PAINT) is a powerful technology that can resolve individual protein clusters in cells and provide information on their molecular composition. Here, we report a step-by-step protocol on how to utilize DNA-PAINT to perform super-resolution imaging of ER-phagy receptors in fixed cells. In addition, we provide a detailed explanation of image generation, cluster analysis, and molecular quantification.	Cells; Organelles; Cellular Structures; Microscopy
Zhang W, Lu CH, Nakamoto ML, Tsai CT, Roy AR, Lee CE, Yang Y, Jahed Z, Li X, and Cui B	Curved adhesions mediate cell attachment to soft matrix fibres in three dimensions.	Nat Cell Biol	2023	Integrin-mediated focal adhesions are the primary architectures that transmit forces between the extracellular matrix (ECM) and the actin cytoskeleton. Although focal adhesions are abundant on rigid and flat substrates that support high mechanical tensions, they are sparse in soft three-dimensional (3D) environments. Here we report curvature-dependent integrin-mediated adhesions called curved adhesions. Their formation is regulated by the membrane curvatures imposed by the topography of ECM protein fibres. Curved adhesions are mediated by integrin $\alpha 5 \beta 1$ and are molecularly distinct from focal adhesions and clathrin lattices. The molecular mechanism involves a previously unknown interaction between integrin $\beta 5$ and a curvature-sensing protein, FCHO2. We find that curved adhesions are prevalent in physiological conditions, and disruption of curved adhesions inhibits the migration of some cancer cell lines in 3D fibre matrices. These findings provide a mechanism for cell anchorage to natural protein fibres and suggest that curved adhesions may serve as a potential therapeutic target.	Cells; Membrane; Cellular Structures; Chemicals and Drugs

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Zhang W, Lu CH, Nakamoto ML, Tsai CT, Roy AR, Lee CE, Yang Y, Jahed Z, Li X, and Cui B	Curved adhesions mediate cell attachment to soft matrix fibres in 3D.	bioRxiv	2023	Mammalian cells adhere to the extracellular matrix (ECM) and sense mechanical cues through integrin-mediated adhesions(1, 2) . Focal adhesions and related structures are the primary architectures that transmit forces between the ECM and the actin cytoskeleton. Although focal adhesions are abundant when cells are cultured on rigid substrates, they are sparse in soft environments that cannot support high mechanical tensions (3) . Here, we report a new class of integrin-mediated adhesions, curved adhesions, whose formation is regulated by membrane curvature instead of mechanical tension. In soft matrices made of protein fibres, curved adhesions are induced by membrane curvatures imposed by the fibre geometry. Curved adhesions are mediated by integrin α Vbeta5 and are molecularly distinct from focal adhesions and clathrin lattices. The molecular mechanism involves a previously unknown interaction between integrin beta5 and a curvature-sensing protein FCHO2. We find that curved adhesions are prevalent in physiologically relevant environments. Disruption of curved adhesions by knocking down integrin beta5 or FCHO2 abolishes the migration of multiple cancer cell lines in 3D matrices. These findings provide a mechanism of cell anchorage to natural protein fibres that are too soft to support the formation of focal adhesions. Given their functional importance for 3D cell migration, curved adhesions may serve as a therapeutic target for future development.	
Yuan Z, and Hansen SB	Cholesterol Regulation of Membrane Proteins Revealed by Two-Color Super-Resolution Imaging.	Membranes (Basel)	2023	Cholesterol and phosphatidylinositol 4,5-bisphosphate (PIP(2)) are hydrophobic molecules that regulate protein function in the plasma membrane of all cells. In this review, we discuss how changes in cholesterol concentration cause nanoscopic (<200 nm) movements of membrane proteins to regulate their function. Cholesterol is known to cluster many membrane proteins (often palmitoylated proteins) with long-chain saturated lipids. Although PIP(2) is better known for gating ion channels, in this review, we will discuss a second independent function as a regulator of nanoscopic protein movement that opposes cholesterol clustering. The understanding of the movement of proteins between nanoscopic lipid domains emerged largely through the recent advent of super-resolution imaging and the establishment of two-color techniques to label lipids separate from proteins. We discuss the labeling techniques for imaging, their strengths and weakness, and how they are used to reveal novel mechanisms for an ion channel, transporter, and enzyme function. Among the mechanisms, we describe substrate and ligand presentation and their ability to activate enzymes, gate channels, and transporters rapidly and potently. Finally, we define cholesterol-regulated proteins (CRP) and discuss the role of PIP(2) in opposing the regulation of cholesterol, as seen through super-resolution imaging.	
Xu D, Jiang W, Wu L, Gaudet RG, Park ES, Su M, Cheppali SK, Cheemarla NR, Kumar P, Uchil PD, Grover JR, Foxman EF, Brown CM, Stansfeld PJ, Bewersdorf J, Mothes W, Karatekin E, Wilen CB, and MacMicking JD	PLSCR1 is a cell-autonomous defence factor against SARS-CoV-2 infection.	Nature	2023	Understanding protective immunity to COVID-19 facilitates preparedness for future pandemics and combats new SARS-CoV-2 variants emerging in the human population. Neutralizing antibodies have been widely studied; however, on the basis of large scale exome sequencing of protected versus severely ill patients with COVID-19, local cell-autonomous defence is also crucial(1-4). Here we identify phospholipid scramblase 1 (PLSCR1) as a potent cell-autonomous restriction factor against live SARS-CoV-2 infection in parallel genome-wide CRISPR-Cas9 screens of human lung epithelia and hepatocytes before and after stimulation with interferon-gamma (IFN γ). IFN γ -induced PLSCR1 not only restricted SARS-CoV-2 USA-WA1/2020, but was also effective against the Delta B.1.617.2 and Omicron BA.1 lineages. Its robust activity extended to other highly pathogenic coronaviruses, was functionally conserved in bats and mice, and interfered with the uptake of SARS-CoV-2 in both the endocytic and the TMPRSS2-dependent fusion routes. Whole-cell 4Pi single-molecule switching nanoscopy together with bipartite nano-reporter assays found that PLSCR1 directly targeted SARS-CoV-2-containing vesicles to prevent spike-mediated fusion and viral escape. A PLSCR1 C-terminal beta-barrel domain-but not lipid scramblase activity-was essential for this fusogenic blockade. Our mechanistic studies, together with reports that COVID-associated PLSCR1 mutations are found in some susceptible people(3,4), identify an anti-coronavirus protein that interferes at a late entry step before viral RNA is released into the host-cell cytosol.	Cells; Viruses; Diseases; Eukaryota
Wing PAC, Schmidt NM, Peters R, Erdmann M, Brown R, Wang H, Swadling L, Newman J, Thakur N, Shionoya K, Morgan SB, Hinks TS, Watashi K, Bailey D, Hansen SB, Davidson AD, Maini MK, and McKeating JA	An ACAT inhibitor suppresses SARS-CoV-2 replication and boosts antiviral T cell activity.	PLoS Pathog	2023	The severity of disease following infection with SARS-CoV-2 is determined by viral replication kinetics and host immunity, with early T cell responses and/or suppression of viraemia driving a favourable outcome. Recent studies uncovered a role for cholesterol metabolism in the SARS-CoV-2 life cycle and in T cell function. Here we show that blockade of the enzyme Acyl-CoA:cholesterol acyltransferase (ACAT) with Avasimibe inhibits SARS-CoV-2 pseudoparticle infection and disrupts the association of ACE2 and GM1 lipid rafts on the cell membrane, perturbing viral attachment. Imaging SARS-CoV-2 RNAs at the single cell level using a viral replicon model identifies the capacity of Avasimibe to limit the establishment of replication complexes required for RNA replication. Genetic studies to transiently silence or overexpress ACAT isoforms confirmed a role for ACAT in SARS-CoV-2 infection. Furthermore, Avasimibe boosts the expansion of functional SARS-CoV-2-specific T cells from the blood of patients sampled during the acute phase of infection. Thus, re-purposing of ACAT inhibitors provides a compelling therapeutic strategy for the treatment of COVID-19 to achieve both antiviral and immunomodulatory effects. Trial registration: NCT04318314.	Cells; Viruses; Immunology; Diseases

Bruker Super-Resolution Publications

Weiss LE, Love JF, Yoon J, Comerici CJ, Milenkovic L, Kanie T, Jackson PK, Stearns T, and Gustavsson AK	Single-molecule imaging in the primary cilium.	Methods Cell Biol	2023	The primary cilium is an important signaling organelle critical for normal development and tissue homeostasis. Its small dimensions and complexity necessitate advanced imaging approaches to uncover the molecular mechanisms behind its function. Here, we outline how single-molecule fluorescence microscopy can be used for tracking molecular dynamics and interactions and for super-resolution imaging of nanoscale structures in the primary cilium. Specifically, we describe in detail how to capture and quantify the 2D dynamics of individual transmembrane proteins PTC1 and SMO and how to map the 3D nanoscale distributions of the inversin compartment proteins INVS, ANKS6, and NPHP3. This protocol can, with minor modifications, be adapted for studies of other proteins and cell lines to further elucidate the structure and function of the primary cilium at the molecular level.	Cells; Signal Transduction; Cellular Structures; Microscopy
Tarasov M, Struckman HL, Olgar Y, Miller A, Demirtas M, Bogdanov V, Terentyeva R, Soltisz AM, Meng X, Min D, Sakuta G, Dunlap J, Duran AD, Foster MP, Davis JP, Terentyev D, Gyorke S, Veeraraghavan R, and Radwanski PB	NaV1.6 dysregulation within myocardial T-tubules by D96V calmodulin enhances proarrhythmic sodium and calcium mishandling.	J Clin Invest	2023	Calmodulin (CaM) plays critical roles in cardiomyocytes, regulating Na ⁺ (NaV) and L-type Ca ²⁺ channels (LTCCs). LTCC dysregulation by mutant CaMs has been implicated in action potential duration (APD) prolongation and arrhythmogenic long QT (LQT) syndrome. Intriguingly, D96V-CaM prolongs APD more than other LQT-associated CaMs despite inducing comparable levels of LTCC dysfunction, suggesting dysregulation of other depolarizing channels. Here, we provide evidence implicating NaV dysregulation within transverse (T) tubules in D96V-CaM-associated arrhythmias. D96V-CaM induced a proarrhythmic late Na ⁺ current (I _{Na}) by impairing inactivation of NaV1.6, but not the predominant cardiac NaV isoform NaV1.5. We investigated arrhythmia mechanisms using mice with cardiac-specific expression of D96V-CaM (cd96V). Super-resolution microscopy revealed close proximity of NaV1.6 and RyR2 within T-tubules. NaV1.6 density within these regions increased in cd96V relative to WT mice. Consistent with NaV1.6 dysregulation by D96V-CaM in these regions, we observed increased late NaV activity in T-tubules. The resulting late I _{Na} promoted aberrant Ca ²⁺ release and prolonged APD in myocytes, leading to LQT and ventricular tachycardia in vivo. Cardiac-specific NaV1.6 KO protected cd96V mice from increased T-tubular late NaV activity and its arrhythmogenic consequences. In summary, we demonstrate that D96V-CaM promoted arrhythmias by dysregulating LTCCs and NaV1.6 within T-tubules and thereby facilitating aberrant Ca ²⁺ release.	Cells; Diseases; Eukaryota; Chemicals and Drugs
Struckman HL, Moise N, King DR, Soltisz A, Buxton A, Dunlap J, Chen Z, Radwanski PB, Weinberg SH, and Veeraraghavan R	Unraveling Chamber-specific Differences in Intercalated Disc Ultrastructure and Molecular Organization and Their Impact on Cardiac Conduction.	bioRxiv	2023	During each heartbeat, the propagation of action potentials through the heart coordinates the contraction of billions of individual cardiomyocytes and is thus, a critical life process. Unsurprisingly, intercalated discs, which are cell-cell contact sites specialized to provide electrical and mechanical coupling between adjacent cardiomyocytes, have been the focus of much investigation. Slowed or disrupted propagation leads to potentially life-threatening arrhythmias in a wide range of pathologies, where intercalated disc remodeling is a common finding. Hence, the importance and urgency of understanding intercalated disc structure and its influence on action potential propagation. Surprisingly, however, conventional modeling approaches cannot predict changes in propagation elicited by perturbations that alter intercalated disc ultrastructure or molecular organization, owing to lack of quantitative structural data at subcellular through nano scales. In order to address this critical gap in knowledge, we sought to quantify intercalated disc structure at these finer spatial scales in the healthy adult mouse heart and relate them to function in a chamber-specific manner as a precursor to understanding the impacts of pathological intercalated disc remodeling. Using super-resolution light microscopy, electron microscopy, and computational image analysis, we provide here the first ever systematic, multiscale quantification of intercalated disc ultrastructure and molecular organization. By incorporating these data into a rule-based model of cardiac tissue with realistic intercalated disc structure, and comparing model predictions of electrical propagation with experimental measures of conduction velocity, we reveal that atrial intercalated discs can support faster conduction than their ventricular counterparts, which is normally masked by inter-chamber differences in myocyte geometry. Further, we identify key ultrastructural and molecular organization features underpinning the ability of atrial intercalated discs to support faster conduction. These data provide the first stepping stone to elucidating chamber-specific impacts of pathological intercalated disc remodeling, as occurs in many arrhythmic diseases.	

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Struckman HL, Moise N, King DR, Soltisz A, Buxton A, Dunlap I, Chen Z, Radwanski PB, Weinberg SH, and Veeraraghavan R	Unraveling Impacts of Chamber-Specific Differences in Intercalated Disc Ultrastructure and Molecular Organization on Cardiac Conduction.	JACC Clin Electrophysiol	2023	<p>BACKGROUND: Propagation of action potentials through the heart coordinates the heartbeat. Thus, intercalated discs, specialized cell-cell contact sites that provide electrical and mechanical coupling between cardiomyocytes, are an important target for study. Impaired propagation leads to arrhythmias in many pathologies, where intercalated disc remodeling is a common finding, hence the importance and urgency of understanding propagation dependence on intercalated disc structure. Conventional modeling approaches cannot predict changes in propagation elicited by perturbations that alter intercalated disc ultrastructure or molecular organization, because of lack of quantitative structural data at subcellular through nano scales. OBJECTIVES: This study sought to quantify intercalated disc structure at these spatial scales in the healthy adult mouse heart and relate them to chamber-specific properties of propagation as a precursor to understanding the effects of pathological intercalated disc remodeling. METHODS: Using super-resolution light microscopy, electron microscopy, and computational image analysis, we provide here the first ever systematic, multiscale quantification of intercalated disc ultrastructure and molecular organization. RESULTS: By incorporating these data into a rule-based model of cardiac tissue with realistic intercalated disc structure, and comparing model predictions of electrical propagation with experimental measures of conduction velocity, we reveal that atrial intercalated discs can support faster conduction than their ventricular counterparts, which is normally masked by interchamber differences in myocyte geometry. Further, we identify key ultrastructural and molecular organization features underpinning the ability of atrial intercalated discs to support faster conduction. CONCLUSIONS: These data provide the first stepping stone to elucidating chamber-specific effects of pathological intercalated disc remodeling, as occurs in many arrhythmic diseases.</p>	Cells; Diseases; Eukaryota

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<p>Sela M, Poley M, Mora-Raimundo P, Kagan S, Avital A, Kaduri M, Chen G, Adir O, Rozenzweig A, Weiss Y, Sade O, Leichtmann-Bardoogo Y, Simchi L, Aga-Mizrachi S, Bell B, Yeretz-Peretz Y, Or AZ, Choudhary A, Rosh I, Cordeiro D, Cohen-Adiv S, Berdichevsky Y, Odeh A, Shklover J, Shainsky-Roitman J, Schroeder JE, Hershkovitz D, Hasson P, Ashkenazi A, Stern S, Laviv T, Ben-Zvi A, Avital A, Ashery U, Maoz BM, and Schroeder A</p>	<p>Brain-Targeted Liposomes Loaded with Monoclonal Antibodies Reduce Alpha-Synuclein Aggregation and Improve Behavioral Symptoms in Parkinson's Disease.</p>	<p>Adv Mater 2023</p>	<p>Monoclonal antibodies (mAbs) hold promise in treating Parkinson's disease (PD), although poor delivery to the brain hinders their therapeutic application. In the current study, it is demonstrated that brain-targeted liposomes (BTL) enhance the delivery of mAbs across the blood-brain-barrier (BBB) and into neurons, thereby allowing the intracellular and extracellular treatment of the PD brain. BTL are decorated with transferrin to improve brain targeting through overexpressed transferrin-receptors on the BBB during PD. BTL are loaded with SynO4, a mAb that inhibits alpha-synuclein (AS) aggregation, a pathological hallmark of PD. It is shown that 100-nm BTL cross human BBB models intact and are taken up by primary neurons. Within neurons, SynO4 is released from the nanoparticles and bound to its target, thereby reducing AS aggregation, and enhancing neuronal viability. In vivo, intravenous BTL administration results in a sevenfold increase in mAbs in brain cells, decreasing AS aggregation and neuroinflammation. Treatment with BTL also improves behavioral motor function and learning ability in mice, with a favorable safety profile. Accordingly, targeted nanotechnologies offer a valuable platform for drug delivery to treat brain neurodegeneration.</p>	<p>Nervous System; Diseases; Eukaryota; Chemicals and Drugs</p>
<p>Sclip A, and Sudhof TC</p>	<p>Combinatorial expression of neurexins and LAR-type phosphotyrosine phosphatase receptors instructs assembly of a cerebellar circuit.</p>	<p>Nat Commun 2023</p>	<p>Synaptic adhesion molecules (SAMs) shape the structural and functional properties of synapses and thereby control the information processing power of neural circuits. SAMs are broadly expressed in the brain, suggesting that they may instruct synapse formation and specification via a combinatorial logic. Here, we generate sextuple conditional knockout mice targeting all members of the two major families of presynaptic SAMs, Neurexins and leukocyte common antigen-related-type receptor phospho-tyrosine phosphatases (LAR-PTPRs), which together account for the majority of known trans-synaptic complexes. Using synapses formed by cerebellar Purkinje cells onto deep cerebellar nuclei as a model system, we confirm that Neurexins and LAR-PTPRs themselves are not essential for synapse assembly. The combinatorial deletion of both neurexins and LAR-PTPRs, however, decreases Purkinje-cell synapses on deep cerebellar nuclei, the major output pathway of cerebellar circuits. Consistent with this finding, combined but not separate deletions of neurexins and LAR-PTPRs impair motor behaviors. Thus, Neurexins and LAR-PTPRs are together required for the assembly of a functional cerebellar circuit.</p>	<p>Cells; Nervous System; Eukaryota; Chemicals and Drugs</p>

Bruker Super-Resolution Publications

Sade O, Boneberg R, Weiss Y, Beldjilali-Labro M, Leichtmann-Bardoogo Y, Talpir I, Gottfried I, Ashery U, Rauti R, and Maoz BM	Super-Resolution-Chip: an in-vitro platform that enables super-resolution microscopy of co-cultures and 3D systems.	Biomed Opt Express	2023	The development of organs-on-a-chip platforms has revolutionized in-vitro cellular culture by allowing cells to be grown in an environment that better mimics human physiology. However, there is still a challenge in integrating those platforms with advanced imaging technology. This is extremely important when we want to study molecular changes and subcellular processes on the level of a single molecule using super-resolution microscopy (SRM), which has a resolution beyond the diffraction limit of light. Currently, existing platforms that include SRM have certain limitations, either as they only support 2D monocultures, without flow or as they demand a lot of production and handling. In this study, we developed a Super-Res-Chip platform, consisting of a 3D-printed chip and a porous membrane, that could be used to co-culture cells in close proximity either in 2D or in 3D while allowing SRM on both sides of the membrane. To demonstrate the functionality of the device, we co-cultured in endothelial and epithelial cells and used direct stochastic optical reconstruction microscopy (dSTORM) to investigate how glioblastoma cells affect the expression of the gap-junction protein Connexin43 in endothelial cells grown in 2D and in 3D. Cluster analysis of Connexin43 distribution revealed no difference in the number of clusters, their size, or radii, but did identify differences in their density. Furthermore, the spatial resolution was high also when the cells were imaged through the membrane (20-30 nm for x-y) and 10-20 nm when imaged directly both for 2D and 3D conditions. Overall, this chip allows to characterize of complex cellular processes on a molecular scale in an easy manner and improved the capacity for imaging in a single molecule resolution complex cellular organization.	
Nakamura DS, Gothie JM, Kornfeld SF, Kothary R, and Kennedy TE	Expression and subcellular localization of mitochondrial docking protein, syntaphilin, in oligodendrocytes and CNS myelin sheath.	Glia	2023	Oligodendrocytes produce lipid-rich myelin sheaths that provide metabolic support to the underlying axon and facilitates saltatory conduction. Oligodendrocyte mitochondria supply the bulk of energy and carbon-chain backbones required for lipid synthesis. The sparsity of mitochondria in the myelin sheath suggests that tight regulation of mitochondrial trafficking is crucial for their efficient distribution in the cell. In particular, retention of mitochondria at axoglial junctions would support local lipid synthesis and membrane remodeling during myelination. How mitochondrial docking in oligodendrocytes is regulated is not known. Our findings indicate that syntaphilin (SNPH), a mitochondrial docking protein that has been characterized in neurons, is expressed by oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes in vitro and present in the myelin sheath in vivo. We have previously reported that bath application of netrin-1 promotes the elaboration of myelin basic protein-positive membranes, and that localized presentation of a netrin-1 coated microbead results in rapid accumulation of mitochondria at the site of oligodendrocyte-bead adhesion. Here we show that netrin-1 increases the redistribution of SNPH to oligodendrocyte processes during the expansion of myelin basic protein-positive membranes and that SNPH clusters at the oligodendrocyte plasma membrane at sites of adhesion with netrin-1-coated beads where mitochondria are retained. These findings suggest roles for SNPH in oligodendrocytes regulating netrin-1-mediated mitochondrial docking and myelin membrane expansion.	Cells; Membrane; Organelles; Nervous System
Mueller BD, Merrill SA, Watanabe S, Liu P, Niu L, Singh A, Maldonado-Catala P, Cherry A, Rich MS, Silva M, Maricq AV, Wang ZW, and Jorgensen EM	CaV1 and CaV2 calcium channels mediate the release of distinct pools of synaptic vesicles.	Elife	2023	Activation of voltage-gated calcium channels at presynaptic terminals leads to local increases in calcium and the fusion of synaptic vesicles containing neurotransmitter. Presynaptic output is a function of the density of calcium channels, the dynamic properties of the channel, the distance to docked vesicles, and the release probability at the docking site. We demonstrate that at <i>Caenorhabditis elegans</i> neuromuscular junctions two different classes of voltage-gated calcium channels, CaV2 and CaV1, mediate the release of distinct pools of synaptic vesicles. CaV2 channels are concentrated in densely packed clusters ~250 nm in diameter with the active zone proteins Neurexin, alpha-Liprin, SYDE, ELKS/CAST, RIM-BP, alpha-Catulin, and MAGI1. CaV2 channels are colocalized with the priming protein UNC-13L and mediate the fusion of vesicles docked within 33 nm of the dense projection. CaV2 activity is amplified by ryanodine receptor release of calcium from internal stores, triggering fusion up to 165 nm from the dense projection. By contrast, CaV1 channels are dispersed in the synaptic varicosity, and are colocalized with UNC-13S. CaV1 and ryanodine receptors are separated by just 40 nm, and vesicle fusion mediated by CaV1 is completely dependent on the ryanodine receptor. Distinct synaptic vesicle pools, released by different calcium channels, could be used to tune the speed, voltage-dependence, and quantal content of neurotransmitter release.	Cells; Synapses; Membrane; Organelles

Bruker Super-Resolution Publications

Mezache L, Soltisz AM, Johnstone SR, Isakson BE, and Veeraraghavan R

[Vascular Endothelial Barrier Protection Prevents Atrial Fibrillation by Preserving Cardiac Nanostructure.](#)

JACC Clin Electrophysiol 2023

BACKGROUND: Atrial fibrillation (AF), the most common cardiac arrhythmia, is widely associated with inflammation, vascular dysfunction, and elevated levels of the vascular leak-inducing cytokine, vascular endothelial growth factor (VEGF). Mechanisms underlying AF are poorly understood and current treatments only manage this progressive disease, rather than arresting the underlying pathology. The authors previously identified edema-induced disruption of sodium channel (NaV1.5)-rich intercalated disk nanodomains as a novel mechanism for AF initiation secondary to acute inflammation. Therefore, we hypothesized that protecting the vascular barrier can prevent vascular leak-induced atrial arrhythmias. OBJECTIVES: In this study the authors tested the hypothesis that protecting the vascular barrier can prevent vascular leak-induced atrial arrhythmias. They identified 2 molecular targets for vascular barrier protection, connexin43 (Cx43) hemichannels and pannexin-1 (Pannx1) channels, which have been implicated in cytokine-induced vascular leak. METHODS: The authors undertook in vivo electrocardiography, electron microscopy, and super-resolution light microscopy studies in mice acutely treated with a clinically relevant level of VEGF. RESULTS: AF incidence was increased in untreated mice exposed to VEGF relative to vehicle control subjects. VEGF also increased the average number of AF episodes. VEGF shifted Na(V)1.5 signal to longer distances from Cx43 gap junctions, measured by a distance transformation-based spatial analysis of 3-dimensional confocal images of intercalated disks. Similar effects were observed with Na(V)1.5 localized near mechanical junctions composed of neural cadherin. Blocking connexin43 hemichannels (alphaCT11 peptide) or Pannx1 channels (Px1L2P peptide) significantly reduced the duration of AF episodes compared with VEGF alone with no treatment. Concurrently, both peptide therapies preserved Na(V)1.5 distance from gap junctions to control levels and reduced mechanical junction-adjacent intermembrane distance in these hearts. Notably, similar antiarrhythmic efficacy was also achieved with clinically-relevant small-molecule inhibitors of Cx43 and Pannx1. CONCLUSIONS: These results highlight vascular barrier protection as an antiarrhythmic strategy following inflammation-induced vascular leak.

Cells; Diseases; Eukaryota; Chemicals and Drugs

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BACKGROUND: Atrial fibrillation (AF), the most common cardiac arrhythmia, is widely associated with inflammation, vascular dysfunction, and elevated levels of the vascular leak-inducing cytokine, vascular endothelial growth factor (VEGF). Mechanisms underlying AF are poorly understood and current treatments only manage this progressive disease, rather than arresting the underlying pathology. The authors previously identified edema-induced disruption of sodium channel (NaV1.5)-rich intercalated disk nanodomains as a novel mechanism for AF initiation secondary to acute inflammation. Therefore, we hypothesized that protecting the vascular barrier can prevent vascular leak-induced atrial arrhythmias. OBJECTIVES: In this study the authors tested the hypothesis that protecting the vascular barrier can prevent vascular leak-induced atrial arrhythmias. They identified 2 molecular targets for vascular barrier protection, connexin43 (Cx43) hemichannels and pannexin-1 (Pannx1) channels, which have been implicated in cytokine-induced vascular leak. METHODS: The authors undertook in vivo electrocardiography, electron microscopy, and super-resolution light microscopy studies in mice acutely treated with a clinically relevant level of VEGF. RESULTS: AF incidence was increased in untreated mice exposed to VEGF relative to vehicle control subjects. VEGF also increased the average number of AF episodes. VEGF shifted Na(V)1.5 signal to longer distances from Cx43 gap junctions, measured by a distance transformation-based spatial analysis of 3-dimensional confocal images of intercalated disks. Similar effects were observed with Na(V)1.5 localized near mechanical junctions composed of neural cadherin. Blocking connexin43 hemichannels (alphaCT11 peptide) or Pannx1 channels (Px1L2P peptide) significantly reduced the duration of AF episodes compared with VEGF alone with no treatment. Concurrently, both peptide therapies preserved Na(V)1.5 distance from gap junctions to control levels and reduced mechanical junction-adjacent intermembrane distance in these hearts. Notably, similar antiarrhythmic efficacy was also achieved with clinically-relevant small-molecule inhibitors of Cx43 and Pannx1. CONCLUSIONS: These results highlight vascular barrier protection as an antiarrhythmic strategy following inflammation-induced vascular leak.

Cells; Diseases; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Lin PY, Chen LY, Jiang M, Trotter JH, Seigneur E, and Sudhof TC	Neurexin-2: An inhibitory neurexin that restricts excitatory synapse formation in the hippocampus.	Sci Adv	2023	Neurexins are widely thought to promote synapse formation and to organize synapse properties. Here we found that in contrast to neurexin-1 and neurexin-3, neurexin-2 unexpectedly restricts synapse formation. In the hippocampus, constitutive or neuron-specific deletions of neurexin-2 nearly doubled the strength of excitatory CA3→CA1 region synaptic connections and markedly increased their release probability. No effect on inhibitory synapses was detected. Stochastic optical reconstruction microscopy (STORM) superresolution microscopy revealed that the neuron-specific neurexin-2 deletion elevated the density of excitatory CA1 region synapses nearly twofold. Moreover, hippocampal neurexin-2 deletions also increased synaptic connectivity in the CA1 region when induced in mature mice and impaired the cognitive flexibility of spatial memory. Thus, neurexin-2 controls the dynamics of hippocampal synaptic circuits by repressing synapse assembly throughout life, a restrictive function that markedly differs from that of neurexin-1 and neurexin-3 and of other synaptic adhesion molecules, suggesting that neurexins evolutionarily diverged into opposing pro- and antisynaptogenic organizers.	
Lee JH, Chiu JH, Ginga NJ, Ahmed T, Thouless MD, Liu Y, and Takayama S	Super-resolution imaging of linearized chromatin in tunable nanochannels.	Nanoscale Horiz	2023	Nanofluidic linearization and optical mapping of naked DNA have been reported in the research literature, and implemented in commercial instruments. However, the resolution with which DNA features can be resolved is still inherently limited by both Brownian motion and diffraction-limited optics. Direct analysis of native chromatin is further hampered by difficulty in electrophoretic manipulation, which is routinely used for DNA analysis. This paper describes the development of a three-layer, tunable, nanochannel system that enables non-electrophoretic linearization and immobilization of native chromatin. Furthermore, through careful selection of self-blinking fluorescent dyes and the design of the nanochannel system, we achieve direct stochastic optical reconstruction microscopy (dSTORM) super-resolution imaging of the linearized chromatin. As an initial demonstration, rDNA chromatin extracted from Tetrahymena is analyzed by multi-color imaging of total DNA, newly synthesized DNA, and newly synthesized histone H3. Our analysis reveals a relatively even distribution of newly synthesized H3 across two halves of the rDNA chromatin with palindromic symmetry, supporting dispersive nucleosome segregation. As a proof-of-concept study, our work achieves super-resolution imaging of native chromatin fibers linearized and immobilized in tunable nanochannels. It opens up a new avenue for collecting long-range and high-resolution epigenetic information as well as genetic information.	Cells; Chromosomes; Cell Nucleus; Cellular Structures
Kopach O, Sylantsev S, Bard L, Michaluk P, Heller JP, Gutierrez Del Arroyo A, Ackland GL, Gourine AV, and Rusakov DA	Human neutrophils communicate remotely via calcium-dependent glutamate-induced glutamate release.	iScience	2023	Neutrophils are white blood cells that are critical to acute inflammatory and adaptive immune responses. Their swarming-pattern behavior is controlled by multiple cellular cascades involving calcium-dependent release of various signaling molecules. Previous studies have reported that neutrophils express glutamate receptors and can release glutamate but evidence of direct neutrophil-neutrophil communication has been elusive. Here, we hold semi-suspended cultured human neutrophils in patch-clamp whole-cell mode to find that calcium mobilization induced by stimulating one neutrophil can trigger an N-methyl-D-aspartate (NMDA) receptor-driven membrane current and calcium signal in neighboring neutrophils. We employ an enzymatic-based imaging assay to image, in real time, glutamate release from neutrophils induced by glutamate released from their neighbors. These observations provide direct evidence for a positive-feedback inter-neutrophil communication that could contribute to mechanisms regulating communal neutrophil behavior.	
Kanie T, Love JF, Fisher SD, Gustavsson AK, and Jackson PK	A hierarchical pathway for assembly of the distal appendages that organize primary cilia.	bioRxiv	2023	Distal appendages are nine-fold symmetric blade-like structures attached to the distal end of the mother centriole. These structures are critical for formation of the primary cilium, by regulating at least four critical steps: ciliary vesicle recruitment, recruitment and initiation of intraflagellar transport (IFT), and removal of CP110. While specific proteins that localize to the distal appendages have been identified, how exactly each protein functions to achieve the multiple roles of the distal appendages is poorly understood. Here we comprehensively analyze known and newly discovered distal appendage proteins (CEP83, SCLT1, CEP164, TTBK2, FBF1, CEP89, KIZ, ANKRD26, PIDD1, LRRC45, NCS1, C3ORF14) for their precise localization, order of recruitment, and their roles in each step of cilia formation. Using CRISPR-Cas9 knockouts, we show that the order of the recruitment of the distal appendage proteins is highly interconnected and a more complex hierarchy. Our analysis highlights two protein modules, CEP83-SCLT1 and CEP164-TTBK2, as critical for structural assembly of distal appendages. Functional assay revealed that CEP89 selectively functions in RAB34(+) ciliary vesicle recruitment, while deletion of the integral components, CEP83-SCLT1-CEP164-TTBK2, severely compromised all four steps of cilium formation. Collectively, our analyses provide a more comprehensive view of the organization and the function of the distal appendage, paving the way for molecular understanding of ciliary assembly.	

Bruker Super-Resolution Publications

Hoffmann C, Rentsch J, Tsunoyama TA, Chhabra A, Aguilar Perez G, Chowdhury R, Trnka F, Korobeinikov AA, Shaib AH, Ganzella M, Giannone G, Rizzoli SO, Kusumi A, Ewers H, and Milovanovic D	Synapsin condensation controls synaptic vesicle sequestering and dynamics.	Nat Commun	2023	Neuronal transmission relies on the regulated secretion of neurotransmitters, which are packed in synaptic vesicles (SVs). Hundreds of SVs accumulate at synaptic boutons. Despite being held together, SVs are highly mobile, so that they can be recruited to the plasma membrane for their rapid release during neuronal activity. However, how such confinement of SVs corroborates with their motility remains unclear. To bridge this gap, we employ ultrafast single-molecule tracking (SMT) in the reconstituted system of native SVs and in living neurons. SVs and synapsin 1, the most highly abundant synaptic protein, form condensates with liquid-like properties. In these condensates, synapsin 1 movement is slowed in both at short (i.e., 60- nm) and long (i.e., several hundred-nm) ranges, suggesting that the SV-synapsin 1 interaction raises the overall packing of the condensate. Furthermore, two-color SMT and super-resolution imaging in living axons demonstrate that synapsin 1 drives the accumulation of SVs in boutons. Even the short intrinsically-disordered fragment of synapsin 1 was sufficient to restore the native SV motility pattern in synapsin triple knock-out animals. Thus, synapsin 1 condensation is sufficient to guarantee reliable confinement and motility of SVs, allowing for the formation of mesoscale domains of SVs at synapses in vivo.	Cells; Synapses; Membrane; Organelles
Guzikowski NJ, and Kavalali ET	Super-resolution imaging of synaptic scaffold proteins in rat hippocampal neurons.	STAR Protoc	2023	Visualizing the nano-organization of the synapse is fundamental to elucidating the structure-function relationship of the nervous system. The advent of super-resolution microscopy provides a tool to assess and quantify the dynamic organization of numerous proteins at the synapse. Here we present a protocol assessing inhibitory synapse scaffold protein, gephyrin, in rat primary hippocampal cultures using dSTORM microscopy. We delineate the steps for artemisinin treatment, immunocytochemistry, dSTORM image acquisition, single-molecule localization, and the analysis of synaptic scaffold protein dynamics. For complete details on the use and execution of this protocol, please refer to Guzikowski and Kavalali (2022).(1).	Cells; Synapses; Membrane; Nervous System
Dolphin AC	Distinct pools of synaptic vesicles are released by different calcium channels.	Cell Calcium	2023	Mueller et al. [1] uncover distinct roles for Ca(V)1 and Ca(V)2 channels in neurotransmitter release at the C. elegans neuromuscular junction. Although nanodomain coupling occurs via clustered Ca(V)2 channels, evidence is also presented that release of a separate vesicular pool is mediated by more peripheral, dispersed Ca(V)1 channels, requiring obligatory coupling with RYR to amplify the Ca(2+) signal.	Cells; Synapses; Organelles; Signal Transduction
Call IM, Bois JL, and Hansen SB	Super-resolution imaging of potassium channels with genetically encoded EGFP.	bioRxiv	2023	The plasma membrane is a well-organized structure of lipids and proteins, segmented into lipid compartments under 200 nm in size. This specific spatial patterning is crucial for the function of proteins and necessitates super-resolution imaging for its elucidation. Here, we establish that the genetically encoded enhanced green fluorescent protein (EGFP), when combined with direct optical reconstruction microscopy (dSTORM), tracks shear- and cholesterol-induced nanoscopic patterning of potassium channels overexpressed in HEK293T cells. Leveraging EGFP in dSTORM (EGFP-STORM), our findings indicate that cholesterol directs the C-terminus of TWIK-related potassium channel (TREK-1) to ceramide-enriched lipid ganglioside (GM1) clusters. In the absence of the C-terminus, the channel associates with phosphatidylinositol 4,5-bisphosphate (PIP(2)) cluster. Similarly, cholesterol derived from astrocytes repositions EGFP-tagged inward-rectifying potassium (Kir) channels into GM1 clusters. Without cholesterol, the channel aligns with PIP(2) lipids. We deduce that cholesterol's interaction with Kir sequesters the channel, separating it from its activating lipid PIP(2). Fundamentally, a genetically encoded EGFP tag should make any protein amenable to dSTORM analysis.	
Azzolin L, Eichenlaub M, Nagel C, Nairn D, Sanchez J, Unger L, Dossel O, Jadidi A, and Loewe A	Personalized ablation vs. conventional ablation strategies to terminate atrial fibrillation and prevent recurrence.	Europace	2023	AIMS: The long-term success rate of ablation therapy is still sub-optimal in patients with persistent atrial fibrillation (AF), mostly due to arrhythmia recurrence originating from arrhythmogenic sites outside the pulmonary veins. Computational modelling provides a framework to integrate and augment clinical data, potentially enabling the patient-specific identification of AF mechanisms and of the optimal ablation sites. We developed a technology to tailor ablations in anatomical and functional digital atrial twins of patients with persistent AF aiming to identify the most successful ablation strategy. METHODS AND RESULTS: Twenty-nine patient-specific computational models integrating clinical information from tomographic imaging and electro-anatomical activation time and voltage maps were generated. Areas sustaining AF were identified by a personalized induction protocol at multiple locations. State-of-the-art anatomical and substrate ablation strategies were compared with our proposed Personalized Ablation Lines (PersonAL) plan, which consists of iteratively targeting emergent high dominant frequency (HDF) regions, to identify the optimal ablation strategy. Localized ablations were connected to the closest non-conductive barrier to prevent recurrence of AF or atrial tachycardia. The first application of the HDF strategy had a success of >98% and isolated only 5-6% of the left atrial myocardium. In contrast, conventional ablation strategies targeting anatomical or structural substrate resulted in isolation of up to 20% of left atrial myocardium. After a second iteration of the HDF strategy, no further arrhythmia episode could be induced in any of the patient-specific models. CONCLUSION: The novel PersonAL in silico technology allows to unveil all AF-perpetuating areas and personalize ablation by leveraging atrial digital twins.	Diseases; Eukaryota

Bruker Super-Resolution Publications

Zhang X, Lin PY, Liakath-Ali K, and Sudhof TC	Teneurins assemble into presynaptic nanoclusters that promote synapse formation via postsynaptic non-teneurin ligands.	Nat Commun	2022	Extensive studies concluded that homophilic interactions between pre- and postsynaptic teneurins, evolutionarily conserved cell-adhesion molecules, encode the specificity of synaptic connections. However, no direct evidence is available to demonstrate that teneurins are actually required on both pre- and postsynaptic neurons for establishing synaptic connections, nor is it known whether teneurins are localized to synapses. Using super-resolution microscopy, we demonstrate that Teneurin-3 assembles into presynaptic nanoclusters of approximately 80 nm in most excitatory synapses of the hippocampus. Presynaptic deletions of Teneurin-3 and Teneurin-4 in the medial entorhinal cortex revealed that they are required for assembly of entorhinal cortex-CA1, entorhinal cortex-subiculum, and entorhinal cortex-dentate gyrus synapses. Postsynaptic deletions of teneurins in the CA1 region, however, had no effect on synaptic connections from any presynaptic input. Our data suggest that different from the current prevailing view, teneurins promote the establishment of synaptic connections exclusively as presynaptic cell-adhesion molecules, most likely via their nanomolar-affinity binding to postsynaptic latrophilins.	Cells; Synapses; Membrane; Nervous System
Zehtabian A, Muller PM, Goisser M, Obendorf L, Janisch L, Humpfer N, Rentsch J, and Ewers H	Precise measurement of nanoscopic septin ring structures with deep learning-assisted quantitative superresolution microscopy.	Mol Biol Cell	2022	The combination of image analysis and superresolution microscopy methods allows for unprecedented insight into the reorganization of macromolecular assemblies in cells. Advances in deep learning (DL)-based object recognition enable the automated processing of large amounts of data, resulting in high accuracy through averaging. However, while the analysis of highly symmetric structures of constant size allows for a resolution approaching the dimensions of structural biology, DL-based image recognition may introduce bias. This prohibits the development of readouts for processes that involve significant changes in size or shape of amorphous macromolecular complexes. Here we address this problem by using changes of septin ring structures in single molecule localization-based superresolution microscopy data as a paradigm. We identify potential sources of bias resulting from different training approaches by rigorous testing of trained models using real or simulated data covering a wide range of possible results. In a quantitative comparison of our models, we find that a trade-off exists between measurement accuracy and the range of recognized phenotypes. Using our thus verified models, we find that septin ring size can be explained by the number of subunits they are assembled from alone. Furthermore, we provide a new experimental system for the investigation of septin polymerization.	Cells; Cellular Structures; Microscopy; Chemicals and Drugs
Yuan Z, Pavel MA, Wang H, Kwachukwu JC, Mediouni S, Jablonski JA, Nettles KW, Reddy CB, Valente ST, and Hansen SB	Hydroxychloroquine blocks SARS-CoV-2 entry into the endocytic pathway in mammalian cell culture.	Commun Biol	2022	Hydroxychloroquine (HCQ), a drug used to treat lupus and malaria, was proposed as a treatment for SARS-coronavirus-2 (SARS-CoV-2) infection, albeit with controversy. In vitro, HCQ effectively inhibits viral entry, but its use in the clinic has been hampered by conflicting results. A better understanding of HCQ's mechanism of actions in vitro is needed. Recently, anesthetics were shown to disrupt ordered clusters of monosialotetrahexosylganglioside1 (GM1) lipid. These same lipid clusters recruit the SARS-CoV-2 surface receptor angiotensin converting enzyme 2 (ACE2) to endocytic lipids, away from phosphatidylinositol 4,5 bisphosphate (PIP(2)) clusters. Here we employed super-resolution imaging of cultured mammalian cells (VeroE6, A549, H1793, and HEK293T) to show HCQ directly perturbs clustering of ACE2 receptor with both endocytic lipids and PIP(2) clusters. In elevated (high) cholesterol, HCQ moves ACE2 nanoscopic distances away from endocytic lipids. In cells with resting (low) cholesterol, ACE2 primarily associates with PIP(2) clusters, and HCQ moves ACE2 away from PIP(2) clusters-erythromycin has a similar effect. We conclude HCQ inhibits viral entry through two distinct mechanisms in high and low tissue cholesterol and does so prior to inhibiting cathepsin-L. HCQ clinical trials and animal studies will need to account for tissue cholesterol levels when evaluating dosing and efficacy.	Cells; Viruses; Lipids; Eukaryota
Yuan Z, Pavel MA, Wang H, Kwachukwu JC, Mediouni S, Jablonski JA, Nettles KW, Reddy CB, Valente ST, and Hansen SB	Author Correction: Hydroxychloroquine blocks SARS-CoV-2 entry into the endocytic pathway in mammalian cell culture.	Commun Biol	2022	?	
Wang CS, Chanaday NL, Monteggia LM, and Kavalali ET	Probing the segregation of evoked and spontaneous neurotransmission via photobleaching and recovery of a fluorescent glutamate sensor.	Elife	2022	Synapses maintain both action potential-evoked and spontaneous neurotransmitter release; however, organization of these two forms of release within an individual synapse remains unclear. Here, we used photobleaching properties of iGluSnFR, a fluorescent probe that detects glutamate, to investigate the subsynaptic organization of evoked and spontaneous release in primary hippocampal cultures. In nonneuronal cells and neuronal dendrites, iGluSnFR fluorescence is intensely photobleached and recovers via diffusion of nonphotobleached probes with a time constant of ~10 s. After photobleaching, while evoked iGluSnFR events could be rapidly suppressed, their recovery required several hours. In contrast, iGluSnFR responses to spontaneous release were comparatively resilient to photobleaching, unless the complete pool of iGluSnFR was activated by glutamate perfusion. This differential effect of photobleaching on different modes of neurotransmission is consistent with a subsynaptic organization where sites of evoked glutamate release are clustered and corresponding iGluSnFR probes are diffusion restricted, while spontaneous release sites are broadly spread across a synapse with readily diffusible iGluSnFR probes.	Cells; Synapses; Membrane; Signal Transduction

Bruker Super-Resolution Publications

<p>Vanover D, Zuria C, Peck HE, Orr-Burks N, Joo JY, Murray J, Holladay N, Hobbs RA, Jung Y, Chaves LCS, Rotolo L, Lifland AW, Olivier AK, Li D, Saunders KO, Sempowski GD, Crowe JE Jr, Haynes BF, Lafontaine ER, Hogan RJ, and Santangelo PJ</p>	<p>Nebulized mRNA-Encoded Antibodies Protect Hamsters from SARS-CoV-2 Infection.</p>	<p>Adv Sci (Weinh)</p>	<p>2022</p>	<p>Despite the success of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccines, there remains a clear need for new classes of preventatives for respiratory viral infections due to vaccine hesitancy, lack of sterilizing immunity, and for at-risk patient populations, including the immunocompromised. While many neutralizing antibodies have been identified, and several approved, to treat COVID-19, systemic delivery, large doses, and high costs have the potential to limit their widespread use, especially in low- and middle-income countries. To use these antibodies more efficiently, an inhalable formulation is developed that allows for the expression of mRNA-encoded, membrane-anchored neutralizing antibodies in the lung to mitigate SARS-CoV-2 infections. First, the ability of mRNA-encoded, membrane-anchored, anti-SARS-CoV-2 antibodies to prevent infections in vitro is demonstrated. Next, it is demonstrated that nebulizer-based delivery of these mRNA-expressed neutralizing antibodies potently abrogates disease in the hamster model. Overall, these results support the use of nebulizer-based mRNA expression of neutralizing antibodies as a new paradigm for mitigating respiratory virus infections.</p>	<p>Viruses; Diseases; Nucleic Acids, Nucleotides, and Nucleosides; Eukaryota</p>
<p>Streiff ME, Corbin AC, Ahmad AA, Hunter C, and Sachse FB</p>	<p>TRPC1 channels underlie stretch-modulated sarcoplasmic reticulum calcium leak in cardiomyocytes.</p>	<p>Front Physiol</p>	<p>2022</p>	<p>Transient receptor potential canonical 1 (TRPC1) channels are Ca(2+)-permeable ion channels expressed in cardiomyocytes. An involvement of TRPC1 channels in cardiac diseases is widely established. However, the physiological role of TRPC1 channels and the mechanisms through which they contribute to disease development are still under investigation. Our prior work suggested that TRPC1 forms Ca(2+) leak channels located in the sarcoplasmic reticulum (SR) membrane. Prior studies suggested that TRPC1 channels in the cell membrane are mechanosensitive, but this was not yet investigated in cardiomyocytes or for SR localized TRPC1 channels. We applied adenoviral transfection to overexpress or suppress TRPC1 expression in neonatal rat ventricular myocytes (NRVMs). Transfections were evaluated with RT-qPCR, western blot, and fluorescent imaging. Single-molecule localization microscopy revealed high colocalization of exogenously expressed TRPC1 and the sarco/endoplasmic reticulum Ca(2+) ATPase (SERCA2). To test our hypothesis that TRPC1 channels contribute to mechanosensitive Ca(2+) SR leak, we directly measured SR Ca(2+) concentration ([Ca(2+)]_{SR}) using adenoviral transfection with a novel ratiometric genetically encoded SR-targeting Ca(2+) sensor. We performed fluorescence imaging to quantitatively assess [Ca(2+)]_{SR} and leak through TRPC1 channels of NRVMs cultured on stretchable silicone membranes. [Ca(2+)]_{SR} was increased in cells with suppressed TRPC1 expression vs. control and Transient receptor potential canonical 1- overexpressing cells. We also detected a significant reduction in [Ca(2+)]_{SR} in cells with Transient receptor potential canonical 1 overexpression when 10% uniaxial stretch was applied. These findings indicate that TRPC1 channels underlie the mechanosensitive modulation of [Ca(2+)]_{SR}. Our findings are critical for understanding the physiological role of TRPC1 channels and support the development of pharmacological therapies for cardiac diseases.</p>	
<p>Shorer Arbel Y, Bronstein Y, Dadosh T, Kamdjou T, Tsuriel S, Shapiro M, Katz BZ, and Herishanu Y</p>	<p>Spatial organization and early signaling of the B-cell receptor in CLL.</p>	<p>Front Immunol</p>	<p>2022</p>	<p>Most chronic lymphocytic leukemia (CLL) clones express B-cell receptors (BcR) of both IgM/IgD isotypes; however, 5%-10% of CLL cases express isotype-switched immunoglobulin G (IgG). The early signaling and spatial patterning of the various BcRs at steady state and after activation are still fully unresolved. Herein, we show higher expression of the BcR signalosome elements and a more robust constitutive cell-intrinsic proximal BcR signaling in CLL with unmutated IGHV expressing IgM isotype (IgM U-CLL), compared with IGHV-mutated CLL (M-CLL) expressing either IgM or IgG isotypes. IgM in U-CLL is frequently located in the membrane plane in polarized patches, occasionally in caps, and sometimes inside the cells. Among M-CLL, IgM is scattered laterally in the membrane plane in a similar pattern as seen in normal B cells, whereas IgG is dispersed around the cell membrane in smaller clusters than in IgM U-CLL. Upon BcR engagement, both IgG and IgM expressing M-CLL showed attenuated signaling and only slight spatial reorganization dynamics of BcR microclusters and internalization, compared with the extensive reorganization and internalization of the BcR in IgM expressing U-CLL. The global gene signature of IgG M-CLL was closely related to that of IgM M-CLL rather than IgM U-CLL. Overall, we report fundamental differences in the basal composition, biochemical status, and spatial organization of the BcR in the three examined immunogenetic CLL subtypes that correlate with their clinical behavior. On the basis of our findings, IgG class-switched M-CLL likely represents the same disease as IgM M-CLL rather than a different biological and/or clinical entity.</p>	<p>Signal Transduction; Diseases; Eukaryota; Chemicals and Drugs</p>

Bruker Super-Resolution Publications

Schwertz H, Rowley JW, Portier I, Middleton EA, Tolley ND, Campbell RA, Eustes AS, Chen K, and Rondina MT	Human platelets display dysregulated sepsis-associated autophagy induced by altered LC3 protein-protein interaction of the Vici-protein EPG5.	Autophagy	2022	<p>Platelets mediate central aspects of host responses during sepsis, an acute profoundly systemic inflammatory response due to infection. Macroautophagy/autophagy, which mediates critical aspects of cellular responses during inflammatory conditions, is known to be a functional cellular process in anucleate platelets, and is essential for normal platelet functions. Nevertheless, how sepsis may alter autophagy in platelets has never been established. Using platelets isolated from septic patients and matched healthy controls, we show that during clinical sepsis, the number of autophagosomes is increased in platelets, most likely due to an accumulation of autophagosomes, some containing mitochondria and indicative of mitophagy. Therefore, autophagy induction or early-stage autophagosome formation (as compared to decreased later-stage autophagosome maturation or autophagosome-late endosome/lysosome fusion) is normal or increased. This was consistent with decreased fusion of autophagosomes with lysosomes in platelets. EPG5 (ectopic P-granules autophagy protein 5 homolog), a protein essential for normal autophagy, expression did increase, while protein-protein interactions between EPG5 and MAP1LC3/LC3 (which orchestrate the fusion of autophagosomes and lysosomes) were significantly reduced in platelets during sepsis. Furthermore, data from a megakaryocyte model demonstrate the importance of TLR4 (toll like receptor 4), LPS-dependent signaling for regulating this mechanism. Similar phenotypes were also observed in platelets isolated from a patient with Vici syndrome: an inherited condition caused by a naturally occurring, loss-of-function mutation in EPG5. Together, we provide evidence that autophagic functions are aberrant in platelets during sepsis, due in part to reduced EPG5-LC3 interactions, regulated by TLR4 engagement, and the resultant accumulation of autophagosomes. Abbreviations: ACTB: beta actin; CLP: cecal ligation and puncture; Co-IP: co-immunoprecipitation; DAP: death associated protein; DMSO: dimethyl sulfoxide; EPG5: ectopic P-granules autophagy protein 5 homolog; ECL: enhanced chemiluminescence; HBSS: Hanks' balanced salt solution; HRP: horseradish peroxidase; ICU: intensive care unit; LPS: lipopolysaccharide; LAMP1: lysosomal associated membrane protein 1; MAP1LC3/LC3: microtubule associated protein 1 light chain 3; MTOR: mechanistic target of rapamycin kinase; MKs: megakaryocytes; PFA: paraformaldehyde; PBS: phosphate-buffered saline; PLA: proximity ligation assay; pRT-PCR: quantitative real-time polymerase chain reaction; RT: room temperature; SQSTM1/p62: sequestosome 1; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TLR4: toll like receptor 4; TEM: transmission electron microscopy; WGA: wheat germ agglutinin.</p>	Cells; Organelles; Cellular Structures; Diseases
Schmerl B, Gimber N, Kurokka B, Stumpf A, Rentsch J, Kunde SA, von Sivers J, Ewers H, Schmitz D, Freund C, Schmoranzler J, Rademacher N, and Shoichet SA	The synaptic scaffold protein MPP2 interacts with GABAA receptors at the periphery of the postsynaptic density of glutamatergic synapses.	PLoS Biol	2022	<p>Recent advances in imaging technology have highlighted that scaffold proteins and receptors are arranged in subsynaptic nanodomains. The synaptic membrane-associated guanylate kinase (MAGUK) scaffold protein membrane protein palmitoylated 2 (MPP2) is a component of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-associated protein complexes and also binds to the synaptic cell adhesion molecule SynCAM 1. Using superresolution imaging, we show that like SynCAM 1-MPP2 is situated at the periphery of the postsynaptic density (PSD). In order to explore MPP2-associated protein complexes, we used a quantitative comparative proteomics approach and identified multiple gamma-aminobutyric acid (GABA)A receptor subunits among novel synaptic MPP2 interactors. In line with a scaffold function for MPP2 in the assembly and/or modulation of intact GABAA receptors, manipulating MPP2 expression had effects on inhibitory synaptic transmission. We further show that GABAA receptors are found together with MPP2 in a subset of dendritic spines and thus highlight MPP2 as a scaffold that serves as an adaptor molecule, linking peripheral synaptic elements critical for inhibitory regulation to central structures at the PSD of glutamatergic synapses.</p>	Cells; Synapses; Membrane; Nervous System
Sando R, Ho ML, Liu X, and Sudhof TC	Engineered synaptic tools reveal localized cAMP signaling in synapse assembly.	J Cell Biol	2022	<p>The physiological mechanisms driving synapse formation are elusive. Although numerous signals are known to regulate synapses, it remains unclear which signaling mechanisms organize initial synapse assembly. Here, we describe new tools, referred to as "SynTAMs" for synaptic targeting molecules, that enable localized perturbations of cAMP signaling in developing postsynaptic specializations. We show that locally restricted suppression of postsynaptic cAMP levels or of cAMP-dependent protein-kinase activity severely impairs excitatory synapse formation without affecting neuronal maturation, dendritic arborization, or inhibitory synapse formation. In vivo, suppression of postsynaptic cAMP signaling in CA1 neurons prevented formation of both Schaffer-collateral and entorhinal-CA1/temporoammonic-path synapses, suggesting a general principle. Retrograde trans-synaptic rabies virus tracing revealed that postsynaptic cAMP signaling is required for continuous replacement of synapses throughout life. Given that postsynaptic latrophilin adhesion-GPCRs drive synapse formation and produce cAMP, we suggest that spatially restricted postsynaptic cAMP signals organize assembly of postsynaptic specializations during synapse formation.</p>	Cells; Synapses; Membrane; Signal Transduction

Bruker Super-Resolution Publications

Polyansky A, Shatz O, Fraiberg M, Shimoni E, Dadosh T, Mari M, Reggiori FM, Qin C, Han X, and Elazar Z	Phospholipid imbalance impairs autophagosome completion.	EMBO J	2022	Autophagy, a conserved eukaryotic intracellular catabolic pathway, maintains cell homeostasis by lysosomal degradation of cytosolic material engulfed in double membrane vesicles termed autophagosomes, which form upon sealing of single-membrane cisternae called phagophores. While the role of phosphatidylinositol 3-phosphate (PI3P) and phosphatidylethanolamine (PE) in autophagosome biogenesis is well-studied, the roles of other phospholipids in autophagy remain rather obscure. Here we utilized budding yeast to study the contribution of phosphatidylcholine (PC) to autophagy. We reveal for the first time that genetic loss of PC biosynthesis via the CDP-DAG pathway leads to changes in lipid composition of autophagic membranes, specifically replacement of PC by phosphatidylserine (PS). This impairs closure of the autophagic membrane and autophagic flux. Consequently, we show that choline-dependent recovery of de novo PC biosynthesis via the CDP-choline pathway restores autophagosome formation and autophagic flux in PC-deficient cells. Our findings therefore implicate phospholipid metabolism in autophagosome biogenesis.	Cells; Organelles; Cellular Structures; Lipids
Martineau RL, Bayles AV, Hung CS, Reyes KG, Helgeson ME, and Gupta MK	Engineering Gelation Kinetics in Living Silk Hydrogels by Differential Dynamic Microscopy Microrheology and Machine Learning.	Adv Biol (Weinh)	2022	Microbes embedded in hydrogels comprise one form of living material. Discovering formulations that balance potentially competing for mechanical and biological properties in living hydrogels-for example, gel time of the hydrogel formulation and viability of the embedded organisms-can be challenging. In this study, a pipeline is developed to automate the characterization of the gel time of hydrogel formulations. Using this pipeline, living materials comprised of enzymatically crosslinked silk and embedded E. coli-formulated from within a 4D parameter space-are engineered to gel within a pre-selected timeframe. Gelation time is estimated using a novel adaptation of microrheology analysis using differential dynamic microscopy (DDM). In order to expedite the discovery of gelation regime boundaries, Bayesian machine learning models are deployed with optimal decision-making under uncertainty. The rate of learning is observed to vary between artificial intelligence (AI)-assisted planning and human planning, with the fastest rate occurring during AI-assisted planning following a round of human planning. For a subset of formulations gelling within a targeted timeframe of 5-15 min, fluorophore production within the embedded cells is substantially similar across treatments, evidencing that gel time can be tuned independent of other material properties-at least over a finite range-while maintaining biological activity.	Bacteria; Microscopy; Eukaryota; Chemicals and Drugs
Kluzek M, Oppenheimer-Shaanan Y, Dadosh T, Morandi MI, Avinoam O, Raanan C, Goldsmith M, Goldberg R, and Klein J	Designer Liposomal Nanocarriers Are Effective Biofilm Eradicators.	ACS Nano	2022	Drug delivery via nanovehicles is successfully employed in several clinical settings, yet bacterial infections, forming microbial communities in the form of biofilms, present a strong challenge to therapeutic treatment due to resistance to conventional antimicrobial therapies. Liposomes can provide a versatile drug-vector strategy for biofilm treatment, but are limited by the need to balance colloidal stability with biofilm penetration. We have discovered a liposomal functionalization strategy, using membrane-embedded moieties of poly[2-(methacryloyloxy)ethyl phosphorylcholine], pMPC, that overcomes this limitation. Such pMPCylation results in liposomal stability equivalent to current functionalization strategies (mostly PEGylation, the present gold-standard), but with strikingly improved cellular uptake and cargo conveyance. Fluorimetry, cryo-electron, and fluorescence microscopies reveal a far-enhanced antibiotic delivery to model Pseudomonas aeruginosa biofilms by pMPC- liposomes, followed by faster cytosolic cargo release, resulting in significantly greater biofilm eradication than either PEGylation or free drug. Moreover, this combination of techniques uncovers the molecular mechanism underlying the enhanced interaction with bacteria, indicating it arises from bridging by divalent ions of the zwitterionic groups on the pMPC moieties to the negatively charged lipopolysaccharide chains emanating from the bacterial membranes. Our results point to pMPCylation as a transformative strategy for liposomal functionalization, leading to next-generation delivery systems for biofilm treatment.	Bacteria; Lipids; Chemicals and Drugs
Katona M, Bartok A, Nichtova Z, Csordas G, Berezhnaya E, Weaver D, Ghosh A, Varnai P, Yule DI, and Hajnoczky G	Capture at the ER-mitochondrial contacts licenses IP(3) receptors to stimulate local Ca(2+) transfer and oxidative metabolism.	Nat Commun	2022	Endoplasmic reticulum-mitochondria contacts (ERMCS) are restructured in response to changes in cell state. While this restructuring has been implicated as a cause or consequence of pathology in numerous systems, the underlying molecular dynamics are poorly understood. Here, we show means to visualize the capture of motile IP(3) receptors (IP3Rs) at ERMCS and document the immediate consequences for calcium signaling and metabolism. IP3Rs are of particular interest because their presence provides a scaffold for ERMCS that mediate local calcium signaling, and their function outside of ERMCS depends on their motility. Unexpectedly, in a cell model with little ERMCS Ca(2+) coupling, IP3Rs captured at mitochondria promptly mediate Ca(2+) transfer, stimulating mitochondrial oxidative metabolism. The Ca(2+) transfer does not require linkage with a pore-forming protein in the outer mitochondrial membrane. Thus, motile IP3Rs can traffic in and out of ERMCS, and, when 'parked', mediate calcium signal propagation to the mitochondria, creating a dynamic arrangement that supports local communication.	Cells; Organelles; Signal Transduction; Cellular Structures

Bruker Super-Resolution Publications

Guzikowski NJ, and Kavalali ET	Nano-organization of spontaneous GABAergic transmission directs its autonomous function in neuronal signaling.	Cell Rep	2022	Earlier studies delineated the precise arrangement of proteins that drive neurotransmitter release and postsynaptic signaling at excitatory synapses. However, spatial organization of neurotransmission at inhibitory synapses remains unclear. Here, we took advantage of the molecularly specific interaction of antimalarial artemisinins and the inhibitory synapse scaffold protein, gephyrin, to probe the functional organization of gamma-aminobutyric acid A receptor (GABA(A)R)-mediated neurotransmission in central synapses. Short-term application of artemisinins severely contracts the size and density of gephyrin and GABA _A gamma2 subunit clusters. This size contraction elicits a neuronal activity-independent increase in Bdnf expression due to a specific reduction in GABAergic spontaneous, but not evoked, neurotransmission. The same functional effect could be mimicked by disruption of microtubules that link gephyrin to the neuronal cytoskeleton. These results suggest that the GABAergic postsynaptic apparatus possesses a concentric center-surround organization, where the periphery of gephyrin clusters selectively maintains spontaneous GABAergic neurotransmission facilitating its autonomous function regulating Bdnf expression.	Cells; Synapses; Membrane; Signal Transduction
Glogger M, Wang D, Kompa J, Balakrishnan A, Hiblot J, Barth HD, Johnsson K, and Heilemann M	Synergizing Exchangeable Fluorophore Labels for Multitarget STED Microscopy.	ACS Nano	2022	Investigating the interplay of cellular proteins with optical microscopy requires multitarget labeling. Spectral multiplexing using high-affinity or covalent labels is limited in the number of fluorophores that can be discriminated in a single imaging experiment. Advanced microscopy methods such as STED microscopy additionally demand balanced excitation, depletion, and emission wavelengths for all fluorophores, further reducing multiplexing capabilities. Noncovalent, weak-affinity labels bypass this "spectral barrier" through label exchange and sequential imaging of different targets. Here, we combine exchangeable HaloTag ligands, weak-affinity DNA hybridization, and hydrophobic and protein-peptide interactions to increase labeling flexibility and demonstrate six-target STED microscopy in single cells. We further show that exchangeable labels reduce photobleaching as well as facilitate long acquisition times and multicolor live-cell and high-fidelity 3D STED microscopy. The synergy of different types of exchangeable labels increases the multiplexing capabilities in fluorescence microscopy, and by that, the information content of microscopy images.	Microscopy; Chemicals and Drugs
Fabre L, Rousset C, Monier K, Da Cruz-Boisson F, Bouvet P, Charreyre MT, Delair T, Fleury E, and Favier A	Fluorescent Polymer-AS1411-Aptamer Probe for dSTORM Super-Resolution Imaging of Endogenous Nucleolin.	Biomacromolecules	2022	Nucleolin is a multifunctional protein involved in essential biological processes. To precisely localize it and unravel its different roles in cells, fluorescence imaging is a powerful tool, especially super-resolution techniques. Here, we developed polymer-aptamer probes, both small and bright, adapted to direct stochastic optical reconstruction microscopy (dSTORM). Well-defined fluorescent polymer chains bearing fluorophores (AlexaFluor647) and a reactive end group were prepared via RAFT polymerization. The reactive end-group was then used for the oriented conjugation with AS1411, a DNA aptamer that recognizes nucleolin with high affinity. Conjugation via strain-promoted alkyne/azide click chemistry (SPAAC) between dibenzylcyclooctyne-ended fluorescent polymer chains and 3'-azido-functionalized nucleic acids proved to be the most efficient approach. In vitro and in cellulo evaluations demonstrated that selective recognition for nucleolin was retained. Their brightness and small size make these polymer-aptamer probes an appealing alternative to immunofluorescence, especially for super-resolution (10-20 nm) nanoscopy. dSTORM imaging demonstrated the ability of our fluorescent polymer- aptamer probe to provide selective and super-resolved detection of cell surface nucleolin.	Microscopy; Nucleic Acids, Nucleotides, and Nucleoproteins; Chemicals and Drugs
Chung KKH, Zhang Z, Kidd P, Zhang Y, Williams ND, Rollins B, Yang Y, Lin C, Baddeley D, and Bewersdorff J	Fluorogenic DNA-PAINT for faster, low-background super-resolution imaging.	Nat Methods	2022	DNA-based points accumulation for imaging in nanoscale topography (DNA-PAINT) is a powerful super-resolution microscopy method that can acquire high-fidelity images at nanometer resolution. It suffers, however, from high background and slow imaging speed, both of which can be attributed to the presence of unbound fluorophores in solution. Here we present two-color fluorogenic DNA-PAINT, which uses improved imager probe and docking strand designs to solve these problems. These self-quenching single-stranded DNA probes are conjugated with a fluorophore and quencher at the terminals, which permits an increase in fluorescence by up to 57-fold upon binding and unquenching. In addition, the engineering of base pair mismatches between the fluorogenic imager probes and docking strands allowed us to achieve both high fluorogenicity and the fast binding kinetics required for fast imaging. We demonstrate a 26-fold increase in imaging speed over regular DNA- PAINT and show that our new implementation enables three-dimensional super-resolution DNA-PAINT imaging without optical sectioning.	Microscopy; DNA; Nucleic Acids, Nucleotides, and Nucleosides; Chemicals and Drugs

Bruker Super-Resolution Publications

An SJ, Stagi M, Gould TJ, Wu Y, Mlodzianoski M, Rivera-Molina F, Toomre D, Strittmatter SM, De Camilli P, Bewersdorf J, and Zenisek D	Multimodal imaging of synaptic vesicles with a single probe.	Cell Rep Methods	2022	A complete understanding of synaptic-vesicle recycling requires the use of multiple microscopy methods to obtain complementary information. However, many currently available probes are limited to a specific microscopy modality, which necessitates the use of multiple probes and labeling paradigms. Given the complexity of vesicle populations and recycling pathways, having new single-vesicle probes that could be used for multiple microscopy techniques would complement existing sets of tools for studying vesicle function. Here, we present a probe based on the membrane-binding C2 domain of cytosolic phospholipase A(2) (cPLA(2)) that fulfills this need. By conjugating the C2 domain with different detectable tags, we demonstrate that a single, modular probe can allow synaptic vesicles to be imaged at multiple levels of spatial and temporal resolution. Moreover, as a general endocytic marker, the C2 domain may also be used to study membrane recycling in many cell types.	Cells; Synapses; Organelles; Nervous System
Albrecht NE, Jiang D, Akhanov V, Hobson R, Speer CM, Robichaux MA, and Samuel MA	Rapid 3D-STORM imaging of diverse molecular targets in tissue.	Cell Rep Methods	2022	Fine-scale molecular architecture is critical for nervous system and other biological functions. Methods to visualize these nanoscale structures would benefit from enhanced accessibility, throughput, and tissue compatibility. Here, we report RAIN-STORM, a rapid and scalable nanoscopic imaging optimization approach that improves three-dimensional visualization for subcellular targets in tissue at depth. RAIN-STORM uses conventional tissue samples and readily available reagents and is suitable for commercial instrumentation. To illustrate the efficacy of RAIN-STORM, we utilized the retina. We show that RAIN-STORM imaging is versatile and provide 3D nanoscopic data for over 20 synapse, neuron, glia, and vasculature targets. Sample preparation is also rapid, with a 1-day turnaround from tissue to image, and parameters are suitable for multiple tissue sources. Finally, we show that this method can be applied to clinical samples to reveal nanoscale features of human cells and synapses. RAIN-STORM thus paves the way for high-throughput studies of nanoscopic targets in tissue.	Cells; Synapses; Membrane; Nervous System
Fujun Luo, Alessandra Scip, Sean Merrill, and Thomas C. Südhof	Neurexins regulate presynaptic GABA(B)-receptors at central synapses.	Nature Communications	2021	Diverse signaling complexes are precisely assembled at the presynaptic active zone for dynamic modulation of synaptic transmission and synaptic plasticity. Presynaptic GABA _B receptors nucleate critical signaling complexes regulating neurotransmitter release at most synapses. However, the molecular mechanisms underlying assembly of GABA _B -receptor signaling complexes remain unclear. Here we show that neurexins are required for the localization and function of presynaptic GABA _B -receptor signaling complexes. At four model synapses, excitatory calyx of Held synapses in the brainstem, excitatory and inhibitory synapses on hippocampal CA1-region pyramidal neurons, and inhibitory basket cell synapses in the cerebellum, deletion of neurexins rendered neurotransmitter release significantly less sensitive to GABA _B -receptor activation. Moreover, deletion of neurexins caused a loss of GABA _B -receptors from the presynaptic active zone of the calyx synapse. These findings extend the role of neurexins at the presynaptic active zone to enabling GABA _B -receptor signaling, supporting the notion that neurexins function as central organizers of active zone signaling complexes.	Synaptic Organization, Control of Signalling
von Diezmann L, and Rog O	Single-Molecule Tracking of Chromatin-Associated Proteins in the C. elegans Gonad.	J Phys Chem B	2021	Biomolecules are distributed within cells by molecular-scale diffusion and binding events that are invisible in standard fluorescence microscopy. These molecular search kinetics are key to understanding nuclear signaling and chromosome organization and can be directly observed by single-molecule tracking microscopy. Here, we report a method to track individual proteins within intact C. elegans gonads and apply it to study the molecular dynamics of the axis, a proteinaceous backbone that organizes meiotic chromosomes. Using either fluorescent proteins or enzymatically ligated dyes, we obtain multisecond trajectories with a localization precision of 15-25 nm in nuclei actively undergoing meiosis. Correlation with a reference channel allows for accurate measurement of protein dynamics, compensating for movements of the nuclei and chromosomes within the gonad. We find that axis proteins exhibit either static binding to chromatin or free diffusion in the nucleoplasm, and we separately quantify the motion parameters of these distinct populations. Freely diffusing axis proteins selectively explore chromatin-rich regions, suggesting they are circumventing the central phase-separated region of the nucleus. This work demonstrates that single-molecule microscopy can infer nanoscale-resolution dynamics within living tissue, expanding the possible applications of this approach.	Cells; Chromosomes; Cell Nucleus; Cellular Structures

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Wang J, Allgeyer ES, Sirinakis G, Zhang Y, Hu K, Lessard MD, Li Y, Diekmann R, Phillips MA, Dobbie IM, Ries J, Booth MJ, and Bewersdorf J	Implementation of a 4Pi-SMS super-resolution microscope.	Nat Protoc	2021	The development of single-molecule switching (SMS) fluorescence microscopy (also called single-molecule localization microscopy) over the last decade has enabled researchers to image cell biological structures at unprecedented resolution. Using two opposing objectives in a so-called 4Pi geometry doubles the available numerical aperture, and coupling this with interferometric detection has demonstrated 3D resolution down to 10 nm over entire cellular volumes. The aim of this protocol is to enable interested researchers to establish 4Pi-SMS super-resolution microscopy in their laboratories. We describe in detail how to assemble the optomechanical components of a 4Pi-SMS instrument, align its optical beam path and test its performance. The protocol further provides instructions on how to prepare test samples of fluorescent beads, operate this instrument to acquire images of whole cells and analyze the raw image data to reconstruct super-resolution 3D data sets. Furthermore, we provide a troubleshooting guide and present examples of anticipated results. An experienced optical instrument builder will require ~12 months from the start of ordering hardware components to acquiring high-quality biological images.	Microscopy; Eukaryota
Taguchi K, Elias BC, Krystofiak E, Qian S, Sant S, Yang H, Fogo AB, and Brooks CR	Quantitative super-resolution microscopy reveals promoting mitochondrial interconnectivity protects against AKI.	Kidney360	2021	BACKGROUND: The root of many kidney diseases in humans can be traced to alterations or damage to subcellular organelles. Mitochondrial fragmentation, endoplasmic reticulum (ER) stress, and lysosomal inhibition, among others, ultimately contribute to kidney injury and are the target of therapeutics in development. Although recent technological advancements allow for the understanding of disease states at the cellular level, investigating changes in subcellular organelles from kidney tissue remains challenging. METHODS: Using structured illumination microscopy, we imaged mitochondria and other organelles from paraffin sections of mouse tissue and human kidney biopsy specimens. The resulting images were 3D rendered to quantify mitochondrial size, content, and morphology. Results were compared with those from transmission electron microscopy and segmentation. RESULTS: Super-resolution imaging reveals kidney tubular epithelial cell mitochondria in rodent and human kidney tissue form large, interconnected networks under basal conditions, which are fragmented with injury. This approach can be expanded to other organelles and cellular structures including autophagosomes, ER, brush border, and cell morphology. We find that, during unilateral ischemia, mitochondrial fragmentation occurs in most tubule cells, and they remain fragmented for >96 hours. Promoting mitochondrial fusion with the fusion promoter M1 preserves mitochondrial morphology and interconnectivity and protects against cisplatin-induced kidney injury. CONCLUSIONS: We provide, for the first time, a nonbiased, semiautomated approach for quantification of the 3D morphology of mitochondria in kidney tissue. Maintaining mitochondrial interconnectivity and morphology protects against kidney injury. Super-resolution imaging has the potential to both drive discovery of novel pathobiologic mechanisms in kidney tissue and broaden the diagnoses that can be made on human biopsy specimens.	Cells; Organelles; Cellular Structures; Microscopy
Sikora R, Bun P, Danglot L, Alqabandi M, Bassereau P, Niedergang F, Galli T, and Zahraoui A	MICAL-L1 is required for cargo protein delivery to the cell surface.	Biol Open	2021	Secreted proteins are transported along intracellular route from the endoplasmic reticulum through the Golgi before reaching the plasma membrane. Small GTPase Rab and their effectors play a key role in membrane trafficking. Using confocal microscopy, we showed that MICAL-L1 was associated with tubulo-vesicular structures and exhibited a significant colocalization with markers of the Golgi apparatus and recycling endosomes. Super resolution STORM microscopy suggested at the molecular level, a very close association of MICAL-L1 and microdomains in the Golgi cisternae. Using a synchronized secretion assay, we report that the shRNA-mediated depletion of MICAL-L1 impaired the delivery of a subset of cargo proteins to the cell surface. The process of membrane tubulation was monitored in vitro, and we observe that recombinant MICAL-L1-RBD domain may contribute to promote PACSINs-mediated membrane tubulation. Interestingly, two hydrophobic residues at the C-terminus of MICAL-L1 appeared to be important for phosphatidic acid binding, and for association with membrane tubules. Our results reveal a new role for MICAL-L1 in cargo delivery to the plasma membrane.	Cells; Membrane; Protein Binding; Cellular Structures

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Scalisi S, Pennacchietti F, Keshavan S, Derr ND, Diaspro A, Pisignano D, Pierzynska-Mach A, Dante S, and Cella Zancchi F	Quantitative Super-Resolution Microscopy to Assess Adhesion of Neuronal Cells on Single-Layer Graphene Substrates.	Membranes (Basel)	2021	Single Layer Graphene (SLG) has emerged as a critically important nanomaterial due to its unique optical and electrical properties and has become a potential candidate for biomedical applications, biosensors, and tissue engineering. Due to its intrinsic 2D nature, SLG is an ideal surface for the development of large-area biosensors and, due to its biocompatibility, can be easily exploited as a substrate for cell growth. The cellular response to SLG has been addressed in different studies with high cellular affinity for graphene often detected. Still, little is known about the molecular mechanism that drives/regulates the cellular adhesion and migration on SLG and SLG-coated interfaces with respect to other substrates. Within this scenario, we used quantitative super-resolution microscopy based on single-molecule localization to study the molecular distribution of adhesion proteins at the nanoscale level in cells growing on SLG and glass. In order to reveal the molecular mechanisms underlying the higher affinity of biological samples on SLG, we exploited stochastic optical reconstruction microscopy (STORM) imaging and cluster analysis, quantifying the super-resolution localization of the adhesion protein vinculin in neurons and clearly highlighting substrate-related correlations. Additionally, a comparison with an epithelial cell line (Chinese Hamster Ovary) revealed a cell dependent mechanism of interaction with SLG.	
Michaluk P, Heller JP, and Rusakov DA	Rapid recycling of glutamate transporters on the astroglial surface.	Elife	2021	Glutamate uptake by astroglial transporters confines excitatory transmission to the synaptic cleft. The efficiency of this mechanism depends on the transporter dynamics in the astrocyte membrane, which remains poorly understood. Here, we visualise the main glial glutamate transporter GLT1 by generating its pH-sensitive fluorescent analogue, GLT1-SEP. Fluorescence recovery after photobleaching-based imaging shows that 70-75% of GLT1-SEP dwell on the surface of rat brain astroglia, recycling with a lifetime of ~22 s. Genetic deletion of the C-terminus accelerates GLT1-SEP membrane turnover while disrupting its surface pattern, as revealed by single-molecule localisation microscopy. Excitatory activity boosts surface mobility of GLT1-SEP, involving its C-terminus, metabotropic glutamate receptors, intracellular Ca(2+), and calcineurin- phosphatase activity, but not the broad-range kinase activity. The results suggest that membrane turnover, rather than lateral diffusion, is the main 'redployment' route for the immobile fraction (20-30%) of surface-expressed GLT1. This finding reveals an important mechanism helping to control extrasynaptic escape of glutamate.	Cells; Nervous System; Chemicals and Drugs
Michaluk P, Heller JP, and Rusakov DA	Rapid recycling of glutamate transporters on the astroglial surface.	Elife	2021	Glutamate uptake by astroglial transporters confines excitatory transmission to the synaptic cleft. The efficiency of this mechanism depends on the transporter dynamics in the astrocyte membrane, which remains poorly understood. Here, we visualise the main glial glutamate transporter GLT1 by generating its pH-sensitive fluorescent analogue, GLT1-SEP. Fluorescence recovery after photobleaching-based imaging shows that 70-75% of GLT1-SEP dwell on the surface of rat brain astroglia, recycling with a lifetime of ~22 s. Genetic deletion of the C-terminus accelerates GLT1-SEP membrane turnover while disrupting its surface pattern, as revealed by single-molecule localisation microscopy. Excitatory activity boosts surface mobility of GLT1-SEP, involving its C-terminus, metabotropic glutamate receptors, intracellular Ca(2+), and calcineurin- phosphatase activity, but not the broad-range kinase activity. The results suggest that membrane turnover, rather than lateral diffusion, is the main 'redployment' route for the immobile fraction (20-30%) of surface-expressed GLT1. This finding reveals an important mechanism helping to control extrasynaptic escape of glutamate.	Cells; Nervous System; Eukaryota; Chemicals and Drugs
Haas KT, and Peaucelle A	Protocol for multicolor three-dimensional dSTORM data analysis using MATLAB-based script package Grafeo.	STAR Protoc	2021	This protocol describes the step-by-step analysis of the multicolor (one-, two-, or three-color) two- and three-dimensional dSTORM (direct Stochastic Optical Reconstruction Microscopy) data using MATLAB-based script package Grafeo. Grafeo primarily uses pointillist data visualization and analysis frameworks, including the nearest neighbors approach, Voronoi tessellation, Delaunay triangulation, Ripley's (K, L) and two-point correlation functions, and graph-based clustering. For complete details on the use and execution of this protocol, please refer to Peaucelle et al. (2020), Haas et al., 2018, Haas et al. (2020b).	Cells

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Gerguri T, Fu X, Kakui Y, Khatri BS, Barrington C, Bates PA, and Uhlmann F	Comparison of loop extrusion and diffusion capture as mitotic chromosome formation pathways in fission yeast.	Nucleic Acids Res	2021	Underlying higher order chromatin organization are Structural Maintenance of Chromosomes (SMC) complexes, large protein rings that entrap DNA. The molecular mechanism by which SMC complexes organize chromatin is as yet incompletely understood. Two prominent models posit that SMC complexes actively extrude DNA loops (loop extrusion), or that they sequentially entrap two DNAs that come into proximity by Brownian motion (diffusion capture). To explore the implications of these two mechanisms, we perform biophysical simulations of a 3.76 Mb-long chromatin chain, the size of the long Schizosaccharomyces pombe chromosome I left arm. On it, the SMC complex condensin is modeled to perform loop extrusion or diffusion capture. We then compare computational to experimental observations of mitotic chromosome formation. Both loop extrusion and diffusion capture can result in native-like contact probability distributions. In addition, the diffusion capture model more readily recapitulates mitotic chromosome axis shortening and chromatin compaction. Diffusion capture can also explain why mitotic chromatin shows reduced, as well as more anisotropic, movements, features that lack support from loop extrusion. The condensin distribution within mitotic chromosomes, visualized by stochastic optical reconstruction microscopy (STORM), shows clustering predicted from diffusion capture. Our results inform the evaluation of current models of mitotic chromosome formation.	Cells; Chromosomes; Cell Nucleus; Cellular Structures
Geertsema HJ, Aimola G, Fabricius V, Fuerste JP, Kaufer BB, and Ewers H	Left-handed DNA-PAINT for improved super-resolution imaging in the nucleus.	Nat Biotechnol	2021	DNA point accumulation in nanoscale topography (DNA-PAINT) increases the resolution and multiplexing capabilities of super-resolution imaging, but cellular DNA interferes with DNA-DNA hybridization between target and probe in the nucleus. Here, we introduce left-handed DNA (L-DNA) oligomers that do not hybridize to natural right-handed DNA (R-DNA) and demonstrate that L-DNA-PAINT has the same specificity and multiplexing capability as R-DNA-PAINT, but improves the imaging of nuclear targets by substantially reducing background signal.	Cells; Cell Nucleus; Organelles; Cellular Structures
Dhanyasi N, VijayRaghavan K, Shilo BZ, and Schejter ED	Microtubules provide guidance cues for myofibril and sarcomere assembly and growth.	Dev Dyn	2021	BACKGROUND: Muscle myofibrils and sarcomeres present exceptional examples of highly ordered cytoskeletal filament arrays, whose distinct spatial organization is an essential aspect of muscle cell functionality. We utilized ultra-structural analysis to investigate the assembly of myofibrils and sarcomeres within developing myotubes of the indirect flight musculature of Drosophila. RESULTS: A temporal sequence composed of three major processes was identified: subdivision of the unorganized cytoplasm of nascent, multi-nucleated myotubes into distinct organelle-rich and filament-rich domains; initial organization of the filament-rich domains into myofibrils harboring nascent sarcomeric units; and finally, maturation of the highly-ordered pattern of sarcomeric thick (myosin-based) and thin (microfilament-based) filament arrays in parallel to myofibril radial growth. Significantly, organized microtubule arrays were present throughout these stages and exhibited dynamic changes in their spatial patterns consistent with instructive roles. Genetic manipulations confirm these notions, and imply specific and critical guidance activities of the microtubule-based cytoskeleton, as well as structural interdependence between the myosin- and actin-based filament arrays. CONCLUSIONS: Our observations highlight a surprisingly significant, behind-the-scenes role for microtubules in establishment of myofibril and sarcomere spatial patterns and size, and provide a detailed account of the interplay between major cytoskeletal elements in generating these essential contractile myogenic units.	Cells; Organelles; Cellular Structures; Eukaryota
Brandao HB, Gabriele M, and Hansen AS	Tracking and interpreting long-range chromatin interactions with super-resolution live-cell imaging.	Curr Opin Cell Biol	2021	Mammalian genomes are organized and regulated through long-range chromatin interactions. Structural loops formed by CTCF-binding factor (CTCF) and cohesin fold the genome into domains, while enhancers interact with promoters across vast genomic distances to regulate gene expression. Although genomics and fixed-cell imaging approaches help illuminate many aspects of chromatin interactions, temporal information is usually lost. Here, we discuss how 3D super-resolution live-cell imaging (SRLCI) can resolve open questions on the dynamic formation and dissolution of chromatin interactions. We discuss SRLCI experimental design, implementation strategies, and data interpretation and highlight associated pitfalls. We conclude that, while technically demanding, SRLCI approaches will likely emerge as a critical tool to dynamically probe 3D genome structure and function and to study enhancer-promoter interactions and chromatin looping.	Cells; Chromosomes; Cell Nucleus; Cellular Structures

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Alawieh A, Chalhoub RM, Mallah K, Langley EF, York M, Broome H, Couch C, Adkins D, and Tomlinson S	Complement Drives Synaptic Degeneration and Progressive Cognitive Decline in the Chronic Phase after Traumatic Brain Injury.	J Neurosci	2021	<p>Cognitive deficits following traumatic brain injury (TBI) remain a major cause of disability and early-onset dementia, and there is increasing evidence that chronic neuroinflammation occurring after TBI plays an important role in this process. However, little is known about the molecular mechanisms responsible for triggering and maintaining chronic inflammation after TBI. Here, we identify complement, and specifically complement-mediated microglial phagocytosis of synapses, as a pathophysiological link between acute insult and a chronic neurodegenerative response that is associated with cognitive decline. Three months after an initial insult, there is ongoing complement activation in the injured brain of male C57BL/6 mice, which drives a robust chronic neuroinflammatory response extending to both hemispheres. This chronic neuroinflammatory response promotes synaptic degeneration and predicts progressive cognitive decline. Synaptic degeneration was driven by microglial phagocytosis of complement-opsonized synapses in both the ipsilateral and contralateral brain, and complement inhibition interrupted the degenerative neuroinflammatory response and reversed cognitive decline, even when therapy was delayed until 2 months after TBI. These findings provide new insight into our understanding of TBI pathology and its management; and whereas previous therapeutic investigations have focused almost exclusively on acute treatments, we show that all phases of TBI, including at chronic time points after TBI, may be amenable to therapeutic interventions, and specifically to complement inhibition. SIGNIFICANCE STATEMENT There is increasing evidence of a chronic neuroinflammatory response after traumatic brain injury (TBI), but little is known about the molecular mechanisms responsible for triggering and maintaining chronic inflammation. We identify complement, and specifically complement-mediated microglial phagocytosis of synapses, as a pathophysiological link between acute insult and a chronic neurodegenerative response, and further that this response is associated with cognitive decline. Complement inhibition interrupted this response and reversed cognitive decline, even when therapy was delayed until 2 months after injury. The data further support the concept that TBI should be considered a chronic rather than an acute disease condition, and have implications for the management of TBI in the chronic phase of injury, specifically with regard to the therapeutic application of complement inhibition.</p>	Cells; Synapses; Membrane; Nervous System
Zhang Y, Schroeder LK, Lessard MD, Kidd P, Chung J, Song Y, Benedetti L, Li Y, Ries J, Grimm JB, Lavis LD, De Camilli P, Rothman JE, Baddeley D, and Bewersdorf J	Nanoscale subcellular architecture revealed by multicolor three-dimensional salvaged fluorescence imaging.	Nat Methods	2020	<p>Combining the molecular specificity of fluorescent probes with three-dimensional imaging at nanoscale resolution is critical for investigating the spatial organization and interactions of cellular organelles and protein complexes. We present a 4Pi single-molecule switching super-resolution microscope that enables ratiometric multicolor imaging of mammalian cells at 5- 10-nm localization precision in three dimensions using 'salvaged fluorescence'. Imaging two or three fluorophores simultaneously, we show fluorescence images that resolve the highly convoluted Golgi apparatus and the close contacts between the endoplasmic reticulum and the plasma membrane, structures that have traditionally been the imaging realm of electron microscopy. The salvaged fluorescence approach is equally applicable in most single-objective microscopes.</p>	Cells; Organelles; Cellular Structures; Eukaryota
Yuan Z, Pavel MA, Wang H, and Hansen SB	Hydroxychloroquine: mechanism of action inhibiting SARS-CoV2 entry.	bioRxiv	2020	<p>Hydroxychloroquine (HCQ) has been proposed in the treatment of SARS-coronavirus 2 (SARS-CoV-2) infection, albeit with much controversy. In vitro, HCQ effectively inhibits viral entry, but its use in the clinic has been hampered by conflicting results. A better understanding of HCQ's mechanism of actions in vitro is needed to resolve these conflicts. Recently, anesthetics were shown to disrupt ordered monolateral tetrahexosyl ganglioside1 (GM1) lipid rafts. These same lipid rafts recruit the SARS-CoV-2 surface receptor angiotensin converting enzyme 2 (ACE2) to an endocytic entry point, away from phosphatidylinositol 4,5 bisphosphate (PIP(2)) domains. Here we employed super resolution imaging of cultured mammalian cells to show HCQ directly perturbs GM1 lipid rafts and inhibits the ability of ACE2 receptor to associate with the endocytic pathway. HCQ also disrupts PIP(2) domains and their ability to cluster and sequester ACE2. Similarly, the antibiotic erythromycin inhibits viral entry and both HCQ and erythromycin decrease the antimicrobial host defense peptide amyloid beta in cultured cells. We conclude HCQ is an anesthetic-like compound that disrupts GM1 lipid rafts similar to anesthetics. The disruption likely decreases viral clustering at both endocytic and putative PIP(2) entry points.</p>	
Yardimci S, Burnham DR, Terry SYA, and Yardimci H	Three-dimensional super-resolution fluorescence imaging of DNA.	Sci Rep	2020	<p>Recent advances in fluorescence super-resolution microscopy are providing important insights into details of cellular structures. To acquire three dimensional (3D) super-resolution images of DNA, we combined binding activated localization microscopy (BALM) using fluorescent double-stranded DNA intercalators and optical astigmatism. We quantitatively establish the advantage of bis- over mono-intercalators before demonstrating the approach by visualizing single DNA molecules stretched between microspheres at various heights. Finally, the approach is applied to the more complex environment of intact and damaged metaphase chromosomes, unravelling their structural features.</p>	Cells; Chromosomes; Cell Nucleus; Protein Binding

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Xie L, Dong P, Chen X, Hsieh TS, Banala S, De Marzio M, English BP, Qi Y, Jung SK, Kieffer-Kwon KR, Legant WR, Hansen AS, Schulmann A, Casellas R, Zhang B, Betzig E, Lavis LD, Chang HY, Tjian R, and Liu Z	3D ATAC-PALM: super-resolution imaging of the accessible genome.	Nat Methods	2020	To image the accessible genome at nanometer scale in situ, we developed three-dimensional assay for transposase-accessible chromatin-photoactivated localization microscopy (3D ATAC-PALM) that integrates an assay for transposase-accessible chromatin with visualization, PALM super-resolution imaging and lattice light-sheet microscopy. Multiplexed with oligopaint DNA-fluorescence in situ hybridization (FISH), RNA-FISH and protein fluorescence, 3D ATAC-PALM connected microscopy and genomic data, revealing spatially segregated accessible chromatin domains (ACDs) that enclose active chromatin and transcribed genes. Using these methods to analyze genetically perturbed cells, we demonstrated that genome architectural protein CTCF prevents excessive clustering of accessible chromatin and decompacts ACDs. These resultshighlight 3D ATAC-PALM as a useful tool to probe the structure and organizing mechanism of the genome.	Microscopy; DNA; Genetic Phenomena; Genetic Structures
Ulisse V, Dey S, Rothbard DE, Zeevi E, Gokhman I, Dadosh T, Minis A, and Yaron A	Regulation of axonal morphogenesis by the mitochondrial protein Efh1.	Life Sci Alliance	2020	During development, neurons adjust their energy balance to meet the high demands of robust axonal growth and branching. The mechanisms that regulate this tuning are largely unknown. Here, we show that sensory neurons lacking liver kinase B1 (Lkb1), a master regulator of energy homeostasis, exhibit impaired axonal growth and branching. Biochemical analysis of these neurons revealed reduction in axonal ATP levels, whereas transcriptome analysis uncovered down-regulation of Efh1 (EF-hand domain family member D1), a mitochondrial Ca(2+)-binding protein. Genetic ablation of Efh1 in mice resulted in reduced axonal morphogenesis as well as enhanced neuronal death. Strikingly, this ablation causes mitochondrial dysfunction and a decrease in axonal ATP levels. Moreover, Efh1 KO sensory neurons display shortened mitochondria at the axonal growth cones, activation of the AMP-activated protein kinase (AMPK)-Ulk (Unc-51-like autophagy-activating kinase 1) pathway and an increase in autophagic flux. Overall, this work uncovers a new mitochondrial regulator that is required for axonal morphogenesis.	Cells; Organelles; Nervous System; Cellular Structures
Struckman HL, Baine S, Thomas J, Mezache L, Mykityn K, Gyorke S, Radwanski PB, and Veeraraghavan R	Super-Resolution Imaging Using a Novel High-Fidelity Antibody Reveals Close Association of the Neuronal Sodium Channel NaV1.6 with Ryanodine Receptors in Cardiac Muscle.	Microsc Microanal	2020	The voltage-gated sodium channel [pore-forming subunit of the neuronal voltage-gated sodium channel (NaV1.6)] has recently been found in cardiac myocytes. Emerging studies indicate a role for NaV1.6 in ionic homeostasis as well as arrhythmogenesis. Little is known about the spatial organization of these channels in cardiac muscle, mainly due to the lack of high-fidelity antibodies. Therefore, we developed and rigorously validated a novel rabbit polyclonal NaV1.6 antibody and undertook super-resolution microscopy studies of NaV1.6 localization in cardiac muscle. We developed and validated a novel rabbit polyclonal antibody against a C-terminal epitope on the neuronal sodium channel 1.6 (NaV1.6). Raw sera showed high affinity in immuno-fluorescence studies, which was improved with affinity purification. The antibody was rigorously validated for specificity via multiple approaches. Lastly, we used this antibody in proximity ligation assay (PLA) and super-resolution Stochastic Optical Reconstruction Microscopy (STORM) studies, which revealed enrichment of NaV1.6 in close proximity to ryanodine receptor (RyR2), a key calcium (Ca2+) cycling protein, in cardiac myocytes. In summary, our novel NaV1.6 antibody demonstrates high degrees of specificity and fidelity in multiple preparations. It enabled multimodal microscopic studies and revealed that over half of the NaV1.6 channels in cardiac myocytes are located within 100 nm of ryanodine receptor Ca2+ release channels.	Microscopy; Eukaryota; Chemicals and Drugs
Roberts R, Smyth JW, Will J, Roberts P, Grek CL, Ghatnekar GS, Sheng Z, Gourdie RG, Lamouille S, and Foster EJ	Development of PLGA nanoparticles for sustained release of a connexin43 mimetic peptide to target glioblastoma cells.	Mater Sci Eng C Mater Biol Appl	2020	Effective therapeutic delivery of peptide and protein drugs is challenged by short in vivo half-lives due to rapid degradation. Sustained release formulations of alphaCT1, a 25 amino acid peptide drug, would afford lower dosing frequency in indications that require long term treatment, such as chronic wounds and cancers. In this study, rhodamine B (RhB) was used as a model drug to develop and optimize a double emulsion-solvent evaporation method of poly(lactic-co-glycolic acid) (PLGA) nanoparticle synthesis. Encapsulation of alphaCT1 in these nanoparticles (NPs) resulted in a sustained in vitro release profile over three weeks, characterized by an initial burst release of approximately 50% of total encapsulated drug over the first three days followed by sustained release over the remaining two and a half weeks. NP uptake by glioblastoma stem cells was through endocytosis and RhB and alphaCT1 were observed in cells after at least 4 days.	Cells; Diseases; Eukaryota; Chemicals and Drugs
Pavel MA, Petersen EN, Wang H, Lerner RA, and Hansen SB	Studies on the mechanism of general anesthesia.	Proc Natl Acad Sci U S A	2020	Inhaled anesthetics are a chemically diverse collection of hydrophobic molecules that robustly activate TWIK-related K(+) channels (TREK-1) and reversibly induce loss of consciousness. For 100 y, anesthetics were speculated to target cellular membranes, yet no plausible mechanism emerged to explain a membrane effect on ion channels. Here we show that inhaled anesthetics (chloroform and isoflurane) activate TREK-1 through disruption of phospholipase D2 (PLD2) localization to lipid rafts and subsequent production of signaling lipid phosphatidic acid (PA). Catalytically dead PLD2 robustly blocks anesthetic TREK-1 currents in whole-cell patch-clamp recordings. Localization of PLD2 renders the TRAAK channel sensitive, a channel that is otherwise anesthetic insensitive. General anesthetics, such as chloroform, isoflurane, diethyl ether, xenon, and propofol, disrupt lipid rafts and activate PLD2. In the whole brain of flies, anesthesia disrupts rafts and PLD(null) flies resist anesthesia. Our results establish a membrane-mediated target of inhaled anesthesia and suggest PA helps set thresholds of anesthetic sensitivity in vivo.	Cells; Membrane; Cellular Structures; Lipids

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<p>Nguyen HQ, Chatteraj S, Castillo D, Nguyen SC, Nir G, Lioutas A, Hershberg EA, Martins NMC, Reginato PL, Hannan M, Beliveau BJ, Church GM, Daugharthy ER, Marti-Renom MA, and Wu CT</p>	<p>3D mapping and accelerated super-resolution imaging of the human genome using in situ sequencing.</p>	<p>Nat Methods</p>	<p>2020</p>	<p>There is a need for methods that can image chromosomes with genome-wide coverage, as well as greater genomic and optical resolution. We introduce OligoFISSEQ, a suite of three methods that leverage fluorescence in situ sequencing (FISSEQ) of barcoded Oligopaint probes to enable the rapid visualization of many targeted genomic regions. Applying OligoFISSEQ to human diploid fibroblast cells, we show how four rounds of sequencing are sufficient to produce 3D maps of 36 genomic targets across six chromosomes in hundreds to thousands of cells, implying a potential to image thousands of targets in only five to eight rounds of sequencing. We also use OligoFISSEQ to trace chromosomes at finer resolution, following the path of the X chromosome through 46 regions, with separate studies showing compatibility of OligoFISSEQ with immunocytochemistry. Finally, we combined OligoFISSEQ with OligoSTORM, laying the foundation for accelerated single-molecule super-resolution imaging of large swaths of, if not entire, human genomes.</p>	<p>Cells; Chromosomes; Cell Nucleus; Cellular Structures</p>
<p>Nguyen HQ, Chatteraj S, Castillo D, Nguyen SC, Nir G, Lioutas A, Hershberg EA, Martins NMC, Reginato PL, Hannan M, Beliveau BJ, Church GM, Daugharthy ER, Marti-Renom MA, and Wu CT</p>	<p>3D mapping and accelerated super-resolution imaging of the human genome using in situ sequencing.</p>	<p>Nat Methods</p>	<p>2020</p>	<p>There is a need for methods that can image chromosomes with genome-wide coverage, as well as greater genomic and optical resolution. We introduce OligoFISSEQ, a suite of three methods that leverage fluorescence in situ sequencing (FISSEQ) of barcoded Oligopaint probes to enable the rapid visualization of many targeted genomic regions. Applying OligoFISSEQ to human diploid fibroblast cells, we show how four rounds of sequencing are sufficient to produce 3D maps of 36 genomic targets across six chromosomes in hundreds to thousands of cells, implying a potential to image thousands of targets in only five to eight rounds of sequencing. We also use OligoFISSEQ to trace chromosomes at finer resolution, following the path of the X chromosome through 46 regions, with separate studies showing compatibility of OligoFISSEQ with immunocytochemistry. Finally, we combined OligoFISSEQ with OligoSTORM, laying the foundation for accelerated single-molecule super-resolution imaging of large swaths of, if not entire, human genomes.</p>	<p>Cells; Chromosomes; Cell Nucleus; Cellular Structures</p>
<p>Mezache L, Struckman HL, Greer-Short A, Baine S, Gyorke S, Radwanski PB, Hund TJ, and Veerarraghavan R</p>	<p>Vascular endothelial growth factor promotes atrial arrhythmias by inducing acute intercalated disk remodeling.</p>	<p>Sci Rep</p>	<p>2020</p>	<p>Atrial fibrillation (AF) is the most common arrhythmia and is associated with inflammation. AF patients have elevated levels of inflammatory cytokines known to promote vascular leak, such as vascular endothelial growth factor A (VEGF). However, the contribution of vascular leak and consequent cardiac edema to the genesis of atrial arrhythmias remains unknown. Previous work suggests that interstitial edema in the heart can acutely promote ventricular arrhythmias by disrupting ventricular myocyte intercalated disk (ID) nanodomains rich in cardiac sodium channels (Na(V)1.5) and slowing cardiac conduction. Interestingly, similar disruption of ID nanodomains has been identified in atrial samples from AF patients. Therefore, we tested the hypothesis that VEGF-induced vascular leak can acutely increase atrial arrhythmia susceptibility by disrupting ID nanodomains and slowing atrial conduction. Treatment of murine hearts with VEGF (30–60 min, at clinically relevant levels) prolonged the electrocardiographic P wave and increased susceptibility to burst pacing-induced atrial arrhythmias. Optical voltage mapping revealed slower atrial conduction following VEGF treatment (10 +/- 0.4 cm/s vs. 21 +/- 1 cm/s at baseline, p < 0.05). Transmission electron microscopy revealed increased intermembrane spacing at ID sites adjacent to gap junctions (GJs; 64 +/- 9 nm versus 17 +/- 1 nm in controls, p < 0.05), as well as sites next to mechanical junctions (MJs; 63 +/- 4 nm versus 27 +/- 2 nm in controls, p < 0.05) in VEGF-treated hearts relative to controls. Importantly, super-resolution microscopy and quantitative image analysis revealed reorganization of Na(V)1.5 away from dense clusters localized near GJs and MJs to a more diffuse distribution throughout the ID. Taken together, these data suggest that VEGF can acutely predispose otherwise normal hearts to atrial arrhythmias by dynamically disrupting Na(V)1.5-rich ID nanodomains and slowing atrial conduction. These data highlight inflammation-induced vascular leak as a potential factor in the development and progression of AF.</p>	<p>Cells; Membrane; Cellular Structures; Microscopy</p>
<p>Mandracchia B, Hua X, Guo C, Son J, Urner T, and Jia S</p>	<p>Fast and accurate sCMOS noise correction for fluorescence microscopy.</p>	<p>Nat Commun</p>	<p>2020</p>	<p>The rapid development of scientific CMOS (sCMOS) technology has greatly advanced optical microscopy for biomedical research with superior sensitivity, resolution, field-of-view, and frame rates. However, for sCMOS sensors, the parallel charge voltage conversion and different responsivity at each pixel induces extra readout and pattern noise compared to charge-coupled devices (CCD) and electron-multiplying CCD (EM-CCD) sensors. This can produce artifacts, deteriorate imaging capability, and hinder quantification of fluorescent signals, thereby compromising strategies to reduce photo-damage to live samples. Here, we propose a content-adaptive algorithm for the automatic correction of sCMOS-related noise (ACsN) for fluorescence microscopy. ACsN combines camera physics and layered sparse filtering to significantly reduce the most relevant noise sources in a sCMOS sensor while preserving the fine details of the signal. The method improves the camera performance, enabling fast, low-light and quantitative optical microscopy with video-rate denoising for a broad range of imaging conditions and modalities.</p>	<p>Cells; Organelles; Cellular Structures; Microscopy</p>

Bruker Super-Resolution Publications

Luppino JM, Park DS, Nguyen SC, Lan Y, Xu Z, Yunker R, and Joyce EF	Cohesin promotes stochastic domain intermingling to ensure proper regulation of boundary-proximal genes.	Nat Genet	2020	The human genome can be segmented into topologically associating domains (TADs), which have been proposed to spatially sequester genes and regulatory elements through chromatin looping. Interactions between TADs have also been suggested, presumably because of variable boundary positions across individual cells. However, the nature, extent and consequence of these dynamic boundaries remain unclear. Here, we combine high-resolution imaging with Oligopaint technology to quantify the interaction frequencies across both weak and strong boundaries. We find that chromatin intermingling across population-defined boundaries is widespread but that the extent of permissibility is locus-specific. Cohesin depletion, which abolishes domain formation at the population level, does not induce ectopic interactions but instead reduces interactions across all boundaries tested. In contrast, WAPL or CTCF depletion increases inter-domain contacts in a cohesin-dependent manner. Reduced chromatin intermingling due to cohesin loss affects the topology and transcriptional bursting frequencies of genes near boundaries. We propose that cohesin occasionally bypasses boundaries to promote incorporation of boundary-proximal genes into neighboring domains.	Cells; Chromosomes; Cell Nucleus; Protein Binding
Liu S, Luttrell LM, Premont RT, and Rockey DC	beta-Arrestin2 is a critical component of the GPCR-eNOS signalosome.	Proc Natl Acad Sci U S A	2020	Endothelial cell nitric oxide (NO) synthase (eNOS), the enzyme responsible for synthesis of NO in endothelial cells, is regulated by complex posttranslational mechanisms. Sinusoidal portal hypertension, a disorder characterized by liver sinusoidal endothelial cell (SEC) injury with resultant reduced eNOS activity and NO production within the liver, has been associated with defects in eNOS protein-protein interactions and posttranslational modifications. We and others have previously identified novel eNOS interactors, including G protein-coupled receptor (GPCR) kinase interactor 1 (GIT1), which we found to play an unexpected stimulatory role in GPCR-mediated eNOS signaling. Here we report that beta-arrestin 2 (beta Arr2), a canonical GPCR signaling partner, localizes in SECs with eNOS in a GIT1/eNOS/NO signaling module. Most importantly, we show that beta-Arr2 stimulates eNOS activity, and that beta-Arr2 expression is reduced and formation of the GIT1/eNOS/NO signaling module is interrupted during liver injury. In beta-Arr2-deficient mice, bile duct ligation injury (BDL) led to significantly reduced eNOS activity and to a dramatic increase in portal hypertension compared to BDL in wild-type mice. Overexpression of beta-Arr2 in injured or beta-Arr2-deficient SECs rescued eNOS function by increasing eNOS complex formation and NO production. We also found that beta-Arr2-mediated GIT1/eNOS complex formation is dependent on Erk1/2 and Src, two kinases known to interact with and be activated by beta-Arr2 in response to GPCR activation. Our data emphasize that beta-Arr2 is an integral component of the GIT1/eNOS/NO signaling pathway and have implications for the pathogenesis of sinusoidal portal hypertension.	Cells; Signal Transduction; Diseases; Eukaryota
Lahav-Mankovski N, Prasad PK, Oppenheimer-Low N, Raviv G, Dadosh T, Unger T, Salame TM, Motiei L, and Margulies D	Decorating bacteria with self-assembled synthetic receptors.	Nat Commun	2020	The responses of cells to their surroundings are mediated by the binding of cell surface proteins (CSPs) to extracellular signals. Such processes are regulated via dynamic changes in the structure, composition, and expression levels of CSPs. In this study, we demonstrate the possibility of decorating bacteria with artificial, self-assembled receptors that imitate the dynamic features of CSPs. We show that the local concentration of these receptors on the bacterial membrane and their structure can be reversibly controlled using suitable chemical signals, in a way that resembles changes that occur with CSP expression levels or posttranslational modifications (PTMs), respectively. We also show that these modifications can endow the bacteria with programmable properties, akin to the way CSP responses can induce cellular functions. By programming the bacteria to glow, adhere to surfaces, or interact with proteins or mammalian cells, we demonstrate the potential to tailor such biomimetic systems for specific applications.	Cells; Membrane; Bacteria; Cellular Structures
Kuznetsova T, Prange KHM, Glass CK, and de Winther MPJ	Transcriptional and epigenetic regulation of macrophages in atherosclerosis.	Nat Rev Cardiol	2020	Monocytes and macrophages provide defence against pathogens and danger signals. These cells respond to stimulation in a fast and stimulus-specific manner by utilizing complex cascaded activation by lineage-determining and signal-dependent transcription factors. The complexity of the functional response is determined by interactions between triggered transcription factors and depends on the microenvironment and interdependent signalling cascades. Dysregulation of macrophage phenotypes is a major driver of various diseases such as atherosclerosis, rheumatoid arthritis and type 2 diabetes mellitus. Furthermore, exposure of monocytes, which are macrophage precursor cells, to certain stimuli can lead to a hypo-inflammatory tolerized phenotype or a hyper-inflammatory trained phenotype in a macrophage. In atherosclerosis, macrophages and monocytes are exposed to inflammatory cytokines, oxidized lipids, cholesterol crystals and other factors. All these stimuli induce not only a specific transcriptional response but also interact extensively, leading to transcriptional and epigenetic heterogeneity of macrophages in atherosclerotic plaques. Targeting the epigenetic landscape of plaque macrophages can be a powerful therapeutic tool to modulate pro-atherogenic phenotypes and reduce the rate of plaque formation. In this Review, we discuss the emerging role of transcription factors and epigenetic remodelling in macrophages in the context of atherosclerosis and inflammation, and provide a comprehensive overview of epigenetic enzymes and transcription factors that are involved in macrophage activation.	Cells; Immunology; Diseases; Genetic Phenomena

Bruker Super-Resolution Publications

Heller JP, Odii T, Zheng K, and Rusakov DA	Imaging tripartite synapses using super-resolution microscopy.	Methods	2020	Astroglia are vital facilitators of brain development, homeostasis, and metabolic support. In addition, they are also essential to the formation and regulation of synaptic circuits. Due to the extraordinary complex, nanoscopic morphology of astrocytes, the underlying cellular mechanisms have been poorly understood. In particular, fine astrocytic processes that can be found in the vicinity of synapses have been difficult to study using traditional imaging techniques. Here, we describe a 3D three-colour super-resolution microscopy approach to unravel the nanostructure of tripartite synapses. The method is based on the SMLM technique direct stochastic optical reconstruction microscopy (dSTORM) which uses conventional fluorophore-labelled antibodies. This approach enables reconstructing the nanoscale localisation of individual astrocytic glutamate transporter (GLT-1) molecules surrounding presynaptic (bassoon) and postsynaptic (Homer1) protein localisations in fixed mouse brain sections. However, the technique is readily adaptable to other types of targets and tissues.	Cells; Synapses; Membrane; Nervous System
Grieco JP, Allen ME, Perry JB, Wang Y, Song Y, Rohani A, Compton SLE, Smyth JW, Swami NS, Brown DA, and Schmelz EM	Progression-Mediated Changes in Mitochondrial Morphology Promotes Adaptation to Hypoxic Peritoneal Conditions in Serous Ovarian Cancer.	Front Oncol	2020	Ovarian cancer is the deadliest gynecological cancer in women, with a survival rate of less than 30% when the cancer has spread throughout the peritoneal cavity. Aggregation of cancer cells increases their viability and metastatic potential; however, there are limited studies that correlate these functional changes to specific phenotypic alterations. In this study, we investigated changes in mitochondrial morphology and dynamics during malignant transition using our MOSE cell model for progressive serous ovarian cancer. Mitochondrial morphology was changed with increasing malignancy from a filamentous network to single, enlarged organelles due to an imbalance of mitochondrial dynamic proteins (fusion: MFN1/OPA1, fission: DRP1/FIS1). These phenotypic alterations aided the adaptation to hypoxia through the promotion of autophagy and were accompanied by changes in the mitochondrial ultrastructure, mitochondrial membrane potential, and the regulation of reactive oxygen species (ROS) levels. The tumor-initiating cells increased mitochondrial fragmentation after aggregation and exposure to hypoxia that correlated well with our previously observed reduced growth and respiration in spheroids, suggesting that these alterations promote viability in non-permissive conditions. Our identification of such mitochondrial phenotypic changes in malignancy provides a model in which to identify targets for interventions aimed at suppressing metastases.	
Gonschior H, Haucke V, and Lehmann M	Super-Resolution Imaging of Tight and Adherens Junctions: Challenges and Open Questions.	Int J Mol Sci	2020	The tight junction (TJ) and the adherens junction (AJ) bridge the paracellular cleft of epithelial and endothelial cells. In addition to their role as protective barriers against bacteria and their toxins they maintain ion homeostasis, cell polarity, and mechano-sensing. Their functional loss leads to pathological changes such as tissue inflammation, ion imbalance, and cancer. To better understand the consequences of such malfunctions, the junctional nanoarchitecture is of great importance since it remains so far largely unresolved, mainly because of major difficulties in dynamically imaging these structures at sufficient resolution and with molecular precision. The rapid development of super-resolution imaging techniques ranging from structured illumination microscopy (SIM), stimulated emission depletion (STED) microscopy, and single molecule localization microscopy (SMLM) has now enabled molecular imaging of biological specimens from cells to tissues with nanometer resolution. Here we summarize these techniques and their application to the dissection of the nanoscale molecular architecture of TJs and AJs. We propose that super-resolution imaging together with advances in genome engineering and functional analyses approaches will create a leap in our understanding of the composition, assembly, and function of TJs and AJs at the nanoscale and, thereby, enable a mechanistic understanding of their dysfunction in disease.	Cells; Membrane; Cellular Structures; Microscopy
Chen YC, Sood C, Marin M, Aaron J, Gratton E, Salaita K, and Melikyan GB	Super-Resolution Fluorescence Imaging Reveals That Serine Incorporator Protein 5 Inhibits Human Immunodeficiency Virus Fusion by Disrupting Envelope Glycoprotein Clusters.	ACS Nano	2020	Serine incorporator protein 5 (SERINC5) is the host antiretroviral factor that reduces HIV-1 infectivity by incorporating into virions and inhibiting the envelope glycoprotein (Env) mediated virus fusion with target cells. We and others have shown that SERINC5 incorporation into virions alters the Env structure and sensitizes the virus to broadly neutralizing antibodies targeting cryptic Env epitopes. We have also found that SERINC5 accelerates the loss of Env function over time compared to control viruses. However, the exact mechanism by which SERINC5 inhibits HIV-1 fusion is not understood. Here, we utilized 2D and 3D super-resolution microscopy to examine the effect of SERINC5 on the distribution of Env glycoproteins on single HIV-1 particles. We find that, in agreement with a previous report, Env glycoproteins form clusters on the surface of mature virions. Importantly, incorporation of SERINC5, but not SERINC2, which lacks antiviral activity, disrupted Env clusters without affecting the overall Env content. We also show that SERINC5 and SERINC2 also form clusters on single virions. Unexpectedly, Env and SERINC molecules exhibited poor codistribution on virions, as evidenced by much greater Env-SERINC pairwise distances compared to Env-Env distances. This observation is inconsistent with the previously reported interaction between Env and SERINC5 and suggests an indirect effect of SERINC5 on Env cluster formation. Collectively, our results reveal a multifaceted mechanism of SERINC5-mediated restriction of HIV-1 fusion that, aside from the effects on individual Env trimers, involves disruption of Env clusters, which likely serve as sites of viral fusion with target cells.	Diseases; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Blanchard EL, Braun MR, Lifland AW, Ludeke B, Noton SL, Vanover D, Zurla C, Fearn R, and Santangelo PJ	Polymerase-tagged respiratory syncytial virus reveals a dynamic rearrangement of the ribonucleocapsid complex during infection.	PLoS Pathog	2020	The ribonucleocapsid complex of respiratory syncytial virus (RSV) is responsible for both viral mRNA transcription and viral replication during infection, though little is known about how this dual function is achieved. Here, we report the use of a recombinant RSV virus with a FLAG-tagged large polymerase protein, L, to characterize and localize RSV ribonucleocapsid structures during the early and late stages of viral infection. Through proximity ligation assays and super-resolution microscopy, viral RNA and proteins in the ribonucleocapsid complex were revealed to dynamically rearrange over time, particularly between 6 and 8 hours post infection, suggesting a connection between the ribonucleocapsid structure and its function. The timing of ribonucleocapsid rearrangement corresponded with an increase in RSV genome RNA accumulation, indicating that this rearrangement is likely involved with the onset of RNA replication and secondary transcription. Additionally, early overexpression of RSV M2-2 from in vitro transcribed mRNA was shown to inhibit virus infection by rearranging the ribonucleocapsid complex. Collectively, these results detail a critical understanding into the localization and activity of RSV L and the ribonucleocapsid complex during RSV infection.	Cells; Viruses; Diseases; Phenomena
Bennett HW, Gustavsson AK, Bayas CA, Petrov PN, Mooney N, Moerner WE, and Jackson PK	Novel fibrillar structure in the inversin compartment of primary cilia revealed by 3D single-molecule superresolution microscopy.	Mol Biol Cell	2020	Primary cilia in many cell types contain a periaxonemal subcompartment called the inversin compartment. Four proteins have been found to assemble within the inversin compartment: INVS, ANKS6, NEK8, and NPHP3. The function of the inversin compartment is unknown, but it appears to be critical for normal development, including left-right asymmetry and renal tissue homeostasis. Here we combine superresolution imaging of human RPE1 cells, a classic model for studying primary cilia in vitro, with a genetic dissection of the protein-protein binding relationships that organize compartment assembly to develop a new structural model. We observe that INVS is the core structural determinant of a compartment composed of novel fibril-like substructures, which we identify here by three-dimensional single-molecule superresolution imaging. We find that NEK8 and ANKS6 depend on INVS for localization to these fibrillar assemblies and that ANKS6-NEK8 density within the compartment is regulated by NEK8. Together, NEK8 and ANKS6 are required downstream of INVS to localize and concentrate NPHP3 within the compartment. In the absence of these upstream components, NPHP3 is redistributed within cilia. These results provide a more detailed structure for the inversin compartment and introduce a new example of a membraneless compartment organized by protein-protein interactions.	Cells; Cellular Structures; Microscopy; Genetic Phenomena
Bednarz K, Alshafie W, Aufmkolk S, Desserteaux T, Markam PS, Storch KF, and Stroth T	Ultradian Secretion of Growth Hormone in Mice: Linking Physiology With Changes in Synapse Parameters Using Super-Resolution Microscopy.	Front Neural Circuits	2020	Neuroendocrine circuits are orchestrated by the pituitary gland in response to hypothalamic hormone-releasing and inhibiting factors to generate an ultradian and/or circadian rhythm of hormone secretion. However, mechanisms that govern this rhythmicity are not fully understood. It has been shown that synaptic transmission in the rodent hypothalamus undergoes cyclical changes in parallel with rhythmic hormone secretion and a growing body of evidence suggests that rapid rewiring of hypothalamic neurons may be the source of these changes. For decades, structural synaptic studies have been utilizing electron microscopy, which provides the resolution suitable for visualizing synapses. However, the small field of view, limited specificity and manual analysis susceptible to bias fuel the search for a more quantitative approach. Here, we apply the fluorescence super-resolution microscopy approach direct Stochastic Optical Reconstruction Microscopy (dSTORM) to quantify and structurally characterize excitatory and inhibitory synapses that contact growth hormone-releasing-hormone (GHRH) neurons during peak and trough values of growth hormone (GH) concentration in mice. This approach relies on a three-color immunofluorescence staining of GHRH and pre- and post-synaptic markers, and a quantitative analysis with a Density-Based Spatial Clustering of Applications with Noise (DBSCAN) algorithm. With this method we confirm our previous findings, using electron microscopy, of increased excitatory synaptic input to GHRH neurons during peak levels of GH. Additionally, we find a shift in synapse numbers during low GH levels, where more inhibitory synaptic inputs are detected. Lastly, we utilize dSTORM to study novel aspects of synaptic structure. We show that more excitatory (but not inhibitory) pre-synaptic clusters associate with excitatory post-synaptic clusters during peaks of GH secretion and that the numbers of post-synaptic clusters increase during high hormone levels. The results presented here provide an opportunity to highlight dSTORM as a valuable quantitative approach to study synaptic structure in the neuroendocrine circuit. Importantly, our analysis of GH circuitry sheds light on the potential mechanism that drives ultradian changes in synaptic transmission and possibly aids in GH pulse generation in mice.	Cells; Synapses; Membrane; Nervous System

Bruker Super-Resolution Publications

Bai JP, Xue N, Lawal O, Nyati A, Santos-Sacchi J, and Navaratnam D	Calcium-induced calcium release in proximity to hair cell BK channels revealed by PKA activation.	Physiol Rep	2020	Large-conductance calcium-activated potassium (BK) channels play a critical role in electrical resonance, a mechanism of frequency selectivity in chicken hair cells. We determine that BK currents are dependent on inward flow of Ca(2+) and intracellular buffering of Ca(2+). Entry of Ca(2+) is further amplified locally by calcium-induced Ca(2+) release (CICR) in close proximity to plasma membrane BK channels. Ca(2+) imaging reveals peripheral clusters of high concentrations of Ca(2+) that are suprathreshold to that needed to activate BK channels. Protein kinase A (PKA) activation increases the size of BK currents likely by recruiting more BK channels due to spatial spread of high Ca(2+) concentrations in turn from increasing CICR. STORM imaging confirms the presence of nanodomains with ryanodine and IP3 receptors in close proximity to the Slo subunit of BK channels. Together, these data require a rethinking of how electrical resonance is brought about and suggest effects of CICR in synaptic release. Both genders were included in this study.	Cells; Signal Transduction; Nervous System; Eukaryota
Appadurai D, Gay L, Moharir A, Lang MJ, Duncan MC, Schmidt O, Teis D, Vu TN, Silva M, Jorgensen EM, and Babst M	Plasma membrane tension regulates eisosome structure and function.	Mol Biol Cell	2020	Eisosomes are membrane furrows at the cell surface of yeast that have been shown to function in two seemingly distinct pathways, membrane stress response and regulation of nutrient transporters. We found that many stress conditions affect both of these pathways by changing plasma membrane tension and thus the morphology and composition of eisosomes. For example, alkaline stress causes swelling of the cell and an endocytic response, which together increase membrane tension, thereby flattening the eisosomes. The flattened eisosomes affect membrane stress pathways and release nutrient transporters, which aids in their down-regulation. In contrast, glucose starvation or hyperosmotic shock causes cell shrinking, which results in membrane slack and the deepening of eisosomes. Deepened eisosomes are able to trap nutrient transporters and protect them from rapid endocytosis. Therefore, eisosomes seem to coordinate the regulation of both membrane tension and nutrient transporter stability.	Cells; Membrane; Cellular Structures; Fungi
Alshafie W, Francis V, Bednarz K, Pan YE, Stroth T, and McPherson PS	Regulated resurfacing of a somatostatin receptor storage compartment fine-tunes pituitary secretion.	J Cell Biol	2020	The surfacing of the glucose transporter GLUT4 driven by insulin receptor activation provides the prototypic example of a homeostasis response dependent on mobilization of an intracellular storage compartment. Here, we generalize this concept to a G protein-coupled receptor, somatostatin receptor subtype 2 (SSTR2), in pituitary cells. Following internalization in corticotropes, SSTR2 moves to a juxtannuclear syntaxin-6-positive compartment, where it remains until the corticotropes are stimulated with corticotropin releasing factor (CRF), whereupon SSTR2 exits the compartment on syntaxin-6-positive vesicular/tubular carriers that depend on Rab10 for their fusion with the plasma membrane. As SSTR2 activation antagonizes CRF-mediated hormone release, this storage/resurfacing mechanism may allow for a physiological homeostatic feedback system. In fact, we find that SSTR2 moves from an intracellular compartment to the cell surface in pituitary gland somatotropes, concomitant with increasing levels of serum growth hormone (GH) during natural GH cycles. Our data thus provide a mechanism by which signaling-mediated plasma membrane resurfacing of SSTR2 can fine-tune pituitary hormone release.	Cells; Signal Transduction; Nervous System; Eukaryota
Ahmad AA, Streiff ME, Hunter C, and Sachse FB	Modulation of Calcium Transients in Cardiomyocytes by Transient Receptor Potential Canonical 6 Channels.	Front Physiol	2020	Transient receptor potential canonical 6 (TRPC6) channels are non-selective cation channels that are thought to underlie mechano-modulation of calcium signaling in cardiomyocytes. TRPC6 channels are involved in development of cardiac hypertrophy and related calcineurin-nuclear factor of activated T cells (NFAT) signaling. However, the exact location and roles of TRPC6 channels remain ill-defined in cardiomyocytes. We used an expression system based on neonatal rat ventricular myocytes (NRVMs) to investigate the location of TRPC6 channels and their role in calcium signaling. NRVMs isolated from 1- to 2-day-old animals were cultured and infected with an adenoviral vector to express enhanced-green fluorescent protein (eGFP) or TRPC6-eGFP. After 3 days, NRVMs were fixed, immunolabeled, and imaged with confocal and super-resolution microscopy to determine TRPC6 localization. Cytosolic calcium transients at 0.5 and 1 Hz pacing rates were recorded in NRVMs using indo-1, a ratio-metric calcium dye. Confocal and super-resolution microscopy suggested that TRPC6-eGFP localized to the sarcolemma. NRVMs infected with TRPC6-eGFP exhibited higher diastolic and systolic cytosolic calcium concentration as well as increased sarcoplasmic reticulum (SR) calcium load compared to eGFP infected cells. We applied a computer model comprising sarcolemmal TRPC6 current to explain our experimental findings. Altogether, our studies indicate that TRPC6 channels play a role in sarcolemmal and intracellular calcium signaling in cardiomyocytes. Our findings support the hypothesis that upregulation or activation of TRPC6 channels, e.g., in disease, leads to sustained elevation of the cytosolic calcium concentration, which is thought to activate calcineurin-NFAT signaling and cardiac hypertrophic remodeling. Also, our findings support the hypothesis that mechanosensitivity of TRPC6 channels modulates cytosolic calcium transients and SR calcium load.	
?	Correction for Liu et al., beta-Arrestin2 is a critical component of the GPCR-eNOS signalosome.	Proc Natl Acad Sci U S A	2020	?	

Bruker Super-Resolution Publications

Yaakov LB, Mutsafi Y, Porat Z, Dadosh T, and Minsky A	Kinetics of Mimivirus Infection Stages Quantified Using Image Flow Cytometry.	Cytometry A	2019	Due to the heterogeneity of viruses and their hosts, a comprehensive view of viral infection is best achieved by analyzing large populations of infected cells. However, information regarding variation in infected cell populations is lost in bulk measurements. Motivated by an interest in the temporal progression of events in virally infected cells, we used image flow cytometry (IFC) to monitor changes in <i>Acanthamoeba polyphaga</i> cells infected with Mimivirus. This first use of IFC to study viral infection required the development of methods to preserve morphological features of adherent amoeba cells prior to detachment and analysis in suspension. It also required the identification of IFC parameters that best report on key events in the Mimivirus infection cycle. The optimized IFC protocol enabled the simultaneous monitoring of diverse processes including generation of viral factories, transport, and fusion of replication centers within the cell, accumulation of viral progeny, and changes in cell morphology for tens of thousands of cells. After obtaining the time windows for these processes, we used IFC to evaluate the effects of perturbations such as oxidative stress and cytoskeletal disruptors on viral infection. Accurate dose-response curves could be generated, and we found that mild oxidative stress delayed multiple stages of virus production, but eventually infection processes occurred with approximately the same amplitudes. We also found that functional actin cytoskeleton is required for fusion of viral replication centers and later for the production of viral progeny. Through this report, we demonstrate that IFC offers a quantitative, high-throughput, and highly robust approach to study viral infection cycles and virus-host interactions. (c) The Authors. Cytometry Part A published by Wiley Periodicals, Inc. on behalf of International Society for Advancement of Cytometry.	Cells; Viruses; Cellular Structures; Diseases
Varsano N, Beghi F, Dadosh T, Elad N, Pereiro E, Haran G, Leiserowitz L, and Addadi L	The Effect of the Phospholipid Bilayer Environment on Cholesterol Crystal Polymorphism.	Chempluschem	2019	Cholesterol crystallization from mixtures of unesterified cholesterol with phospholipids and cholesterol esters is believed to be a key event in atherosclerosis progression. Not much is understood, however, about the influence of the lipid environment on cholesterol crystallization. Here we study cholesterol monohydrate crystal formation from mixed bilayers with palmitoyl-oleoyl-phosphatidylcholine (POPC), dipalmitoyl-phosphatidylcholine (DPPC) and sphingomyelin. We show that disordered phospholipids and sphingomyelin stabilize the formation of crystal plates of the triclinic cholesterol monohydrate polymorph, whereas saturated glycerolipids stabilize helical and tubular crystals of the metastable monoclinic polymorph. We followed the subsequent transformation of these helical crystals into the stable triclinic plates. Discovering the relations between membrane lipid composition and cholesterol crystal polymorphism may provide important clues to the understanding of cholesterol crystal formation in atherosclerosis.	Lipids; Chemicals and Drugs
Varsano N, Beghi F, Dadosh T, Elad N, Pereiro E, Haran G, Leiserowitz L, and Addadi L	The Effect of the Phospholipid Bilayer Environment on Cholesterol Crystal Polymorphism.	Chempluschem	2019	Invited for this month's cover are the group of Prof. Lia Addadi at the Weizmann Institute of Science, Israel and collaborators at the Università Degli Studi di Milano, Italy, and the ALBA Synchrotron Light Source, Spain. The front cover shows how cholesterol crystals form in macrophage cells and in lipid bilayers of different compositions. Cholesterol monohydrate stable triclinic crystals form in vitro as rhomb-shaped plates, whereas the monoclinic crystals fold into tubular or helical shapes. Read the full text of the article at 10.1002/cplu.201800632 .	Lipids; Eukaryota; Chemicals and Drugs
Schroeder LK, Barentine AES, Merta H, Schweighofer S, Zhang Y, Baddeley D, Bewersdorf J, and Bahmanyar S	Dynamic nanoscale morphology of the ER surveyed by STED microscopy.	J Cell Biol	2019	The endoplasmic reticulum (ER) is composed of interconnected membrane sheets and tubules. Superresolution microscopy recently revealed densely packed, rapidly moving ER tubules mistaken for sheets by conventional light microscopy, highlighting the importance of revisiting classical views of ER structure with high spatiotemporal resolution in living cells. In this study, we use live-cell stimulated emission depletion (STED) microscopy to survey the architecture of the ER at 50-nm resolution. We determine the nanoscale dimensions of ER tubules and sheets for the first time in living cells. We demonstrate that ER sheets contain highly dynamic, subdiffraction-sized holes, which we call nanoholes, that coexist with uniform sheet regions. Reticulon family members localize to curved edges of holes within sheets and are required for their formation. The luminal tether Climp63 and microtubule cytoskeleton modulate their nanoscale dynamics and organization. Thus, by providing the first quantitative analysis of ER membrane structure and dynamics at the nanoscale, our work reveals that the ER in living cells is not limited to uniform sheets and tubules; instead, we suggest the ER contains a continuum of membrane structures that includes dynamic nanoholes in sheets as well as clustered tubules.	Cells; Membrane; Organelles; Cellular Structures

Bruker Super-Resolution Publications

<p>Poleshko A, Smith CL, Nguyen SC, Sivaramakrishnan P, Wong KG, Murray JI, Lakadamyali M, Joyce EF, Jain R, and Epstein JA</p>	<p>H3K9me2 orchestrates inheritance of spatial positioning of peripheral heterochromatin through mitosis.</p>	<p>Elife</p>	<p>2019</p>	<p>Cell-type-specific 3D organization of the genome is unrecognizable during mitosis. It remains unclear how essential positional information is transmitted through cell division such that a daughter cell recapitulates the spatial genome organization of the parent. Lamina-associated domains (LADs) are regions of repressive heterochromatin positioned at the nuclear periphery that vary by cell type and contribute to cell-specific gene expression and identity. Here we show that histone 3 lysine 9 dimethylation (H3K9me2) is an evolutionarily conserved, specific mark of nuclear peripheral heterochromatin and that it is retained through mitosis. During mitosis, phosphorylation of histone 3 serine 10 temporarily shields the H3K9me2 mark allowing for dissociation of chromatin from the nuclear lamina. Using high-resolution 3D immuno-oligoFISH, we demonstrate that H3K9me2-enriched genomic regions, which are positioned at the nuclear lamina in interphase cells prior to mitosis, re-associate with the forming nuclear lamina before mitotic exit. The H3K9me2 modification of peripheral heterochromatin ensures that positional information is safeguarded through cell division such that individual LADs are re-established at the nuclear periphery in daughter nuclei. Thus, H3K9me2 acts as a 3D architectural mitotic guidepost. Our data establish a mechanism for epigenetic memory and inheritance of spatial organization of the genome.</p>	<p>Cells; Chromosomes; Cell Nucleus; Cellular Structures</p>
<p>Plecita-Hlavata L, Engstova H, Jezek J, Holendova B, Tauber J, Petraskova L, Kren V, and Jezek P</p>	<p>Potential of Mitochondria-Targeted Antioxidants to Prevent Oxidative Stress in Pancreatic beta-cells.</p>	<p>Oxid Med Cell Longev</p>	<p>2019</p>	<p>Pancreatic beta-cells are vulnerable to oxidative stress due to their low content of redox buffers, such as glutathione, but possess a rich content of thioredoxin, peroxiredoxin, and other proteins capable of redox relay, transferring redox signaling. Consequently, it may be predicted that cytosolic antioxidants could interfere with the cytosolic redox signaling and should not be recommended for any potential therapy. In contrast, mitochondrial matrix-targeted antioxidants could prevent the primary oxidative stress arising from the primary superoxide sources within the mitochondrial matrix, such as at the flavin (I(F)) and ubiquinone (I(Q)) sites of superoxide formation within respiratory chain complex I and the outer ubiquinone site (III(Q)) of complex III. Therefore, using time-resolved confocal fluorescence monitoring with MitoSOX Red, we investigated various effects of mitochondria-targeted antioxidants in model pancreatic beta-cells (insulinoma INS-1E cells) and pancreatic islets. Both SkQ1 (a mitochondria-targeted plastoquinone) and a suppressor of complex III site Q electron leak (S3QEL) prevented superoxide production released to the mitochondrial matrix in INS-1E cells with stimulatory glucose, where SkQ1 also exhibited an antioxidant role for UCP2-silenced cells. SkQ1 acted similarly at nonstimulatory glucose but not in UCP2- silenced cells. Thus, UCP2 can facilitate the antioxidant mechanism based on SkQ1(+) fatty acid anion(-) pairing. The elevated superoxide formation induced by antimycin A was largely prevented by S3QEL, and that induced by rotenone was decreased by SkQ1 and S3QEL and slightly by S1QEL, acting at complex I site Q. Similar results were obtained with the MitoB probe, for the LC-MS-based assessment of the 4 hr accumulation of reactive oxygen species within the mitochondrial matrix but for isolated pancreatic islets. For 2 hr INS-1E incubations, some samples were influenced by the cell death during the experiment. Due to the frequent dependency of antioxidant effects on metabolic modes, we suggest a potential use of mitochondria-targeted antioxidants for the treatment of prediabetic states after cautious nutrition-controlled tests. Their targeted delivery might eventually attenuate the vicious spiral leading to type 2 diabetes.</p>	<p>Cells; Membrane; Organelles; Cellular Structures</p>
<p>Pilely K, Bakke SS, Palarasah Y, Skjoeft MO, Bartels ED, Espevik T, and Garred P</p>	<p>Alpha-cyclodextrin inhibits cholesterol crystal-induced complement-mediated inflammation: A potential new compound for treatment of atherosclerosis.</p>	<p>Atherosclerosis</p>	<p>2019</p>	<p>BACKGROUND AND AIMS: Cholesterol crystal (CC)-induced inflammation is a critical step in the development of atherosclerosis. CCs activate the complement system and induce an inflammatory response resulting in phagocytosis of the CCs, production of reactive oxygen species (ROS) and release of cytokines. The cyclodextrin 2-hydroxypropyl-beta-cyclodextrin has been found to reduce CC-induced complement activation and induce regression of established atherosclerotic plaques in a mouse model of atherosclerosis, thus inhibition of complement with cyclodextrins is a potential new strategy for treatment of inflammation during atherosclerosis. We hypothesized that other cyclodextrins, like alpha- cyclodextrin, may have related functions. METHODS: The effect of cyclodextrins on CC-induced complement activation, phagocytosis, and production of ROS from granulocytes and monocytes was investigated by flow cytometry and ELISA. RESULTS: We showed that alpha-cyclodextrin strongly inhibited CC-induced complement activation by inhibiting binding of the pattern recognition molecules C1q (via IgM) and ficolin-2. The reduced CC-induced complement activation mediated by alpha-cyclodextrin resulted in reduced phagocytosis and reduced ROS production in monocytes and granulocytes. Alpha- cyclodextrin was the most effective inhibitor of CC-induced complement activation, with the reduction in deposition of complement activation products being significantly different from the reduction induced by 2-hydroxypropyl-beta- cyclodextrin. We also found that alpha-cyclodextrin was able to dissolve CCs. CONCLUSIONS: This study identified alpha- cyclodextrin as a potential candidate in the search for therapeutics to prevent CC-induced inflammation in atherosclerosis.</p>	<p>Cells; Immunology; Diseases; Lipids</p>

Bruker Super-Resolution Publications

Pavel MA, Chung HW, Petersen EN, and Hansen SB	Polymodal Mechanism for TWIK-Related K+ Channel Inhibition by Local Anesthetic.	Anesth Analg	2019	<p>BACKGROUND: Local anesthetics cause reversible block of pain and robustly inhibit TWIK-related K channel (TREK-1) currents. Before local anesthesia onset, injection of local anesthetics can cause unwanted transient pain. TREK-1 is an anesthetic-sensitive potassium channel that when inhibited produces pain. A disordered C-terminal loop of TREK-1 is thought to contribute to anesthetic sensitivity, but the molecular basis for TREK-1 inhibition by local anesthetics is unknown. Phospholipase D2 (PLD2) is an enzyme that produces phosphatidic acid (PA) required for TREK-1 activation and also binds to the channel's C terminus. METHODS: Here, we use biophysical and cellular techniques to characterize direct and indirect lipid-mediated mechanism for TREK-1 inhibition (respectively). We characterized direct binding of local anesthetic to TREK-1 by reconstituting the purified channel into artificial membranes and measuring ion flux. We characterized indirect PA-mediated inhibition of TREK-1 by monitoring lipid production in live whole cells using a fluorescent PLD2 product release assay and ion channel current using live whole-cell patch-clamp electrophysiology. We monitored anesthetic-induced nanoscale translocation of PLD2 to TREK-1 channels with super-resolution direct stochastic reconstruction microscopy (dSTORM). RESULTS: We find local anesthetics tetracaine, lidocaine, and bupivacaine directly bind to and inhibit PLD2 enzymatic activity. The lack of PLD2 activity indirectly inhibited TREK-1 currents. Select local anesthetics also partially blocked the open pore of TREK-1 through direct binding. The amount of pore block was variable with tetracaine greater than bupivacaine and lidocaine exhibiting a minor effect. Local anesthetics also disrupt lipid rafts, a mechanism that would normally activate PLD2 were it not for their direct inhibition of enzyme catalysis. CONCLUSIONS: We propose a mechanism of TREK-1 inhibition comprised of (1) primarily indirect PLD2-dependent inhibition of lipid catalysis and (2) limited direct inhibition for select local anesthetics through partial open pore block. The inhibition through PLD2 explains how the C terminus can regulate the channel despite being devoid of structure and putative binding sites for local anesthetics.</p>	Cells; Membrane; Signal Transduction; Cellular Structures
Nidorf SM, and Thompson PL	Why Colchicine Should Be Considered for Secondary Prevention of Atherosclerosis: An Overview.	Clin Ther	2019	<p>PURPOSE: Colchicine is a widely available, inexpensive drug with a range of antiinflammatory properties that may make it suitable for the secondary prevention of atherosclerosis. This review examines how past and contemporary approaches to antiinflammatory therapy for atherosclerosis have led to a better understanding of the nature of the disease and sets out the reasons why colchicine has the potential to become a cornerstone therapy in its management. METHODS: We performed a literature search using PubMed, the Cochrane library, and clinical trial registries to identify completed and ongoing clinical studies on colchicine in coronary artery disease, and a PubMed search to identify publications on the mechanism of action of colchicine relevant to atherosclerosis. FINDINGS: A large body of data confirms that inflammation plays a pivotal role in atherosclerosis. The translation of this extensive knowledge into improved clinical outcomes has until recently been elusive. Findings from statin trials support the possibility that targeting inflammation may be beneficial, but this evidence has been inconclusive. Direct inhibition of atherosclerotic inflammation is being explored in current clinical trials. Targeted inhibition of interleukin 1beta with canakinumab provided the proof of principle that limiting inflammation can improve outcomes in atherosclerotic vascular disease, but long-term treatment with a monoclonal antibody is unlikely to have widespread uptake. Other approaches using agents with a wider set of targets are being explored. Findings from observational studies suggest that methotrexate may reduce cardiovascular risk in patients with rheumatoid arthritis, but CIRT (Cardiovascular Inflammation Reduction Trial) demonstrated that methotrexate provided no cardiovascular benefit in patients with atherosclerotic vascular disease. Recent demonstration that cholesterol crystals trigger the NLRP3 (nucleotide oligomerization domain-, leucine-rich repeat-, and pyrin domain-containing protein 3) inflammasome and the release of inflammatory cytokines that also drive uric acid crystal-induced inflammation indicates that the multiple actions of colchicine that make it effective in gout may be relevant to preventing inflammation and limiting inflammatory injury in atherosclerosis. The ongoing LoDoCo2 (Low Dose Colchicine2) and COLCOT (Colchicine Cardiovascular Outcomes Trial) trials and several other planned large-scale rigorous trials will determine the long-term tolerability and efficacy of low-dose colchicine for secondary prevention in patients with coronary disease. IMPLICATION: Colchicine holds promise as an important, accessible drug that could be successfully repurposed for the secondary prevention of atherosclerotic cardiovascular disease should its tolerability and cardiovascular benefits be confirmed in ongoing clinical trials.</p>	Diseases; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Morgan E, Doh J, Beatty K, and Reich N	VIPER(nano): Improved Live Cell Intracellular Protein Tracking.	ACS Appl Mater Interfaces	2019	Tracking intracellular proteins in live cells has many challenges. The most widely used method, fluorescent protein fusions, can track proteins in their native cellular environment and has led to significant discoveries in cell biology. Fusion proteins add steric bulk to the target protein and can negatively affect native protein function. The use of exogenous probes such as antibodies or protein labels is problematic because these cannot cross the plasma membrane on their own and thus cannot label intracellular targets in cells. We developed a labeling platform, VIPER(nano), for live cell imaging of intracellular proteins using a peptide fusion tag (CoilE) to the protein of interest and delivery of a fluorescently labeled probe peptide (CoilR). CoilR and CoilE form an alpha-helical heterodimer with the protein of interest, rendering a labeled protein. Delivery of CoilR into the cell uses hollow gold nanoshells (HGNs) as the primary delivery vehicle. The technology relies on the conjugation and light-activated release of the CoilR peptide on the surface of the HGNs. We demonstrate light-activated VIPER(nano) delivery and labeling with two intracellular proteins, localized either in the mitochondria or the nucleus. This technology has the ability to study intracellular protein dynamics and spatial tracking while lessening the steric bulk of tags associated with the protein of interest.	Cells; Cellular Structures; Eukaryota; Chemicals and Drugs
Mockl L, Pedram K, Roy AR, Krishnan V, Gustavsson AK, Dorigo O, Bertozzi CR, and Moerner WE	Quantitative Super-Resolution Microscopy of the Mammalian Glycocalyx.	Dev Cell	2019	The mammalian glycocalyx is a heavily glycosylated extramembrane compartment found on nearly every cell. Despite its relevance in both health and disease, studies of the glycocalyx remain hampered by a paucity of methods to spatially classify its components. We combine metabolic labeling, bioorthogonal chemistry, and super-resolution localization microscopy to image two constituents of cell-surface glycans, N-acetylgalactosamine (GalNAc) and sialic acid, with 10-20 nm precision in 2D and 3D. This approach enables two measurements: glycocalyx height and the distribution of individual sugars distal from the membrane. These measurements show that the glycocalyx exhibits nanoscale organization on both cell lines and primary human tumor cells. Additionally, we observe enhanced glycocalyx height in response to epithelial-to-mesenchymal transition and to oncogenic KRAS activation. In the latter case, we trace increased height to an effector gene, GALNT7. These data highlight the power of advanced imaging methods to provide molecular and functional insights into glycocalyx biology.	Cells; Membrane; Cellular Structures; Microscopy
Melton EM, Li H, Benson J, Sohn P, Huang LH, Song BL, Li BL, Chang CCY, and Chang TY	Myeloid Acat1/Spat1 KO attenuates pro-inflammatory responses in macrophages and protects against atherosclerosis in a model of advanced lesions.	J Biol Chem	2019	Cholesterol esters are a key ingredient of foamy cells in atherosclerotic lesions; their formation is catalyzed by two enzymes: acyl-CoA:cholesterol acyltransferases (ACATs; also called sterol O-acyltransferases, or SOATs) ACAT1 and ACAT2. ACAT1 is present in all body cells and is the major isoenzyme in macrophages. Whether blocking ACAT1 benefits atherosclerosis has been under debate for more than a decade. Previously, our laboratory developed a myeloid-specific Acat1 knockout (KO) mouse (Acat1(-M/-M)), devoid of ACAT1 only in macrophages, microglia, and neutrophils. In previous work using the ApoE KO (ApoE(-/-)) mouse model for early lesions, Acat1(-M/-M) significantly reduced lesion macrophage content and suppressed atherosclerosis progression. In advanced lesions, cholesterol crystals become a prominent feature. Here we evaluated the effects of Acat1(-M/-M) in the ApoE KO mouse model for more advanced lesions and found that mice lacking myeloid Acat1 had significantly reduced lesion cholesterol crystal contents. Acat1(-M/-M) also significantly reduced lesion size and macrophage content without increasing apoptotic cell death. Cell culture studies showed that inhibiting ACAT1 in macrophages caused cells to produce less proinflammatory responses upon cholesterol loading by acetyl low-density lipoprotein. In advanced lesions, Acat1(-M/-M) reduced but did not eliminate foamy cells. In advanced plaques isolated from ApoE(-/-) mice, immunostainings showed that both ACAT1 and ACAT2 are present. In cell culture, both enzymes are present in macrophages and smooth muscle cells and contribute to cholesterol ester biosynthesis. Overall, our results support the notion that targeting ACAT1 or targeting both ACAT1 and ACAT2 in macrophages is a novel strategy to treat advanced lesions.	Cells; Immunology; Diseases; Genetic Phenomena
Marro SG, Chanda S, Yang N, Janas JA, Valperga G, Trotter J, Zhou B, Merrill S, Yousif I, Shelby H, Vogel H, Kalani MYS, Sudhof TC, and Wernig M	Neurologin-4 Regulates Excitatory Synaptic Transmission in Human Neurons.	Neuron	2019	The autism-associated synaptic-adhesion gene Neurologin-4 (NLGN4) is poorly conserved evolutionarily, limiting conclusions from Nlgn4 mouse models for human cells. Here, we show that the cellular and subcellular expression of human and murine Neurologin-4 differ, with human Neurologin-4 primarily expressed in cerebral cortex and localized to excitatory synapses. Overexpression of NLGN4 in human embryonic stem cell-derived neurons resulted in an increase in excitatory synapse numbers but a remarkable decrease in synaptic strength. Human neurons carrying the syndromic autism mutation NLGN4-R704C also formed more excitatory synapses but with increased functional synaptic transmission due to a postsynaptic mechanism, while genetic loss of NLGN4 did not significantly affect synapses in the human neurons analyzed. Thus, the NLGN4-R704C mutation represents a change-of-function mutation. Our work reveals contrasting roles of NLGN4 in human and mouse neurons, suggesting that human evolution has impacted even fundamental cell biological processes generally assumed to be highly conserved.	Cells; Synapses; Membrane; Signal Transduction

Bruker Super-Resolution Publications

Koganitsky A, Tworowski D, Dadosh T, Cecchini G, and Eisenbach M	A Mechanism of Modulating the Direction of Flagellar Rotation in Bacteria by Fumarate and Fumarate Reductase.	J Mol Biol	2019	Fumarate, an electron acceptor in anaerobic respiration of Escherichia coli, has an additional function of assisting the flagellarmotor to shift from counterclockwise to clockwise rotation, with a consequent modulation of the bacterial swimming behavior. Fumarate transmits its effect to the motor via the fumarate reductase complex (FrdABCD), shown to bind to FliG- one of the motor's switch proteins. How binding of the FrdABCD respiratory enzyme to FliG enhances clockwise rotation and how fumarate is involved in this activity have remained puzzling. Here we show that the FrdA subunit in the presence of fumarate is sufficient for binding to FliG and for clockwise enhancement. We further demonstrate by in vitro binding assays and super-resolution microscopy in vivo that the mechanism by which fumarate-occupied FrdA enhances clockwise rotation involves its preferential binding to the clockwise state of FliG (FliG(cw)). Continuum electrostatics combined with docking analysis and conformational sampling endorsed the experimental conclusions and suggested that the FrdA-FliG(cw) interaction is driven by the positive electrostatic potential generated by FrdA and the negatively charged areas of FliG. They further demonstrated that fumarate changes FrdA's conformation to one that can bind to FliG(cw). These findings also show that the reason for the failure of the succinate dehydrogenase flavoprotein SdhA (an almost-identical analog of FrdA shown to bind to FliG equally well) to enhance clockwise rotation is that it has no binding preference for FliG(cw). We suggest that this mechanism is physiologically important as it can modulate the magnitude of DeltaG(0) between the clockwise and counterclockwise states of the motor to tune the motor to the growth conditions of the bacteria.	Cells; Bacteria; Protein Binding; Cellular Structures
Heller JP, and Rusakov DA	A Method to Visualize the Nanoscopic Morphology of Astrocytes In Vitro and In Situ.	Methods Mol Biol	2019	In recent years it has become apparent that astroglia are not only essential players in brain development, homeostasis, and metabolic support but are also important for the formation and regulation of synaptic circuits. Fine astrocytic processes that can be found in the vicinity of synapses undergo considerable structural plasticity associated with age- and use-dependent changes in neural circuitries. However, due to the extraordinary complex, essentially nanoscopic morphology of astroglia, the underlying cellular mechanisms remain poorly understood. Here we detail a super-resolution microscopy approach, based on the single-molecule localisation microscopy (SMLM) technique direct stochastic optical reconstruction microscopy (dSTORM) to visualize astroglial morphology on the nanoscale. This approach enables visualization of key morphological changes that occur in nanoscopic astrocyte processes, whose characteristic size falls below the diffraction limit of conventional optical microscopy.	Cells; Nervous System; Microscopy; Eukaryota
Escribano-Lopez I, Banuls C, Diaz-Morales N, Iannantuoni F, Rovira-Llopis S, Gomis R, Rocha M, Hernandez-Mijares A, Murphy MP, and Victor VM	The Mitochondria-Targeted Antioxidant MitoQ Modulates Mitochondrial Function and Endoplasmic Reticulum Stress in Pancreatic beta Cells Exposed to Hyperglycaemia.	Cell Physiol Biochem	2019	BACKGROUND/AIMS: Mitochondria-targeted antioxidants such as mitoquinone (MitoQ) have demonstrated protective effects against oxidative damage in several diseases. The increase in reactive oxygen species (ROS) production during glucose metabolism in beta cells can be exacerbated under hyperglycaemic conditions such as type 2 diabetes (T2D), thus contributing to beta cell function impairment. In the present work, we aimed to evaluate the effect of MitoQ on insulin secretion, oxidative stress, endoplasmic reticulum (ER) stress and nuclear factor kappa B (NFkappaB) signalling in a pancreatic beta cell line under normoglycaemic (NG, 11.1 mM glucose), hyperglycaemic (HG, 25 mM glucose) and lipidic (palmitic acid (PA), 0.5mM) conditions. METHODS: We incubated the pancreatic beta cell line INS-1E with or without MitoQ (0.5microM) under NG, HG and PA conditions. We then assessed the following parameters: glucose-induced insulin secretion, O(2) consumption (with a Clark-type electrode); mitochondrial function, oxidative stress parameters and calcium levels (by fluorescence microscopy); ER stress markers and NFkappaB-p65 protein levels (by western blotting). RESULTS: MitoQ increased insulin secretion and prevented the enhancement of ROS production and O(2) consumption and decrease in GSH levels that are characteristic under HG conditions. MitoQ also reduced protein levels of ER stress markers (GRP78 and P- eIF2alpha) and the proinflammatory nuclear transcription factor NFkappaB-p65, both of which increased under HG. MitoQ did not significantly alter ER stress markers under lipidic conditions. CONCLUSION: Our findings suggest that treatment with MitoQ modulates mitochondrial function, which in turn ameliorates endoplasmic reticulum stress and NFkappaB activation, thereby representing potential benefits for pancreatic beta cell function.	Cells; Organelles; Signal Transduction; Cellular Structures

Bruker Super-Resolution Publications

<p>Dlaskova A, Spacek T, Engstova H, Spackova J, Schrofel A, Holendova B, Smolkova K, Plecita- Hlavata L, and Jezek P</p>	<p>Mitochondrial cristae narrowing upon higher 2-oxoglutarate load.</p>	<p>Biochim Biophys Acta Bioenerg 2019</p>	<p>Hypoxia causes mitochondrial cristae widening, enabled by the ~20% degradation of Mic60/mitofilin, with concomitant clustering of the MICOS complex, reflecting the widening of crista junctions (outlets) (Plecita-Hlavata et al. FASEB J., 2016 30:1941-1957). Attempting to accelerate metabolism by the addition of membrane-permeant dimethyl-2-oxoglutarate (dm2OG) to HepG2 cells pre-adapted to hypoxia, we found cristae narrowing by transmission electron microscopy. Glycolytic HepG2 cells, which downregulate hypoxic respiration, instantly increased respiration with dm2OG. Changes in intracristal space (ICS) morphology were also revealed by 3D super-resolution microscopy using Eos-conjugated ICS-located lactamase-β. Cristae topology was resolved in detail by focused-ion beam/scanning electron microscopy (FIB/SEM). The spatial relocations of key cristae-shaping proteins were indicated by immunocytochemical stochastic 3D super-resolution microscopy (dSTORM), while analyzing inter-antibody-distance histograms: i) ATP-synthase dimers exhibited a higher fraction of shorter inter-distances between bound F(1)-α primary Alexa-Fluor-647-conjugated antibodies, indicating cristae narrowing. ii) Mic60/mitofilin clusters (established upon hypoxia) decayed, restoring isotropic random Mic60/mitofilin distribution (a signature of normoxia). iii) outer membrane SAMM50 formed more focused clusters. Less abundant fractions of higher ATP-synthase oligomers of hypoxic samples on blue-native electrophoresis became more abundant fractions at the high dm2OG load and at normoxia. This indicates more labile ATP-synthase dimeric rows established at crista rims upon hypoxia, strengthened at normoxia or dm2OG-substrate load. Hypothetically, the increased Krebs substrate load stimulates the cross-linking/strengthening of rows of ATP-synthase dimers at the crista rims, making them sharper. Crista narrowing ensures a more efficient coupling of proton pumping to ATP synthesis. We demonstrated that cristae morphology changes even within minutes.</p>	<p>Cells; Membrane; Organelles; Cellular Structures</p>
<p>Dimou E, Cosentino K, Platonova E, Ros U, Sadeghi M, Kashyap P, Katsinelos T, Wegehngel S, Noe F, Garcia-Saez AJ, Ewers H, and Nickel W</p>	<p>Single event visualization of unconventional secretion of FGF2.</p>	<p>J Cell Biol 2019</p>	<p>FGF2 is exported from cells by an unconventional secretory mechanism. Here, we directly visualized individual FGF2 membrane translocation events at the plasma membrane using live cell TIRF microscopy. This process was dependent on both PI(4,5)P(2)-mediated recruitment of FGF2 at the inner leaflet and heparan sulfates capturing FGF2 at the outer plasma membrane leaflet. By simultaneous imaging of both FGF2 membrane recruitment and the appearance of FGF2 at the cell surface, we revealed the kinetics of FGF2 membrane translocation in living cells with an average duration of approximately 200 ms. Furthermore, we directly demonstrated FGF2 oligomers at the inner leaflet of living cells with a FGF2 dimer being the most prominent species. We propose this dimer to represent a key intermediate in the formation of higher FGF2 oligomers that form membrane pores and put forward a kinetic model explaining the mechanism by which membrane-inserted FGF2 oligomers serve as dynamic translocation intermediates during unconventional secretion of FGF2.</p>	<p>Cells; Membrane; Cellular Structures; Microscopy</p>
<p>Collot M, Ashokkumar P, Anton H, Boutant E, Faklaris O, Galli T, Mely Y, Danglot L, and Klymchenko AS</p>	<p>MemBright: A Family of Fluorescent Membrane Probes for Advanced Cellular Imaging and Neuroscience.</p>	<p>Cell Chem Biol 2019</p>	<p>The proper staining of the plasma membrane (PM) is critical in bioimaging as it delimits the cell. Herein, we developed MemBright, a family of six cyanine-based fluorescent turn-on PM probes that emit from orange to near infrared when reaching the PM, and enable homogeneous and selective PM staining with excellent contrast in mono- and two-photon microscopy. These probes are compatible with long-term live-cell imaging and immunostaining. Moreover, MemBright label neurons in a brighter manner than surrounding cells, allowing identification of neurons in acute brain tissue sections and neuromuscular junctions without any use of transfection or transgenic animals. In addition, MemBright probes were used in super-resolution imaging to unravel the neck of dendritic spines. 3D multicolor dSTORM in combination with immunostaining revealed en-passant synapse displaying endogenous glutamate receptors clustered at the axonal-dendritic contact site. MemBright probes thus constitute a universal toolkit for cell biology and neuroscience biomembrane imaging with a variety of microscopy techniques. VIDEO ABSTRACT.</p>	<p>Cells; Membrane; Nervous System; Cellular Structures</p>

Bruker Super-Resolution Publications

Clement M, Chen X, Chenoweth HL, Teng Z, Thome S, Newland SA, Harrison J, Yu X, Finigan AJ, Mallat Z, and Li X	MARK4 (Microtubule Affinity-Regulating Kinase 4)-Dependent Inflammasome Activation Promotes Atherosclerosis-Brief Report.	Arterioscler Thromb Vasc Biol	2019	OBJECTIVE: MARK4 (microtubule affinity-regulating kinase 4) regulates NLRP3 (nucleotide-binding oligomerization domain,leucine-rich repeat, and pyrin domain containing 3) inflammasome activation. The aim of the study is to examine the role of MARK4 in hematopoietic cells during atherosclerosis. METHODS AND RESULTS: We show increased MARK4 expression in human atherosclerotic lesions compared with adjacent areas. MARK4 is coexpressed with NLRP3, and they colocalize in areas enriched in CD68-positive but alpha-SMA (alpha-smooth muscle actin)-negative cells. Expression of MARK4 and NLRP3 in the atherosclerotic lesions is associated with the production of active IL (interleukin)-1beta and IL-18. To directly assess the role of hematopoietic MARK4 in NLRP3 inflammasome activation and atherosclerotic plaque formation, Ldlr (low-density lipoprotein receptor)-deficient mice were lethally irradiated and reconstituted with either wild-type or Mark4-deficient bone marrow cells, and were subsequently fed a high-fat diet and cholesterol diet for 9 weeks. Mark4 deficiency in bone marrow cells led to a significant reduction of lesion size, together with decreased circulating levels of IL-18 and IFN-gamma (interferon-gamma). Furthermore, Mark4 deficiency in primary murine bone marrow-derived macrophages prevented cholesterol crystal-induced NLRP3 inflammasome activation, as revealed by reduced caspase-1 activity together with reduced production of IL-1beta and IL-18. CONCLUSIONS: MARK4-dependent NLRP3 inflammasome activation in the hematopoietic cells regulates the development of atherosclerosis.	Cells; Diseases; Eukaryota; Chemicals and Drugs
Bonilla IM, Belevych AE, Baine S, Stepanov A, Mezache L, Bodnar T, Liu B, Volpe P, Priori S, Weisleder N, Sakuta G, Carnes CA, Radwanski PB, Veeraghavan R, and Gyorke S	Enhancement of Cardiac Store Operated Calcium Entry (SOCE) within Novel Intercalated Disk Microdomains in Arrhythmic Disease.	Sci Rep	2019	Store-operated Ca(2+) entry (SOCE), a major Ca(2+) signaling mechanism in non-mycyte cells, has recently emerged as a component of Ca(2+) signaling in cardiac myocytes. Though it has been reported to play a role in cardiac arrhythmias and to be upregulated in cardiac disease, little is known about the fundamental properties of cardiac SOCE, its structural underpinnings or effector targets. An even greater question is how SOCE interacts with canonical excitation-contraction coupling (ECC). We undertook a multiscale structural and functional investigation of SOCE in cardiac myocytes from healthy mice (wild type; WT) and from a genetic murine model of arrhythmic disease (catecholaminergic ventricular tachycardia; CPVT). Here we provide the first demonstration of local, transient Ca(2+) entry (LoCE) events, which comprise cardiac SOCE. Although infrequent in WT myocytes, LoCEs occurred with greater frequency and amplitude in CPVT myocytes. CPVT myocytes also evidenced characteristic arrhythmogenic spontaneous Ca(2+) waves under cholinergic stress, which were effectively prevented by SOCE inhibition. In a surprising finding, we report that both LoCEs and their underlying protein machinery are concentrated at the intercalated disk (ID). Therefore, localization of cardiac SOCE in the ID compartment has important implications for SOCE-mediated signaling, arrhythmogenesis and intercellular mechanical and electrical coupling in health and disease.	Cells; Organelles; Signal Transduction; Cellular Structures
Baumer Y, McCurdy S, Jin X, Weatherby TM, Dey AK, Mehta NN, Yap JK, Kruth HS, and Boisvert WA	Ultramorphological analysis of plaque advancement and cholesterol crystal formation in Ldlr knockout mouse atherosclerosis.	Atherosclerosis	2019	BACKGROUND AND AIMS: The low-density lipoprotein receptor-deficient (Ldlr(-/-)) mouse has been utilized by cardiovascularresearchers for more than two decades to study atherosclerosis. However, there has not yet been a systematic effort to document the ultrastructural changes that accompany the progression of atherosclerotic plaque in this model. METHODS: Employing several different staining and microscopic techniques, including immunohistochemistry, as well as electron and polarized microscopy, we analyzed atherosclerotic lesion development in Ldlr(-/-) mice fed an atherogenic diet over time. RESULTS: Lipid-like deposits occurred in the subendothelial space after only one week of atherogenic diet. At two weeks, cholesterol crystals (CC) formed and increased thereafter. Lipid, CC, vascular smooth muscles cells, and collagen progressively increased over time, while after 4 weeks, relative macrophage content decreased. Accelerated accumulation of plate- and needle-shaped CC accompanied plaque core necrosis. Lastly, CC were surrounded by cholesterol microdomains, which co-localized with CC through all stages of atherosclerosis, indicating that the cholesterol microdomains may be a source of CC. CONCLUSIONS: Here, we have documented, for the first time in a comprehensive way, atherosclerotic plaque morphology and composition from early to advanced stages in the Ldlr(-/-) mouse, one of the most commonly used animal models utilized in atherosclerosis research.	Cells; Immunology; Microscopy; Diseases
Bartok A, Weaver D, Golenar T, Nichtova Z, Katona M, Bansaghi S, Alzayady KJ, Thomas VK, Ando H, Mikoshiba K, Joseph SK, Yule DI, Csordas G, and Hajnoczky G	IP(3) receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer.	Nat Commun	2019	Contact sites of endoplasmic reticulum (ER) and mitochondria locally convey calcium signals between the IP(3) receptors(IP3R) and the mitochondrial calcium uniporter, and are central to cell survival. It remains unclear whether IP3Rs also have a structural role in contact formation and whether the different IP3R isoforms have redundant functions. Using an IP3R- deficient cell model rescued with each of the three IP3R isoforms and an array of super-resolution and ultrastructural approaches we demonstrate that IP3Rs are required for maintaining ER-mitochondrial contacts. This role is independent of calcium fluxes. We also show that, while each isoform can support contacts, type 2 IP3R is the most effective in delivering calcium to the mitochondria. Thus, these studies reveal a non-canonical, structural role for the IP3Rs and direct attention towards the type 2 IP3R that was previously neglected in the context of ER-mitochondrial calcium signaling.	Cells; Organelles; Signal Transduction; Cellular Structures

Bruker Super-Resolution Publications

Anbalagan S, Blechman J, Gliksberg M, Gordon L, Rotkopf R, Dadosh T, Shimoni E, and Levkowitz G	Robo2 regulates synaptic oxytocin content by affecting actin dynamics.	Elife	2019	The regulation of neuropeptide level at the site of release is essential for proper neurophysiological functions. We focused on a prominent neuropeptide, oxytocin (OXT) in the zebrafish as an in vivo model to visualize and quantify OXT content at the resolution of a single synapse. We found that OXT-loaded synapses were enriched with polymerized actin. Perturbation of actin filaments by either cytochalasin-D or conditional Cofilin expression resulted in decreased synaptic OXT levels. Genetic loss of robo2 or slit3 displayed decreased synaptic OXT content and robo2 mutants displayed reduced mobility of the actin probe Lifeact-EGFP in OXT synapses. Using a novel transgenic reporter allowing real-time monitoring of OXT-loaded vesicles, we show that robo2 mutants display slower rate of vesicles accumulation. OXT-specific expression of dominant-negative Cdc42, which is a key regulator of actin dynamics and a downstream effector of Robo2, led to a dose-dependent increase in OXT content in WT, and a dampened effect in robo2 mutants. Our results link Slit3-Robo2-Cdc42, which controls local actin dynamics, with the maintenance of synaptic neuropeptide levels.	Cells; Synapses; Membrane; Signal Transduction
Zahavi EE, Steinberg N, Altman T, Chein M, Joshi Y, Gradus-Pery T, and Perslon E	The receptor tyrosine kinase TrkB signals without dimerization at the plasma membrane.	Sci Signal	2018	Tropomyosin-related tyrosine kinase B (TrkB) is the receptor for brain-derived neurotrophic factor (BDNF) and provides critical signaling that supports the development and function of the mammalian nervous system. Like other receptor tyrosine kinases (RTKs), TrkB is thought to signal as a dimer. Using cell imaging and biochemical assays, we found that TrkB acted as a monomeric receptor at the plasma membrane regardless of its binding to BDNF and initial activation. Dimerization occurred only after the internalization and accumulation of TrkB monomers within BDNF-containing endosomes. We further showed that dynamin-mediated endocytosis of TrkB-BDNF was required for the effective activation of the kinase AKT but not of the kinase ERK1/2. Thus, we report a previously uncharacterized mode of monomeric signaling for an RTK and a specific role for the endosome in TrkB homodimerization.	Cells; Membrane; Cellular Structures; Eukaryota
Winer H, Fraiberg M, Abada A, Dadosh T, Tamim-Yecheskel BC, and Elazar Z	Autophagy differentially regulates TNF receptor Fn14 by distinct mammalian Atg8 proteins.	Nat Commun	2018	Autophagy, a conserved membrane trafficking process, sequesters cytoplasmic components into autophagosomes and targets them for lysosomal degradation. The TNF receptor Fn14 participates in multiple intracellular signaling pathways and is strongly induced upon tissue injury and solid tumorigenesis. While Fn14 is a short-lived protein, the regulation of its levels is largely obscure. Here we uncover a role for autophagy in Fn14 turnover, wherein specific core autophagy Atg8 proteins play distinct roles: Fn14 accumulates in the ERGIC in absence of GABARAP but within endosomes in the vicinity of autophagic membranes in absence of GATE-16. Moreover, GABARAP regulates overall cellular levels of Fn14, whereas GATE-16 regulates TWEAK signaling by Fn14 and thereby NF-kappaB activity. These findings not only implicate different Atg8 proteins in distinct roles within the mechanism of selective autophagic regulation of Fn14, but may also provide a more general view of their role in mediating autophagosome biogenesis from different membrane sources.	Cells; Organelles; Signal Transduction; Cellular Structures
Wan G, Fields BD, Spracklin G, Shukla A, Phillips CM, and Kennedy S	Spatiotemporal regulation of liquid-like condensates in epigenetic inheritance.	Nature	2018	Non-membrane-bound organelles such as nucleoli, processing bodies, Cajal bodies and germ granules form by the spontaneous self-assembly of specific proteins and RNAs. How these biomolecular condensates form and interact is poorly understood. Here we identify two proteins, ZNFX-1 and WAGO-4, that localize to Caenorhabditis elegans germ granules (P granules) in early germline blastomeres. Later in germline development, ZNFX-1 and WAGO-4 separate from P granules to define an independent liquid-like condensate that we term the Z granule. In adult germ cells, Z granules assemble into ordered tri-condensate assemblages with P granules and Mutator foci, which we term PZM granules. Finally, we show that one biological function of ZNFX-1 and WAGO-4 is to interact with silencing RNAs in the C. elegans germline to direct transgenerational epigenetic inheritance. We speculate that the temporal and spatial ordering of liquid droplet organelles may help cells to organize and coordinate the complex RNA processing pathways that underlie gene-regulatory systems, such as RNA-directed transgenerational epigenetic inheritance.	Cells; Organelles; Cellular Structures; Genetic Phenomena
Veeraraghavan R, Hoeker GS, Alvarez-Laviada A, Hoagland D, Wan X, King DR, Sanchez-Alonso J, Chen C, Jourdan J, Isom LL, Deschenes I, Smyth JW, Gorelik J, Poelzing S, and Gourdie RG	The adhesion function of the sodium channel beta subunit (beta1) contributes to cardiac action potential propagation.	Elife	2018	Computational modeling indicates that cardiac conduction may involve ephaptic coupling - intercellular communication involving electrochemical signaling across narrow extracellular clefts between cardiomyocytes. We hypothesized that beta1(SCN1B) -mediated adhesion scaffolds trans-activating Na(V)1.5 (SCN5A) channels within narrow (<30 nm) perinexal clefts adjacent to gap junctions (GJs), facilitating ephaptic coupling. Super-resolution imaging indicated preferential beta1 localization at the perinexus, where it co-localizes with Na(V)1.5. Smart patch clamp (SPC) indicated greater sodium current density I(Na) at perinexi, relative to non-junctional sites. A novel, rationally designed peptide, betaadp1, potently and selectively inhibited beta1-mediated adhesion, in electric cell-substrate impedance sensing studies. betaadp1 significantly widened perinexi in guinea pig ventricles, and selectively reduced perinexal I(Na), but not whole cell I(Na), in myocyte monolayers. In optical mapping studies, betaadp1 precipitated arrhythmogenic conduction slowing. In summary, beta1-mediated adhesion at the perinexus facilitates action potential propagation between cardiomyocytes, and may represent a novel target for anti-arrhythmic therapies.	Cells; Membrane; Cellular Structures; Diseases

Bruker Super-Resolution Publications

Varsano N, Beghi F, Elad N, Pereiro E, Dadosh T, Pinkas I, Perez-Berna AJ, Jin X, Kruth HS, Leiserowitz L, and Addadi L	Two polymorphic cholesterol monohydrate crystal structures form in macrophage culture models of atherosclerosis.	Proc Natl Acad Sci U S A	2018	The formation of atherosclerotic plaques in the blood vessel walls is the result of LDL particle uptake, and consequently of cholesterol accumulation in macrophage cells. Excess cholesterol accumulation eventually results in cholesterol crystal deposition, the hallmark of mature atheromas. We followed the formation of cholesterol crystals in J774A.1 macrophage cells with time, during accumulation of LDL particles, using a previously developed correlative cryo-soft X-ray tomography (cryo-SXT) and stochastic optical reconstruction microscopy (STORM) technique. We show, in the initial accumulation stages, formation of small quadrilateral crystal plates associated with the cell plasma membrane, which may subsequently assemble into large aggregates. These plates match crystals of the commonly observed cholesterol monohydrate triclinic structure. Large rod-like cholesterol crystals form at a later stage in intracellular locations. Using cryotransmission electron microscopy (cryo-TEM) and cryoelectron diffraction (cryo-ED), we show that the structure of the large elongated rods corresponds to that of monoclinic cholesterol monohydrate, a recently determined polymorph of the triclinic crystal structure. These monoclinic crystals form with an unusual hollow cylinder or helical architecture, which is preserved in the mature rod-like crystals. The rod-like morphology is akin to that observed in crystals isolated from atheromas. We suggest that the crystals in the atherosclerotic plaques preserve in their morphology the memory of the structure in which they were formed. The identification of the polymorph structure, besides explaining the different crystal morphologies, may serve to elucidate mechanisms of cholesterol segregation and precipitation in atherosclerotic plaques.	Cells; Immunology; Microscopy; Diseases
Tiwari PM, Vanover D, Lindsay KE, Bawage SS, Kirschman JL, Bhosle S, Lifland AW, Zurla C, and Santangelo PJ	Engineered mRNA-expressed antibodies prevent respiratory syncytial virus infection.	Nat Commun	2018	The lung is a critical prophylaxis target for clinically important infectious agents, including human respiratory syncytial virus (RSV) and influenza. Here, we develop a modular, synthetic mRNA-based approach to express neutralizing antibodies directly in the lung via aerosol, to prevent RSV infections. First, we express palivizumab, which reduces RSV F copies by 90.8%. Second, we express engineered, membrane-anchored palivizumab, which prevents detectable infection in transfected cells, reducing in vitro titer and in vivo RSV F copies by 99.7% and 89.6%, respectively. Finally, we express an anchored or secreted high-affinity, anti-RSV F, camelid antibody (RSV aVHH and sVHH). We demonstrate that RSV aVHH, but not RSV sVHH, significantly inhibits RSV 7 days post transfection, and we show that RSV aVHH is present in the lung for at least 28 days. Overall, our data suggests that expressing membrane-anchored broadly neutralizing antibodies in the lungs could potentially be a promising pulmonary prophylaxis approach.	Cells; Membrane; Viruses; Cellular Structures
Nir G, Farabella I, Perez Estrada C, Ebeling CG, Beliveau BJ, Sasaki HM, Lee SD, Nguyen SC, McCole RB, Chatteraj S, Erceg J, AlHaj Abed J, Martins NMC, Nguyen HQ, Hannan MA, Russell S, Durand NC, Rao SSP, Kishi JY, Soler-Vila P, Di Pierro M, Onuchic JN, Callahan SP, Schreiner JM, Stuckey JA, Yin P, Aiden EL, Marti-Renom MA, and Wu CT	Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling.	PLOS Genet	2018	Chromosome organization is crucial for genome function. Here, we present a method for visualizing chromosomal DNA at super-resolution and then integrating Hi-C data to produce three-dimensional models of chromosome organization. Using the super-resolution microscopy methods of OligoSTORM and OligoDNA-PAINT, we trace 8 megabases of human chromosome 19, visualizing structures ranging in size from a few kilobases to over a megabase. Focusing on chromosomal regions that contribute to compartments, we discover distinct structures that, in spite of considerable variability, can predict whether such regions correspond to active (A-type) or inactive (B-type) compartments. Imaging through the depths of entire nuclei, we capture pairs of homologous regions in diploid cells, obtaining evidence that maternal and paternal homologous regions can be differentially organized. Finally, using restraint-based modeling to integrate imaging and Hi-C data, we implement a method-integrative modeling of genomic regions (IMGR)-to increase the genomic resolution of our traces to 10 kb.	Cells; Chromosomes; Cell Nucleus; Cellular Structures
Lagache T, Grassart A, Dallongeville S, Faklaris O, Sauvonnnet N, Dufour A, Danglot L, and Olivo-Marin JC	Mapping molecular assemblies with fluorescence microscopy and object-based spatial statistics.	Nat Commun	2018	Elucidating protein functions and molecular organisation requires to localise precisely single or aggregated molecules and analyse their spatial distributions. We develop a statistical method SODA (Statistical Object Distance Analysis) that uses either micro- or nanoscopy to significantly improve on standard co-localisation techniques. Our method considers cellular geometry and densities of molecules to provide statistical maps of isolated and associated (coupled) molecules. We use SODA with three-colour structured-illumination microscopy (SIM) images of hippocampal neurons, and statistically characterise spatial organisation of thousands of synapses. We show that presynaptic synapsin is arranged in asymmetric triangle with the 2 postsynaptic markers homer and PSD95, indicating a deeper localisation of homer. We then determine stoichiometry and distance between localisations of two synaptic vesicle proteins with 3D-STORM. These findings give insights into the protein organisation at the synapse, and prove the efficiency of SODA to quantitatively assess the geometry of molecular assemblies.	

Bruker Super-Resolution Publications

James CC, Zeitz MJ, Calhoun PJ, Lamouille S, and Smyth JW	Altered translation initiation of Gja1 limits gap junction formation during epithelial-mesenchymal transition.	Mol Biol Cell	2018	Epithelial-mesenchymal transition (EMT) is activated during development, wound healing, and pathologies including fibrosis and cancer metastasis. Hallmarks of EMT are remodeling of intercellular junctions and adhesion proteins, including gap junctions. The GJA1 mRNA transcript encoding the gap junction protein connexin43 (Cx43) has been demonstrated to undergo internal translation initiation, yielding truncated isoforms that modulate gap junctions. The PI3K/Akt/mTOR pathway is central to translation regulation and is activated during EMT, leading us to hypothesize that altered translation initiation would contribute to gap junction loss. Using TGF-beta-induced EMT as a model, we find reductions in Cx43 gap junctions despite increased transcription and stabilization of Cx43 protein. Biochemical experiments reveal suppression of the internally translated Cx43 isoform, GJA1-20k in a Smad3 and ERK-dependent manner. Ectopic expression of GJA1-20k does not halt EMT, but is sufficient to rescue gap junction formation. GJA1-20k localizes to the Golgi apparatus, and using superresolution localization microscopy we find retention of GJA1-43k at the Golgi in mesenchymal cells lacking GJA1-20k. NativePAGE demonstrates that levels of GJA1-20k regulate GJA1-43k hexamer oligomerization, a limiting step in Cx43 trafficking. These findings reveal alterations in translation initiation as an unexplored mechanism by which the cell regulates Cx43 gap junction formation during EMT.	
Gunasinghe SD, Shiota T, Stubenrauch CJ, Schulze KE, Webb CT, Fulcher AJ, Dunstan RA, Hay ID, Naderer T, Whelan DR, Bell TDM, Elgass KD, Strugnell RA, and Lithgow T	The WD40 Protein BamB Mediates Coupling of BAM Complexes into Assembly Precincts in the Bacterial Outer Membrane.	Cell Rep	2018	The beta-barrel assembly machinery (BAM) complex is essential for localization of surface proteins on bacterial cells, but the mechanism by which it functions is unclear. We developed a direct stochastic optical reconstruction microscopy (dSTORM) methodology to view the BAM complex in situ. Single-cell analysis showed that discrete membrane precincts housing several BAM complexes are distributed across the E. coli surface, with a nearest neighbor distance of approximately 200 nm. The auxiliary lipoprotein subunit BamB was crucial for this spatial distribution, and in situ crosslinking shows that BamB makes intimate contacts with BamA and BamB in neighboring BAM complexes within the precinct. The BAM complex precincts swell when outer membrane protein synthesis is maximal, visual proof that the precincts are active in protein assembly. This nanoscale interrogation of the BAM complex in situ suggests a model whereby bacterial outer membranes contain highly organized assembly precincts to drive integral protein assembly.	Cells; Membrane; Bacteria; Cellular Structures
Goliand I, Adar-Levor S, Segal I, Nachmias D, Dadosh T, Kozlov MM, and Elia N	Resolving ESCRT-III Spirals at the Intercellular Bridge of Dividing Cells Using 3D STORM.	Cell Rep	2018	The ESCRT machinery mediates membrane fission in a variety of processes in cells. According to current models, ESCRT-III proteins drive membrane fission by assembling into helical filaments on membranes. Here, we used 3D STORM imaging of endogenous ESCRT-III component IST1 to reveal the evolution of the structural organization of ESCRT-III in mammalian cytokinetic abscission. Using this approach, ESCRT-III ring and spiral assemblies were resolved and characterized at different stages of abscission. Visualization of IST1 structures in cells lacking the microtubule-severing enzyme spastin and in cells depleted of specific ESCRT-III components or the ATPase VPS4 demonstrated the contribution of these components to the organization and function of ESCRTs in cells. This work provides direct evidence that ESCRT-III proteins form helical filaments to mediate their function in cells and raises new mechanistic scenarios for ESCRT-driven cytokinetic abscission.	Cells; Membrane; Signal Transduction; Cellular Structures
Dlaskova A, Engstova H, Spacek T, Kahancova A, Pavluch V, Smolkova K, Spackova J, Bartos M, Hlavata LP, and Jezek P	3D super-resolution microscopy reflects mitochondrial cristae alternations and mtDNA nucleoid size and distribution.	Biochim Biophys Acta Bioenerg	2018	3D super-resolution microscopy based on the direct stochastic optical reconstruction microscopy (dSTORM) with primary Alexa-Fluor-647-conjugated antibodies is a powerful method for accessing changes of objects that could be normally resolved only by electron microscopy. Despite the fact that mitochondrial cristae yet to become resolved, we have indicated changes in cristae width and/or morphology by dSTORM of ATP-synthase F(1) subunit alpha (F(1)alpha). Obtained 3D images were analyzed with the help of Ripley's K-function modeling spatial patterns or transferring them into distance distribution function. Resulting histograms of distances frequency distribution provide most frequent distances (MFD) between the localized single antibody molecules. In fasting state of model pancreatic beta-cells, INS-1E, MFD between F(1)alpha were ~80 nm at 0 and 3 mM glucose, whereas decreased to 61 nm and 57 nm upon glucose-stimulated insulin secretion (GSIS) at 11 mM and 20 mM glucose, respectively. Shorter F(1)alpha interdistances reflected cristae width decrease upon GSIS, since such repositioning of F(1)alpha correlated to average 20 nm and 15 nm cristae width at 0 and 3 mM glucose, and 9 nm or 8 nm after higher glucose simulating GSIS (11, 20 mM glucose, respectively). Also, submitochondrial entities such as nucleoids of mtDNA were resolved e.g. after bromo-deoxyuridine (BrdU) pretreatment using anti-BrdU dSTORM. MFD in distances distribution histograms reflected an average nucleoid diameter (<100 nm) and average distances between nucleoids (~1000 nm). Double channel PALM/dSTORM with Eos-lactamase-beta plus anti-TFAM dSTORM confirmed the latter average inter-nucleoid distance. In conclusion, 3D single molecule (dSTORM) microscopy is a reasonable tool for studying mitochondrion.	Cells; Membrane; Cellular Structures; Microscopy

Bruker Super-Resolution Publications

Deo P, Chow SH, Hay ID, Kleinfeld O, Costin A, Elgass KD, Jiang JH, Ramm G, Gabriel K, Dougan G, Lithgow T, Heinz E, and Naderer T	Outer membrane vesicles from <i>Neisseria gonorrhoeae</i> target PorB to mitochondria and induce apoptosis.	PLoS Pathog	2018	<p><i>Neisseria gonorrhoeae</i> causes the sexually transmitted disease gonorrhoea by evading innate immunity. Colonizing themucosa of the reproductive tract depends on the bacterial outer membrane porin, PorB, which is essential for ion and nutrient uptake. PorB is also targeted to host mitochondria and regulates apoptosis pathways to promote infections. How PorB traffics from the outer membrane of <i>N. gonorrhoeae</i> to mitochondria and whether it modulates innate immune cells, such as macrophages, remains unclear. Here, we show that <i>N. gonorrhoeae</i> secretes PorB via outer membrane vesicles (OMVs). Purified OMVs contained primarily outer membrane proteins including oligomeric PorB. The porin was targeted to mitochondria of macrophages after exposure to purified OMVs and wild type <i>N. gonorrhoeae</i>. This was associated with loss of mitochondrial membrane potential, release of cytochrome c, activation of apoptotic caspases and cell death in a time- dependent manner. Consistent with this, OMV-induced macrophage death was prevented with the pan-caspase inhibitor, Q- VD-PH. This shows that <i>N. gonorrhoeae</i> utilizes OMVs to target PorB to mitochondria and to induce apoptosis in macrophages, thus affecting innate immunity.</p>	Cells; Membrane; Bacteria; Organelles
Bergman JP, Bovyn MJ, Doval FF, Sharma A, Gudheti MV, Gross SP, Allard JF, and Vershinin MD	Cargo navigation across 3D microtubule intersections.	Proc Natl Acad Sci U S A	2018	<p>The eukaryotic cell's microtubule cytoskeleton is a complex 3D filament network. Microtubules cross at a wide variety of separation distances and angles. Prior studies in vivo and in vitro suggest that cargo transport is affected by intersection geometry. However, geometric complexity is not yet widely appreciated as a regulatory factor in its own right, and mechanisms that underlie this mode of regulation are not well understood. We have used our recently reported 3D microtubule manipulation system to build filament crossings de novo in a purified in vitro environment and used them to assay kinesin-1-driven model cargo navigation. We found that 3D microtubule network geometry indeed significantly influences cargo routing, and in particular that it is possible to bias a cargo to pass or switch just by changing either filament spacing or angle. Furthermore, we captured our experimental results in a model which accounts for full 3D geometry, stochastic motion of the cargo and associated motors, as well as motor force production and force-dependent behavior. We used a combination of experimental and theoretical analysis to establish the detailed mechanisms underlying cargo navigation at microtubule crossings.</p>	Cells; Protein Binding; Cellular Structures; Eukaryota
Artzi L, Dadoth T, Milrot E, Morais S, Levin-Zaidman S, Morag E, and Bayer EA	Colocalization and Disposition of Cellulosomes in <i>Clostridium clariflavum</i> as Revealed by Correlative Superresolution Imaging.	mBio	2018	<p>Cellulosomes are multienzyme complexes produced by anaerobic, cellulolytic bacteria for highly efficient breakdown of plantcell wall polysaccharides. <i>Clostridium clariflavum</i> is an anaerobic, thermophilic bacterium that produces the largest assembled cellulosome complex in nature to date, comprising three types of scaffoldins: a primary scaffoldin, ScaA; an adaptor scaffoldin, ScaB; and a cell surface anchoring scaffoldin, ScaC. This complex can contain 160 polysaccharide-degrading enzymes. In previous studies, we proposed potential types of cellulosome assemblies in <i>C. clariflavum</i> and demonstrated that these complexes are released into the extracellular medium. In the present study, we explored the disposition of the highly structured, four-tiered cell-anchored cellulosome complex of this bacterium. Four separate, integral cellulosome components were subjected to immunolabeling: ScaA, ScaB, ScaC, and the cellulosome's most prominent enzyme, GH48. Imaging of the cells by correlating scanning electron microscopy and three-dimensional (3D) superresolution fluorescence microscopy revealed that some of the protuberance-like structures on the cell surface represent cellulosomes and that the components are highly colocalized and organized by a defined hierarchy on the cell surface. The display of the cellulosome on the cell surface was found to differ between cells grown on soluble or insoluble substrates. Cell growth on microcrystalline cellulose and wheat straw exhibited dramatic enhancement in the amount of cellulosomes displayed on the bacterial cell surface. IMPORTANCE Conversion of plant biomass into soluble sugars is of high interest for production of fermentable industrial materials, such as biofuels. Biofuels are a very attractive alternative to fossil fuels, both for recycling of agricultural wastes and as a source of sustainable energy. Cellulosomes are among the most efficient enzymatic degraders of biomass known to date, due to the incorporation of a multiplicity of enzymes into a potent, multifunctional nanomachine. The intimate association with the bacterial cell surface is inherent in its efficient action on lignocellulosic substrates, although this property has not been properly addressed experimentally. The dramatic increase in cellulosome performance on recalcitrant feedstocks is critical for the design of cost-effective processes for efficient biomass degradation.</p>	Cells; Bacteria; Cellular Structures; Microscopy

Bruker Super-Resolution Publications

Takakura H, Zhang Y, Erdmann RS, Thompson AD, Lin Y, McNellis B, Rivera-Molina F, Uno SN, Kamiya M, Urano Y, Rothman JE, Bewersdorf J, Schepartz A, and Toomre D	Long time-lapse nanoscopy with spontaneously blinking membrane probes.	Nat Biotechnol	2017	Imaging cellular structures and organelles in living cells by long time-lapse super-resolution microscopy is challenging, as it requires dense labeling, bright and highly photostable dyes, and non-toxic conditions. We introduce a set of high-density, environment-sensitive (HIDE) membrane probes, based on the membrane-permeable silicon-rhodamine dye HMSiR, that assemble in situ and enable long time-lapse, live-cell nanoscopy of discrete cellular structures and organelles with high spatiotemporal resolution. HIDE-enabled nanoscopy movies span tens of minutes, whereas movies obtained with labeled proteins span tens of seconds. Our data reveal 2D dynamics of the mitochondria, plasma membrane and filopodia, and the 2D and 3D dynamics of the endoplasmic reticulum, in living cells. HIDE probes also facilitate acquisition of live-cell, two-color, super-resolution images, expanding the utility of nanoscopy to visualize dynamic processes and structures in living cells.	Cells; Cellular Structures; Microscopy; Eukaryota
Spacek T, Pavluch V, Alan L, Capkova N, Engstova H, Dlaskova A, Berkova Z, Saudek F, and Jezek P	Nkx6.1 decline accompanies mitochondrial DNA reduction but subtle nucleoid size decrease in pancreatic islet beta-cells of diabetic Goto Kakizaki rats.	Sci Rep	2017	Hypertrophic pancreatic islets (PI) of Goto Kakizaki (GK) diabetic rats contain a lower number of beta-cells vs. non-diabetic Wistar rat PI. Remaining beta-cells contain reduced mitochondrial (mt) DNA per nucleus (copy number), probably due to declining mtDNA replication machinery, decreased mt biogenesis or enhanced mitophagy. We confirmed mtDNA copy number decrease down to <30% in PI of one-year-old GK rats. Studying relations to mt nucleoid sizes, we employed 3D superresolution fluorescent photoactivable localization microscopy (FPALM) with lentivirally transduced Eos conjugate of mt single-stranded-DNA-binding protein (mtSSB) or transcription factor TFAM; or by 3D immunocytochemistry. mtSSB (binding transcription or replication nucleoids) contoured "nucleoids" which were smaller by 25% (less diameters >150 nm) in GK beta-cells. Eos-TFAM-visualized nucleoids, composed of 72% localized TFAM, were smaller by 10% (immunohistochemically by 3%). A theoretical ~70% decrease in cell nucleoid number (spatial density) was not observed, rejecting model of single mtDNA per nucleoid. The beta-cell maintenance factor Nkx6.1 mRNA and protein were declining with age (>12-fold, 10 months) and decreasing with fasting hyperglycemia in GK rats, probably predetermining the impaired mtDNA replication (copy number decrease), while spatial expansion of mtDNA kept nucleoids with only smaller sizes than those containing much higher mtDNA in non-diabetic beta-cells.	Cells; Organelles; Cellular Structures; Diseases
Rohlenova K, Sachaphibulkij K, Stursa J, Bezawork Geleta A, Blecha J, Endaya B, Werner L, Cerny J, Zabalova R, Goodwin J, Spacek T, Alizadeh Peddar E, Yan B, Nguyen MN, Vondrusova M, Sobol M, Jezek P, Hozak P, Truksa J, Rohlena J, Dong LF, and Neuzil J	Selective Disruption of Respiratory Supercomplexes as a New Strategy to Suppress Her2(high) Breast Cancer.	Antioxid Redox Signal	2017	AIMS: Expression of the HER2 oncogene in breast cancer is associated with resistance to treatment, and Her2 may regulate bioenergetics. Therefore, we investigated whether disruption of the electron transport chain (ETC) is a viable strategy to eliminate Her2(high) disease. RESULTS: We demonstrate that Her2(high) cells and tumors have increased assembly of respiratory supercomplexes (SCs) and increased complex I-driven respiration in vitro and in vivo. They are also highly sensitive to MitoTam, a novel mitochondrial-targeted derivative of tamoxifen. Unlike tamoxifen, MitoTam efficiently suppresses experimental Her2(high) tumors without systemic toxicity. Mechanistically, MitoTam inhibits complex I-driven respiration and disrupts respiratory SCs in Her2(high) background in vitro and in vivo, leading to elevated reactive oxygen species production and cell death. Intriguingly, higher sensitivity of Her2(high) cells to MitoTam is dependent on the mitochondrial fraction of Her2. INNOVATION: Oncogenes such as HER2 can restructure ETC, creating a previously unrecognized therapeutic vulnerability exploitable by SC-disrupting agents such as MitoTam. CONCLUSION: We propose that the ETC is a suitable therapeutic target in Her2(high) disease. Antioxid. Redox Signal. 26, 84-103.	Cells; Organelles; Protein Binding; Cellular Structures
Rivkin N, Chapnik E, Mildner A, Barshtein G, Porat Z, Kartvelishvily E, Dadosh T, Birger Y, Amir G, Yedgar S, Izraeli S, Jung S, and Hornstein E	Erythrocyte survival is controlled by microRNA-142.	Haematologica	2017	Hematopoietic-specific microRNA-142 is a critical regulator of various blood cell lineages, but its role in erythrocytes is unexplored. Herein, we characterize the impact of microRNA-142 on erythrocyte physiology and molecular cell biology, using a mouse loss-of-function allele. We report that microRNA-142 is required for maintaining the typical erythrocyte biconcave shape and structural resilience, for the normal metabolism of reactive oxygen species, and for overall lifespan. microRNA-142 further controls ACTIN filament homeostasis and membrane skeleton organization. The analyses presented reveal previously unappreciated functions of microRNA-142 and contribute to an emerging view of small RNAs as key players in erythropoiesis. Finally, the work herein demonstrates how a housekeeping network of cytoskeletal regulators can be reshaped by a single micro-RNA denominator in a cell type specific manner.	Cells; Nucleic Acids, Nucleotides, and Nucleosides; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Milrot E, Shimoni E, Dadosh T, Rechav K, Unger T, Van Etten JL, and Minsky A	Structural studies demonstrating a bacteriophage-like replication cycle of the eukaryote-infecting <i>Paramecium bursaria chlorella virus-1</i>.	PLoS Pathog	2017	A fundamental stage in viral infection is the internalization of viral genomes in host cells. Although extensively studied, the mechanisms and factors responsible for the genome internalization process remain poorly understood. Here we report our observations, derived from diverse imaging methods on genome internalization of the large dsDNA <i>Paramecium bursaria chlorella virus-1</i> (PBCV-1). Our studies reveal that early infection stages of this eukaryotic-infecting virus occurs by a bacteriophage-like pathway, whereby PBCV-1 generates a hole in the host cell wall and ejects its dsDNA genome in a linear, base-pair-by-base-pair process, through a membrane tunnel generated by the fusion of the virus internal membrane with the host membrane. Furthermore, our results imply that PBCV-1 DNA condensation that occurs shortly after infection probably plays a role in genome internalization, as hypothesized for the infection of some bacteriophages. The subsequent perforation of the host photosynthetic membranes presumably enables trafficking of viral genomes towards host nuclei. Previous studies established that at late infection stages PBCV-1 generates cytoplasmic organelles, termed viral factories, where viral assembly takes place, a feature characteristic of many large dsDNA viruses that infect eukaryotic organisms. PBCV-1 thus appears to combine a bacteriophage-like mechanism during early infection stages with a eukaryotic-like infection pathway in its late replication cycle.	Viruses; Microscopy; Diseases; DNA
Kopek BG, Paez-Segala MG, Shtengel G, Sochacki KA, Sun MG, Wang Y, Xu CS, van Engelenburg SB, Taraska JW, Looger LL, and Hess HF	Diverse protocols for correlative super-resolution fluorescence imaging and electron microscopy of chemically fixed samples.	Nat Protoc	2017	Our groups have recently developed related approaches for sample preparation for super-resolution imaging within endogenous cellular environments using correlative light and electron microscopy (CLEM). Four distinct techniques for preparing and acquiring super-resolution CLEM data sets for aldehyde-fixed specimens are provided, including Tokuyasu cryosectioning, whole-cell mount, cell unroofing and platinum replication, and resin embedding and sectioning. The choice of the best protocol for a given application depends on a number of criteria that are discussed in detail. Tokuyasu cryosectioning is relatively rapid but is limited to small, delicate specimens. Whole-cell mount has the simplest sample preparation but is restricted to surface structures. Cell unroofing and platinum replication creates high-contrast, 3D images of the cytoplasmic surface of the plasma membrane but is more challenging than whole-cell mount. Resin embedding permits serial sectioning of large samples but is limited to osmium-resistant probes, and is technically difficult. Expected results from these protocols include super-resolution localization (approximately 10-50 nm) of fluorescent targets within the context of electron microscopy ultrastructure, which can help address cell biological questions. These protocols can be completed in 2-7 d, are compatible with a number of super-resolution imaging protocols, and are broadly applicable across biology.	Microscopy; Chemicals and Drugs
Kannan M, Bayam E, Wagner C, Rinaldi B, Kretz PF, Tilly P, Roos M, McGillewie L, Bar S, Minocha S, Chevalier C, Po C, Chelly J, Mandel JL, Borgatti R, Piton A, Kinnear C, Loos B, Adams DJ, Herault Y, Collins SC, Friant S, Godin JD, and Yalcin B	WD40-repeat 47, a microtubule-associated protein, is essential for brain development and autophagy.	Proc Natl Acad Sci U S A	2017	The family of WD40-repeat (WDR) proteins is one of the largest in eukaryotes, but little is known about their function in brain development. Among 26 WDR genes assessed, we found 7 displaying a major impact in neuronal morphology when inactivated in mice. Remarkably, all seven genes showed corpus callosum defects, including thicker (Atg16l1, Coro1c, Dmnl2, and Herc1), thinner (Kif21b and Wdr89), or absent corpus callosum (Wdr47), revealing a common role for WDR genes in brain connectivity. We focused on the poorly studied WDR47 protein sharing structural homology with LIS1, which causes lissencephaly. In a dosage-dependent manner, mice lacking Wdr47 showed lethality, extensive fiber defects, microcephaly, thinner cortices, and sensory motor gating abnormalities. We showed that WDR47 shares functional characteristics with LIS1 and participates in key microtubule-mediated processes, including neural stem cell proliferation, radial migration, and growth cone dynamics. In absence of WDR47, the exhaustion of late cortical progenitors and the consequent decrease of neurogenesis together with the impaired survival of late-born neurons are likely yielding to the worsening of the microcephaly phenotype postnatally. Interestingly, the WDR47-specific C-terminal to LisH (CTLH) domain was associated with functions in autophagy described in mammals. Silencing WDR47 in hypothalamic GT1-7 neuronal cells and yeast models independently recapitulated these findings, showing conserved mechanisms. Finally, our data identified superior cervical ganglion-10 (SCG10) as an interacting partner of WDR47. Taken together, these results provide a starting point for studying the implications of WDR proteins in neuronal regulation of microtubules and autophagy.	Cells; Nervous System; Cellular Structures; Genetic Phenomena

Bruker Super-Resolution Publications

<p>Joensuu M, Martinez-Marmol R, Padmanabhan P, Glass NR, Durisic N, Pelekanos M, Mollazade M, Balistreri G, Amor R, Cooper-White JJ, Goodhill GJ, and Meunier FA</p>	<p>Visualizing endocytic recycling and trafficking in live neurons by subdiffractional tracking of internalized molecules.</p>	<p>Nat Protoc</p>	<p>2017</p>	<p>Our understanding of endocytic pathway dynamics is restricted by the diffraction limit of light microscopy. Although super-resolution techniques can overcome this issue, highly crowded cellular environments, such as nerve terminals, can also dramatically limit the tracking of multiple endocytic vesicles such as synaptic vesicles (SVs), which in turn restricts the analytical dissection of their discrete diffusional and transport states. We recently introduced a pulse-chase technique for subdiffractional tracking of internalized molecules (sdTIM) that allows the visualization of fluorescently tagged molecules trapped in individual signaling endosomes and SVs in presynapses or axons with 30- to 50-nm localization precision. We originally developed this approach for tracking single molecules of botulinum neurotoxin type A, which undergoes activity-dependent internalization and retrograde transport in autophagosomes. This method was then adapted to localize the signaling endosomes containing cholera toxin subunit-B that undergo retrograde transport in axons and to track SVs in the crowded environment of hippocampal presynapses. We describe (i) the construction of a custom-made microfluidic device that enables control over neuronal orientation; (ii) the 3D printing of a perfusion system for sdTIM experiments performed on glass-bottom dishes; (iii) the dissection, culturing and transfection of hippocampal neurons in microfluidic devices; and (iv) guidance on how to perform the pulse-chase experiments and data analysis. In addition, we describe the use of single-molecule-tracking analytical tools to reveal the average and the heterogeneous single-molecule mobility behaviors. We also discuss alternative reagents and equipment that can, in principle, be used for sdTIM experiments and describe how to adapt sdTIM to image nanocluster formation and/or tubulation in early endosomes during sorting events. The procedures described in this protocol take approximately 1 week.</p>	<p>Cells; Nervous System; Genetic Phenomena; Eukaryota</p>
<p>Heller JP, and Rusakov DA</p>	<p>The Nanoworld of the Tripartite Synapse: Insights from Super-Resolution Microscopy.</p>	<p>Front Cell Neurosci</p>	<p>2017</p>	<p>Synaptic connections between individual nerve cells are fundamental to the process of information transfer and storage in the brain. Over the past decades a third key partner of the synaptic machinery has been unveiled: ultrathin processes of electrically passive astroglia which often surround pre- and postsynaptic structures. The recent advent of super-resolution (SR) microscopy has begun to uncover the dynamic nanoworld of synapses and their astroglial environment. Here we overview and discuss the current progress in our understanding of the synaptic nanoenvironment, as gleaned from the imaging methods that go beyond the diffraction limit of conventional light microscopy. We argue that such methods are essential to achieve a new level of comprehension pertinent to the principles of signal integration in the brain.</p>	
<p>Heller JP, Michaluk P, Sugao K, and Rusakov DA</p>	<p>Probing nano-organization of astroglia with multi-color super-resolution microscopy.</p>	<p>J Neurosci Res</p>	<p>2017</p>	<p>Astroglia are essential for brain development, homeostasis, and metabolic support. They also contribute actively to the formation and regulation of synaptic circuits, by successfully handling, integrating, and propagating physiological signals of neural networks. The latter occurs mainly by engaging a versatile mechanism of internal Ca(2+) fluctuations and regenerative waves prompting targeted release of signaling molecules into the extracellular space. Astroglia also show substantial structural plasticity associated with age- and use-dependent changes in neural circuitry. However, the underlying cellular mechanisms are poorly understood, mainly because of the extraordinary complex morphology of astroglial compartments on the nanoscopic scale. This complexity largely prevents direct experimental access to astroglial processes, most of which are beyond the diffraction limit of optical microscopy. Here we employed super-resolution microscopy (direct stochastic optical reconstruction microscopy; dSTORM), to visualize astroglial organization on the nanoscale, in culture and in thin brain slices, as an initial step to understand the structural basis of astrocytic nano-physiology. We were able to follow nanoscopic morphology of GFAP-enriched astrocytes, which adopt a flattened shape in culture and a sponge-like structure in situ, with GFAP fibers of varied diameters. We also visualized nanoscopic astrocytic processes using the ubiquitous cytosolic astrocyte marker proteins S100beta and glutamine synthetase. Finally, we overexpressed and imaged membrane-targeted pHluorin and lymphocyte-specific protein tyrosine kinase (N-terminal domain)-green fluorescent protein (lck-GFP), to better understand the molecular cascades underlying some common astroglia-targeted fluorescence imaging techniques. The results provide novel, albeit initial, insights into the cellular organization of astroglia on the nanoscale, paving the way for function-specific studies. (c) 2017 Wiley Periodicals, Inc.</p>	<p>Cells; Nervous System; Microscopy; Eukaryota</p>

Bruker Super-Resolution Publications

<p>Graber TE, Freemantle E, Anadolu MN, Hebert-Seropian S, MacAdam RL, Shin U, Hoang HD, Alain T, Lacaille JC, and Sossin WS</p>	<p>UPF1 Governs Synaptic Plasticity through Association with a STAU2 RNA Granule.</p>	<p>J Neurosci</p>	<p>2017</p>	<p>Neuronal mRNAs can be packaged in reversibly stalled polysome granules before their transport to distant synaptic locales. Stimulation of synaptic metabotropic glutamate receptors (mGluRs) reactivates translation of these particular mRNAs to produce plasticity-related protein; a phenomenon exhibited during mGluR-mediated LTD. This form of plasticity is deregulated in Fragile X Syndrome, a monogenic form of autism in humans, and understanding the stalling and reactivation mechanism could reveal new approaches to therapies. Here, we demonstrate that UPF1, known to stall peptide release during nonsense-mediated RNA decay, is critical for assembly of stalled polysomes in rat hippocampal neurons derived from embryos of either sex. Moreover, UPF1 and its interaction with the RNA binding protein STAU2 are necessary for proper transport and local translation from a prototypical RNA granule substrate and for mGluR-LTD in hippocampal neurons. These data highlight a new, neuronal role for UPF1, distinct from its RNA decay functions, in regulating transport and/or translation of mRNAs that are critical for synaptic plasticity. SIGNIFICANCE STATEMENT The elongation and/or termination steps of mRNA translation are emerging as important control points in mGluR-LTD, a form of synaptic plasticity that is compromised in a severe monogenic form of autism, Fragile X Syndrome. Deciphering the molecular mechanisms controlling this type of plasticity may thus open new therapeutic opportunities. Here, we describe a new role for the ATP-dependent helicase UPF1 and its interaction with the RNA localization protein STAU2 in mediating mGluR-LTD through the regulation of mRNA translation complexes stalled at the level of elongation and/or termination.</p>	<p>Cells; Synapses; Membrane; Organelles</p>
<p>German CL, Gudheti MV, Fleckenstein AE, and Jorgensen EM</p>	<p>Brain Slice Staining and Preparation for Three-Dimensional Super-Resolution Microscopy.</p>	<p>Methods Mol Biol</p>	<p>2017</p>	<p>Localization microscopy techniques such as photoactivation localization microscopy (PALM), fluorescent PALM (FPALM), ground state depletion (GSD), and stochastic optical reconstruction microscopy (STORM) provide the highest precision for single-molecule localization currently available. However, localization microscopy has been largely limited to cell cultures due to the difficulties that arise in imaging thicker tissue sections. Sample fixation and antibody staining, background fluorescence, fluorophore photostability, light scattering in thick sections, and sample movement create significant challenges for imaging intact tissue. We have developed a sample preparation and image acquisition protocol to address these challenges in rat brain slices. The sample preparation combined multiple fixation steps, saponin permeabilization, and tissue clarification. Together, these preserve intracellular structures, promote antibody penetration, reduce background fluorescence and light scattering, and allow acquisition of images deep in a 30 µm thick slice. Image acquisition challenges were resolved by overlaying samples with a permeable agarose pad and custom-built stainless-steel imaging adapter, and sealing the imaging chamber. This approach kept slices flat, immobile, bathed in imaging buffer, and prevented buffer oxidation during imaging. Using this protocol, we consistently obtained single-molecule localizations of synaptic vesicle and active zone proteins in three dimensions within individual synaptic terminals of the striatum in rat brain slices. These techniques may be easily adapted to the preparation and imaging of other tissues, substantially broadening the application of super-resolution imaging.</p>	<p>Nervous System; Microscopy; Eukaryota; Chemicals and Drugs</p>
<p>Batsir S, Geiger B, and Kam Z</p>	<p>Dynamics of the sealing zone in cultured osteoclasts.</p>	<p>Cytoskeleton (Hoboken)</p>	<p>2017</p>	<p>Bone resorption by osteoclasts (OCs) depends on the formation and stability of the sealing zone (SZ), a peripheral belt of actin and integrin-based podosomes. Recent studies demonstrated that the SZ is a highly dynamic structure, undergoing cycles of assembly and disassembly. In this study, we explored the mechanisms underlying the regulation of SZ stability and reorganization in OCs cultured on glass slides, and forming an SZ-like podosome belt (SZL). By monitoring this belt in cultured RAW264.7 cells expressing GFP-tagged actin, we show here that SZL stability is usually locally regulated, and its dissociation, occurring mostly in concave segments, is manifested in the loss of both podosome coherence, and actin belt continuity. Double labeling of cells for actin and tubulin indicated that microtubules (MTs) are mostly confined by the inner aspect of the stable SZL-associated actin belt. However, in unstable regions of the SZL, MTs tend to extend radially, across the SZL, toward the cell edge. Disruption of MTs by nocodazole induces SZ disassembly, without affecting individual podosome stability. Inspection of the MT network indicates that it is enriched along stable SZL regions, while bypassing disorganized regions. These results suggest that the SZL is stabilized by MTs flanking its inner aspect, while disruption or misalignment of MTs leads to SZL destabilization. We further demonstrate that the MT-associated protein dynamin2 is involved in the regulation of SZL stability, and dynamin2 knockdown or inactivation cause SZL destabilization.</p>	<p>Cells; Eukaryota; Chemicals and Drugs</p>

Bruker Super-Resolution Publications

Banisch TU, Maimon I, Dadosh T, and Gilboa L	Escort cells generate a dynamic compartment for germline stem cell differentiation via combined Stat and Erk signalling.	Development	2017	Two different compartments support germline stem cell (GSC) self-renewal and their timely differentiation: the classical niche provides maintenance cues, while a differentiation compartment, formed by somatic escort cells (ECs), is required for proper GSC differentiation. ECs extend long protrusions that invade between tightly packed germ cells, and alternate between encapsulating and releasing them. How ECs achieve this dynamic balance has not been resolved. By combining live imaging and genetic analyses in <i>Drosophila</i> , we have characterized EC shapes and their dynamic changes. We show that germ cell encapsulation by ECs is a communal phenomenon, whereby EC-EC contacts stabilise an extensive meshwork of protrusions. We further show that Signal Transducer and Activator of Transcription (Stat) and Epidermal Growth Factor Receptor (Egfr) signalling sustain EC protrusiveness and flexibility by combinatorially affecting the activity of different RhoGTPases. Our results reveal how a complex signalling network can determine the shape of a cell and its dynamic behaviour. It also explains how the differentiation compartment can establish extensive contacts with germ cells, while allowing a continual posterior movement of differentiating GSC daughters.	Cells; Signal Transduction; Eukaryota; Chemicals and Drugs
Arbel-Goren R, Shapira Y, and Stavans J	Method for Labeling Transcripts in Individual Escherichia coli Cells for Single-molecule Fluorescence In Situ Hybridization Experiments.	J Vis Exp	2017	A method is described for labeling individual messenger RNA (mRNA) transcripts in fixed bacteria for use in single-molecule fluorescence in situ hybridization (smFISH) experiments in <i>E. coli</i> . smFISH allows the measurement of cell-to-cell variability in mRNA copy number of genes of interest, as well as the subcellular location of the transcripts. The main steps involved are fixation of the bacterial cell culture, permeabilization of cell membranes, and hybridization of the target transcripts with sets of commercially available short fluorescently-labeled oligonucleotide probes. smFISH can allow the imaging of the transcripts of multiple genes in the same cell, with limitations imposed by the spectral overlap between different fluorescent markers. Following completion of the protocol illustrated below, cells can be readily imaged using a microscope coupled with a camera suitable for low-intensity fluorescence. These images, together with cell contours obtained from segmentation of phase contrast frames, or from cell membrane staining, allow the calculation of the mRNA copy number distribution of a sample of cells using open-source or custom-written software. The labeling method described here can also be applied to image transcripts with stochastic optical reconstruction microscopy (STORM).	Bacteria; Genetic Phenomena
?	Erratum: Graber et al., "UPF1 Governs Synaptic Plasticity through Association with a STAU2 RNA Granule".	J Neurosci	2017	[This corrects the article on p. 9116 in vol. 37, PMID: 28821679].	
Zalli D, Neff L, Nagano K, Shin NY, Witke W, Gori F, and Baron R	The Actin-Binding Protein Cofilin and Its Interaction With Cortactin Are Required for Podosome Patterning in Osteoclasts and Bone Resorption In Vivo and In Vitro.	J Bone Miner Res	2016	The adhesion of osteoclasts (OCs) to bone and bone resorption require the assembly of specific F-actin adhesion structures, the podosomes, and their dense packing into a sealing zone. The OC-specific formation of the sealing zone requires the interaction of microtubule (MT) + ends with podosomes. Here, we deleted cofilin, a cortactin (CTTN)- and actin-binding protein highly expressed in OCs, to determine if it acts downstream of the MT-CTTN axis to regulate actin polymerization in podosomes. Conditional deletion of cofilin in OCs in mice, driven by the cathepsin K promoter (Ctsk-Cre), impaired bone resorption in vivo, increasing bone density. In vitro, OCs were not able to organize podosomes into peripheral belts. The MT network was disorganized, MT stability was decreased, and cell migration impaired. Active cofilin stabilizes MTs and allows podosome belt formation, whereas MT disruption deactivates cofilin via phosphorylation. Cofilin interacts with CTTN in podosomes and phosphorylation of either protein disrupts this interaction, which is critical for belt stabilization and for the maintenance of MT dynamic instability. Accordingly, active cofilin was required to rescue the OC cytoskeletal phenotype in vitro. These findings suggest that the patterning of podosomes into a sealing zone involves the dynamic interaction between cofilin, CTTN, and the MTs + ends. This interaction is critical for the functional organization of OCs and for bone resorption. (c) 2016 American Society for Bone and Mineral Research.	Cells; Protein Binding; Cellular Structures; Diseases
Xie W, Horn HF, and Wright GD	Superresolution Microscopy of the Nuclear Envelope and Associated Proteins.	Methods Mol Biol	2016	Superresolution microscopy is undoubtedly one of the most exciting technologies since the invention of the optical microscope. Capable of nanometer-scale resolution to surpass the diffraction limit and coupled with the versatile labeling techniques available, it is revolutionizing the study of cell biology. Our understanding of the nucleus, the genetic and architectural center of the cell, has gained great advancements through the application of various superresolution microscopy techniques. This chapter describes detailed procedures of multichannel superresolution imaging of the mammalian nucleus, using structured illumination microscopy and single-molecule localization microscopy.	Cells; Membrane; Cell Nucleus; Cellular Structures

Bruker Super-Resolution Publications

Wegel E, Gohler A, Lagerholm BC, Wainman A, Uphoff S, Kaufmann R, and Dobbie IM	Imaging cellular structures in super-resolution with SIM, STED and Localisation Microscopy: A practical comparison.	Sci Rep	2016	Many biological questions require fluorescence microscopy with a resolution beyond the diffraction limit of light. Super-resolution methods such as Structured Illumination Microscopy (SIM), Stimulated Emission Depletion (STED) microscopy and Single Molecule Localisation Microscopy (SMLM) enable an increase in image resolution beyond the classical diffraction-limit. Here, we compare the individual strengths and weaknesses of each technique by imaging a variety of different subcellular structures in fixed cells. We chose examples ranging from well separated vesicles to densely packed three dimensional filaments. We used quantitative and correlative analyses to assess the performance of SIM, STED and SMLM with the aim of establishing a rough guideline regarding the suitability for typical applications and to highlight pitfalls associated with the different techniques.	Cells; Cellular Structures; Immunology; Microscopy
Veeraraghavan R, and Gourdie RG	Stochastic optical reconstruction microscopy-based relative localization analysis (STORM-RLA) for quantitative nanoscale assessment of spatial protein organization.	Mol Biol Cell	2016	The spatial association between proteins is crucial to understanding how they function in biological systems. Colocalization analysis of fluorescence microscopy images is widely used to assess this. However, colocalization analysis performed on two-dimensional images with diffraction-limited resolution merely indicates that the proteins are within 200-300 nm of each other in the xy-plane and within 500-700 nm of each other along the z-axis. Here we demonstrate a novel three-dimensional quantitative analysis applicable to single-molecule positional data: stochastic optical reconstruction microscopy-based relative localization analysis (STORM-RLA). This method offers significant advantages: 1) STORM imaging affords 20-nm resolution in the xy-plane and <50 nm along the z-axis; 2) STORM-RLA provides a quantitative assessment of the frequency and degree of overlap between clusters of colabeled proteins; and 3) STORM-RLA also calculates the precise distances between both overlapping and nonoverlapping clusters in three dimensions. Thus STORM-RLA represents a significant advance in the high-throughput quantitative assessment of the spatial organization of proteins.	Microscopy; Chemicals and Drugs
Veeraraghavan R, Lin J, Keener JP, Gourdie R, and Poelzing S	Potassium channels in the Cx43 gap junction perinexus modulate ephaptic coupling: an experimental and modeling study.	Pflugers Arch	2016	It was recently demonstrated that cardiac sodium channels (Nav1.5) localized at the perinexus, an intercalated disc (ID) nanodomain associated with gap junctions (GJ), may contribute to electrical coupling between cardiac myocytes via an ephaptic mechanism. Impairment of ephaptic coupling by acute interstitial edema (AIE)-induced swelling of the perinexus was associated with arrhythmogenic, anisotropic conduction slowing. Given that Kir2.1 has also recently been reported to localize at intercalated discs, we hypothesized that Kir2.1 channels may reside within the perinexus and that inhibiting them may mitigate arrhythmogenic conduction slowing observed during AIE. Using gated stimulated emission depletion (gSTED) and stochastic optical reconstruction microscopy (STORM) super-resolution microscopy, we indeed find that a significant proportion of Kir2.1 channels resides within the perinexus. Moreover, whereas Nav1.5 inhibition during AIE exacerbated arrhythmogenic conduction slowing, inhibiting Kir2.1 channels during AIE preferentially increased transverse conduction velocity-decreasing anisotropy and ameliorating arrhythmia risk compared to AIE alone. Comparison of our results with a nanodomain computer model identified enrichment of both Nav1.5 and Kir2.1 at intercalated discs as key factors underlying the experimental observations. We demonstrate that Kir2.1 channels are localized within the perinexus alongside Nav1.5 channels. Further, targeting Kir2.1 modulates intercellular coupling between cardiac myocytes, anisotropy of conduction, and arrhythmia propensity in a manner consistent with a role for ephaptic coupling in cardiac conduction. For over half a century, electrical excitation in the heart has been thought to occur exclusively via gap junction-mediated ionic current flow between cells. Further, excitation was thought to depend almost exclusively on sodium channels with potassium channels being involved mainly in returning the cell to rest. Here, we demonstrate that sodium and potassium channels co-reside within nanoscale domains at cell-to-cell contact sites. Experimental and computer modeling results suggest a role for these channels in electrical coupling between cardiac muscle cells via an ephaptic mechanism working in tandem with gap junctions. This new insight into the mechanism of cardiac electrical excitation could pave the way for novel therapies against cardiac rhythm disturbances.	Cells; Membrane; Cellular Structures; Diseases

Bruker Super-Resolution Publications

Varsano N, Dadosh T, Kapishnikov S, Pereiro E, Shimoni E, Jin X, Kruth HS, Leiserowitz L, and Addadi L	Development of Correlative Cryo-soft X-ray Tomography and Stochastic Reconstruction Microscopy. A Study of Cholesterol Crystal Early Formation in Cells.	J Am Chem Soc	2016	We have developed a high resolution correlative method involving cryo-soft X-ray tomography (cryo-SXT) and stochastic optical reconstruction microscopy (STORM), which provides information in three dimensions on large cellular volumes at 70 nm resolution. Cryo-SXT morphologically identified and localized aggregations of carbon-rich materials. STORM identified specific markers on the desired epitopes, enabling colocalization between the identified objects, in this case cholesterol crystals, and the cellular environment. The samples were studied under ambient and cryogenic conditions without dehydration or heavy metal staining. The early events of cholesterol crystal development were investigated in relation to atherosclerosis, using as model macrophage cell cultures enriched with LDL particles. Atherosclerotic plaques build up in arteries in a slow process involving cholesterol crystal accumulation. Cholesterol crystal deposition is a crucial stage in the pathological cascade. Our results show that cholesterol crystals can be identified and imaged at a very early stage on the cell plasma membrane and in intracellular locations. This technique can in principle be applied to other biological samples where specific molecular identification is required in conjunction with high resolution 3D-imaging.	Cells; Immunology; Microscopy; Lipids
Plecita-Hlavata L, Engstova H, Alan L, Spacek T, Dlaskova A, Smolkova K, Spackova J, Tauber J, Stradalova V, Malinsky J, Lessard M, Bewersdorf J, and Jezek P	Hypoxic HepG2 cell adaptation decreases ATP synthase dimers and ATP production in inflated cristae by mitofilin down-regulation concomitant to MICOS clustering.	FASEB J	2016	The relationship of the inner mitochondrial membrane (IMM) cristae structure and intracristal space (ICS) to oxidative phosphorylation (oxphos) is not well understood. Mitofilin (subunit Mic60) of the mitochondrial contact site and cristae organizing system (MICOS) IMM complex is attached to the outer membrane (OMM) via the sorting and assembly machinery/topogenesis of mitochondrial outer membrane beta-barrel proteins (SAM/TOB) complex and controls the shape of the cristae. ATP synthase dimers determine sharp cristae edges, whereas trimeric OPA1 tightens ICS outlets. Metabolism is altered during hypoxia, and we therefore studied cristae morphology in HepG2 cells adapted to 5% oxygen for 72 h. Three dimensional (3D), super-resolution biplane fluorescence photoactivation localization microscopy with Eos-conjugated, ICS-located lactamase-beta indicated hypoxic ICS expansion with an unchanged OMM (visualized by Eos-mitochondrial fission protein-1). 3D direct stochastic optical reconstruction microscopy immunocytochemistry revealed foci of clustered mitofilin (but not MICOS subunit Mic19) in contrast to its even normoxic distribution. Mitofilin mRNA and protein decreased by approximately 20%. ATP synthase dimers vs monomers and state-3/state-4 respiration ratios were lower during hypoxia. Electron microscopy confirmed ICS expansion (maximum in glycolytic cells), which was absent in reduced or OMM-detached cristae of OPA1- and mitofilin-silenced cells, respectively. Hypoxic adaptation is reported as rounding sharp cristae edges and expanding cristae width (ICS) by partial mitofilin/Mic60 down-regulation. Mitofilin-depleted MICOS detaches from SAM while remaining MICOS with mitofilin redistributes toward higher interdistances. This phenomenon causes partial oxphos dormancy in glycolytic cells via disruption of ATP synthase dimers.-Plecita-Hlavata, L, Engstova, H., Alan, L., Spacek, T., Dlaskova, A., Smolkova, K., Spackova, J., Tauber, J., Stradalova, V., Malinsky, J., Lessard, M., Bewersdorf, J., Jezek, P. Hypoxic HepG2 cell adaptation decreases ATP synthase dimers and ATP production in inflated cristae by mitofilin down-regulation concomitant to MICOS clustering.	Cells; Organelles; Cellular Structures; Genetic Phenomena
Petersen EN, Chung HW, Nayeibosadri A, and Hansen SB	Kinetic disruption of lipid rafts is a mechanosensor for phospholipase D.	Nat Commun	2016	The sensing of physical force, mechanosensation, underlies two of five human senses-touch and hearing. How transduction of force in a membrane occurs remains unclear. We asked if a biological membrane could employ kinetic energy to transduce a signal absent tension. Here we show that lipid rafts are dynamic compartments that inactivate the signalling enzyme phospholipase D2 (PLD2) by sequestering the enzyme from its substrate. Mechanical disruption of the lipid rafts activates PLD2 by mixing the enzyme with its substrate to produce the signalling lipid phosphatidic acid (PA). We calculate a latency time of <650 μs for PLD activation by mixing. Our results establish a fast, non-tension mechanism for mechanotransduction where disruption of ordered lipids initiates a mechanosensitive signal for cell growth through mechanical mixing.	Cells; Membrane; Signal Transduction; Cellular Structures
Lo CY, Chen S, Creed SJ, Kang M, Zhao N, Tang BZ, and Elgass KD	Novel super-resolution capable mitochondrial probe, MitoRed AIE, enables assessment of real-time molecular mitochondrial dynamics.	Sci Rep	2016	Mitochondria and mitochondrial dynamics play vital roles in health and disease. With the intricate nanometer-scale structure and rapid dynamics of mitochondria, super-resolution microscopy techniques possess great un-tapped potential to significantly contribute to understanding mitochondrial biology and kinetics. Here we present a novel mitochondrial probe (MitoRed AIE) suitable for live mitochondrial dynamics imaging and single particle tracking (SPT), together with a multi-dimensional data analysis approach to assess local mitochondrial (membrane) fluidity. The MitoRed AIE probe localizes primarily to mitochondrial membranes, with 95 ms fluorophore on-time delivering 106 photons/ms, characteristics which we exploit to demonstrate live cell 100 fps 3D time-lapse tracking of mitochondria. Combining our experimental and analytical approaches, we uncover mitochondrial dynamics at unprecedented time scales. This approach opens up a new regime into high spatio-temporal resolution dynamics in many areas of mitochondrial biology.	Cells; Membrane; Organelles; Cellular Structures

Bruker Super-Resolution Publications

Hartley JM, Zhang R, Gudheti M, Yang J, and Kopecek J	Tracking and quantifying polymer therapeutic distribution on a cellular level using 3D dSTORM.	J Control Release	2016	We used a single-molecule localization technique called direct stochastic optical reconstruction microscopy (dSTORM) to quantify both colocalization and spatial distribution on a cellular level for two conceptually different N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer conjugates. Microscopy images were acquired of entire cells with resolutions as high as 25nm revealing the nanoscale distribution of the fluorescently labeled therapeutic components. Drug- free macromolecular therapeutics consisting of two self-assembling nanoconjugates showed slight increase in nanoclusters on the cell surface with time. Additionally, dSTORM provided high resolution images of the nanoscale organization of the self- assembling conjugates at the interface between two cells. A conjugate designed for treating ovarian cancer showed that the model drug (Cy3) and polymer bound to Cy5 were colocalized at an early time point before the model drug was enzymatically cleaved from the polymer. Using spatial descriptive statistics it was found that the drug was randomly distributed after 24h while the polymer bound dye remained in clusters. Four different fluorescent dyes were used and two different therapeutic systems were tested to demonstrate the versatility and possible general applicability of dSTORM for use in studying drug delivery systems.	Cells; Microscopy; Diseases; Eukaryota
De La Fuente S, Fernandez-Sanz C, Vail C, Agra EJ, Holmstrom K, Sun J, Mishra J, Williams D, Finkel T, Murphy E, Joseph SK, Sheu SS, and Csordas G	Strategic Positioning and Biased Activity of the Mitochondrial Calcium Uniporter in Cardiac Muscle.	J Biol Chem	2016	Control of myocardial energetics by Ca(2+) signal propagation to the mitochondrial matrix includes local Ca(2+) delivery from sarcoplasmic reticulum (SR) ryanodine receptors (RyR2) to the inner mitochondrial membrane (IMM) Ca(2+) uniporter (mtCU). mtCU activity in cardiac mitochondria is relatively low, whereas the IMM surface is large, due to extensive cristae folding. Hence, stochastically distributed mtCU may not suffice to support local Ca(2+) transfer. We hypothesized that mtCU concentrated at mitochondria-SR associations would promote the effective Ca(2+) transfer. mtCU distribution was determined by tracking MCU and EMRE, the proteins essential for channel formation. Both proteins were enriched in the IMM-outer mitochondrial membrane (OMM) contact point submitochondrial fraction and, as super-resolution microscopy revealed, located more to the mitochondrial periphery (inner boundary membrane) than inside the cristae, indicating high accessibility to cytosol-derived Ca(2+) inputs. Furthermore, MCU immunofluorescence distribution was biased toward the mitochondria-SR interface (RyR2), and this bias was promoted by Ca(2+) signaling activity in intact cardiomyocytes. The SR fraction of heart homogenate contains mitochondria with extensive SR associations, and these mitochondria are highly enriched in EMRE. Size exclusion chromatography suggested for EMRE- and MCU-containing complexes a wide size range and also revealed MCU-containing complexes devoid of EMRE (thus disabled) in the mitochondrial but not the SR fraction. Functional measurements suggested more effective mtCU-mediated Ca(2+) uptake activity by the mitochondria of the SR than of the mitochondrial fraction. Thus, mtCU "hot spots" can be formed at the cardiac muscle mitochondria-SR associations via localization and assembly bias, serving local Ca(2+) signaling and the excitation-energetics coupling.	Cells; Membrane; Organelles; Signal Transduction
Ben Shoham A, Rot C, Stern T, Krief S, Akiva A, Dadosh T, Sabany H, Lu Y, Kadler KE, and Zelzer E	Deposition of collagen type I onto skeletal endothelium reveals a new role for blood vessels in regulating bone morphology.	Development	2016	Recently, blood vessels have been implicated in the morphogenesis of various organs. The vasculature is also known to be essential for endochondral bone development, yet the underlying mechanism has remained elusive. We show that a unique composition of blood vessels facilitates the role of the endothelium in bone mineralization and morphogenesis. Immunostaining and electron microscopy showed that the endothelium in developing bones lacks basement membrane, which normally isolates the blood vessel from its surroundings. Further analysis revealed the presence of collagen type I on the endothelial wall of these vessels. Because collagen type I is the main component of the osteoid, we hypothesized that the bone vasculature guides the formation of the collagenous template and consequently of the mature bone. Indeed, some of the bone vessels were found to undergo mineralization. Moreover, the vascular pattern at each embryonic stage prefigured the mineral distribution pattern observed one day later. Finally, perturbation of vascular patterning by overexpressing Vegf in osteoblasts resulted in abnormal bone morphology, supporting a role for blood vessels in bone morphogenesis. These data reveal the unique composition of the endothelium in developing bones and indicate that vascular patterning plays a role in determining bone shape by forming a template for deposition of bone matrix.	Cells; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Alan L, Spacek T, and Jezek P	Delaunay algorithm and principal component analysis for 3D visualization of mitochondrial DNA nucleoids by Biplane FPALM/dSTORM.	Eur Biophys J	2016	Data segmentation and object rendering is required for localization super-resolution microscopy, fluorescent photoactivation localization microscopy (FPALM), and direct stochastic optical reconstruction microscopy (dSTORM). We developed and validated methods for segmenting objects based on Delaunay triangulation in 3D space, followed by facet culling. We applied them to visualize mitochondrial nucleoids, which confine DNA in complexes with mitochondrial (mt) transcription factor A (TFAM) and gene expression machinery proteins, such as mt single-stranded-DNA-binding protein (mtSSB). Eos2-conjugated TFAM visualized nucleoids in HepG2 cells, which was compared with dSTORM 3D-immunocytochemistry of TFAM, mtSSB, or DNA. The localized fluorophores of FPALM/dSTORM data were segmented using Delaunay triangulation into polyhedron models and by principal component analysis (PCA) into general PCA ellipsoids. The PCA ellipsoids were normalized to the smoothed volume of polyhedrons or by the net unsmoothed Delaunay volume and remodeled into rotational ellipsoids to obtain models, termed DVRE. The most frequent size of ellipsoid nucleoid model imaged via TFAM was 35 x 45 x 95 nm; or 35 x 45 x 75 nm for mtDNA cores; and 25 x 45 x 100 nm for nucleoids imaged via mtSSB. Nucleoids encompassed different point density and wide size ranges, speculatively due to different activity stemming from different TFAM/mtDNA stoichiometry/density. Considering twofold lower axial vs. lateral resolution, only bulky DVRE models with an aspect ratio >3 and tilted toward the xy-plane were considered as two proximal nucleoids, suspicious occurring after division following mtDNA replication. The existence of proximal nucleoids in mtDNA-dSTORM 3D images of mtDNA "doubling"-supported possible direct observations of mt nucleoid division after mtDNA replication.	Cells; Microscopy; DNA; Genetic Phenomena
Yang J, Zhang R, Christopher Radford D, and Kopecek J	Design and synthesis of FRET-trackable HPMA-based biodegradable conjugates for drug/gene delivery.	J Control Release	2015	?	
Pengo T, Holden SJ, and Manley S	PALMsiever: a tool to turn raw data into results for single-molecule localization microscopy.	Bioinformatics	2015	During the past decade, localization microscopy (LM) has transformed into an accessible, commercially available technique for life sciences. However, data processing can be challenging to the non-specialist and care is still needed to produce meaningful results. PALMsiever has been developed to provide a user-friendly means of visualizing, filtering and analyzing LM data. It includes drift correction, clustering, intelligent line profiles, many rendering algorithms and 3D data visualization. It incorporates the main analysis and data processing modalities used by experts in the field, as well as several new features we developed, and makes them broadly accessible. It can easily be extended via plugins and is provided as free of charge open-source software.	Cells; Cellular Structures; Microscopy; Eukaryota
Olejar T, Pajuelo-Reguera D, Alan L, Dlaskova A, and Jezek P	Coupled aggregation of mitochondrial single-strand DNA-binding protein tagged with Eos fluorescent protein visualizes synchronized activity of mitochondrial nucleoids.	Mol Med Rep	2015	Oligomer aggregation of green-to-red phot-convertible- fluorescent protein Eos (EosFP) is a natural feature of the wild-type variant. The aim of the present study was to follow up mitochondrial nucleoid behavior under natural conditions of living cells transfected with mitochondrial single strand DNA binding protein (mtSSB) conjugated with EosFP. HEPG2 and SH SY5Y cells were subjected to lentiviral transfection and subsequently immunostained with anti DNA, anti transcription factor A, mitochondrial (TFAM) or anti translocase of the inner membrane 23 antibodies. Fluorescent microscopy, conventional confocal microscopy, superresolution biplane fluorescence photo-activation localization microscopy and direct stochastic optical reconstruction microscopy were used for imaging. In the two cell types, apparent couples of equally sized mt-SSB EosFP visualized dots were observed. During the time course of the ongoing transfection procedure, however, a small limited number of large aggregates of mtSSB- EosFP tagged protein started to form in the cells, which exhibited a great localization with the noted coupled- positions. Antibody staining and 3D immunocytochemistry confirmed that nucleoid components such as TFAM and DNA were co-localized with these aggregates. Furthermore, the observed reduction of the mtDNA copy number in mtSSB EosFP transfected cells suggested a possible impairment of nucleoid function. In conclusion, the present study demonstrated that coupled nucleoids are synchronized by mtSSB EosFP overexpression and visualized through their equal binding capacity to mtSSB EosFP tagged protein. This observation suggested parallel replication and transcription activity of nucleoid couples native from a parental one. Preserved coupling in late stages of artificial EosFP mediated aggregation of tagged proteins suggested a rational manner of mitochondrial branching that may be cell-type specifically dependent on hierarchical nucleoid replication.	Cells; Organelles; Protein Binding; Cellular Structures

Bruker Super-Resolution Publications

MacDonald L, Baldini G, and Storrie B	Does super-resolution fluorescence microscopy obsolete previous microscopic approaches to protein co-localization?	Methods Mol Biol	2015	Conventional microscopy techniques, namely, the confocal microscope or deconvolution processes, are resolution limited to approximately 200-250 nm by the diffraction properties of light as developed by Ernst Abbe in 1873. This diffraction limit is appreciably above the size of most multi-protein complexes, which are typically 20-50 nm in diameter. In the mid-2000s, biophysicists moved beyond the diffraction barrier by structuring the illumination pattern and then applying mathematical principles and algorithms to allow a resolution of approximately 100 nm, sufficient to address protein subcellular co-localization questions. This "breaking" of the diffraction barrier, affording resolution beyond 200 nm, is termed super-resolution microscopy. More recent approaches include single-molecule localization (such as photoactivated localization microscopy (PALM)/stochastic optical reconstruction microscopy (STORM)) and point spread function engineering (such as stimulated emission depletion (STED) microscopy). In this review, we explain basic principles behind currently commercialized super-resolution setups and address advantages and considerations in applying these techniques to protein co-localization in biological systems.	Microscopy; Chemicals and Drugs
Kaplan C, and Ewers H	Optimized sample preparation for single-molecule localization-based superresolution microscopy in yeast.	Nat Protoc	2015	Single-molecule localization-based superresolution microscopy methods allow the resolution of cellular structures in the range of tens of nanometers. However, these techniques are of limited use in current yeast labeling protocols, owing to problems with structural preservation. Here we describe an optimized sample preparation protocol that enables single-molecule localization microscopy at high resolution combined with improved structural preservation in <i>Saccharomyces cerevisiae</i> . The protocol uses small binders called nanobodies and an enzymatic labeling strategy to deliver organic dyes to the target protein. These small binders readily penetrate through the yeast cell wall and thus eliminate the requirement for its prior degradation, and they allow structural preservation. In addition, the small size of the binders reduces the distance of the dye to the target protein, and thus it reduces the localization error. The preparation of <i>S. cerevisiae</i> cells for superresolution imaging takes 2-4 h to perform. Researchers should have skills in yeast molecular biology, immunolabeling techniques and access to a microscope equipped for single-molecule imaging.	Cells; Cellular Structures; Fungi; Microscopy
Hujaya SD, Marchioli G, van Apeldoorn AA, Paulusse JM, Karperien M, and Engbersen JF	Cell-transfecting multilayered surfaces from poly(amido amine)s.	J Control Release	2015	?	
Hujaya SD, Engbersen JF, and Paulusse JM	Multilayered thin films from poly(amido amine)s and DNA.	Acta Biomater	2015	Dip-coated multilayered thin films of poly(amido amine)s (PAAs) and DNA have been developed to provide surfaces with cell-transfecting capabilities. Three types of PAAs, differing in side chain functional groups, were synthesized and characterized for their properties in forming multilayered structures with ultrasonicated calf thymus DNA (CTDNA) as model DNA. All three polymers display a multilayer build-up in linear profiles as demonstrated by UV spectroscopy. More highly charged side chains were found to provide the lowest deposition of DNA. Surface profiles of the obtained films were investigated by atomic force microscopy (AFM) and static water contact angle measurements to reveal complete surface coverage after at least four layer pair depositions, where alternating patterns of surface profiles were observed depending on whether the cationic polymer or the anionic DNA layer was on top. The stability of the formed surfaces was investigated in vitro under physiological and reductive conditions. Owing to the presence of disulfide bonds in the PAA main chain, the films were readily degraded in the presence of 1mM of DTT in vitro. Under non-reductive physiological conditions, two of the thicker films underwent thermodynamic rearrangement, which resulted in release of approximately half of the incorporated material within 1h, which was caused by the physiological salt concentration. Further, this unpacking phenomenon proved useful in transfecting COS-7 cells seeded on top of these multilayers containing functional plasmid DNA encoding for green fluorescence protein (GFP). Two out of the three different multilayers facilitated good COS-7 cell attachment, proliferation, and transfection in vitro within 2d ays of culture. Fluorescence staining further revealed the presence of DNA-containing released film material among cultured cells. The present work demonstrates the possibility of coating surfaces with thin films that are conveniently adjustable in thickness and amount of active agent to provide cell-transfecting functionality. In this manner transfection can be achieved by simply culturing cells on a multilayer-coated surface in their optimal culturecondition (in the presence of serum) and without the need of removing the transfection agent to avoid cytotoxicity.	Cells; DNA; Genetic Phenomena; Nucleic Acids, Nucleotides, and Nucleosides

Bruker Super-Resolution Publications

Brown MS, Grubb J, Zhang A, Rust MJ, and Bishop DK	Small Rad51 and Dmc1 Complexes Often Co-occupy Both Ends of a Meiotic DNA Double Strand Break.	PLoS Genet	2015	The Eukaryotic RecA-like proteins Rad51 and Dmc1 cooperate during meiosis to promote recombination between homologous chromosomes by repairing programmed DNA double strand breaks (DSBs). Previous studies showed that Rad51 and Dmc1 form partially overlapping co-foci. Here we show these Rad51-Dmc1 co-foci are often arranged in pairs separated by distances of up to 400 nm. Paired co-foci remain prevalent when DSBs are dramatically reduced or when strand exchange or synapsis is blocked. Super-resolution dSTORM microscopy reveals that individual foci observed by conventional light microscopy are often composed of two or more substructures. The data support a model in which the two tracts of ssDNA formed by a single DSB separate from one another by distances of up to 400 nm, with both tracts often bound by one or more short (about 100 nt) Rad51 filaments and also by one or more short Dmc1 filaments.	Fungi; DNA; Genetic Phenomena; Genetic Structures
Akiyama H, Ramirez NG, Gudheti MV, and Gummuluru S	CD169-mediated trafficking of HIV to plasma membrane invaginations in dendritic cells attenuates efficacy of anti-gp120 broadly neutralizing antibodies.	PLoS Pathog	2015	Myeloid dendritic cells (DCs) can capture HIV-1 via the receptor CD169/Siglec-1 that binds to the ganglioside, GM3, in the virus particle membrane. In turn, HIV-1 particles captured by CD169, an I-type lectin, whose expression on DCs is enhanced upon maturation with LPS, are protected from degradation in CD169+ virus-containing compartments (VCCs) and disseminated to CD4(+) T cells, a mechanism of DC-mediated HIV-1 trans-infection. In this study, we describe the mechanism of VCC formation and its role in immune evasion mechanisms of HIV-1. We find HIV-1-induced formation of VCCs is restricted to myeloid cells, and that the cytoplasmic tail of CD169 is dispensable for HIV-1 trafficking and retention within VCCs and subsequent trans-infection to CD4(+) T cells. Interestingly, introduction of a di-aromatic endocytic motif in the cytoplasmic tail of CD169 that results in endocytosis of HIV-1 particles, suppressed CD169-mediated HIV-1 trans-infection. Furthermore, super-resolution microscopy revealed close association of CD169 and HIV-1 particles in surface-accessible but deep plasma membrane invaginations. Intriguingly, HIV-1 particles in deep VCCs were inefficiently accessed by anti-gp120 broadly neutralizing antibodies, VRC01 and NIH45-46 G54W, and thus were less susceptible to neutralization. Our study suggests that HIV-1 capture by CD169 can provide virus evasion from both innate (phagocytosis) and adaptive immune responses.	Cells; Membrane; Viruses; Cellular Structures
?	Correction: CD169-Mediated Trafficking of HIV to Plasma Membrane Invaginations in Dendritic Cells Attenuates Efficacy of Anti-gp120 Broadly Neutralizing Antibodies.	PLoS Pathog	2015	?	
Zhang R, Yang J, Sima M, Zhou Y, and Kopecek J	Sequential combination therapy of ovarian cancer with degradable N-(2-hydroxypropyl)methacrylamide copolymer paclitaxel and gemcitabine conjugates.	Proc Natl Acad Sci U S A	2014	For rapid and effective clinical translation, polymer-based anticancer therapeutics need long circulating conjugates that produce a sustained concentration gradient between the vasculature and solid tumor. To this end, we designed second-generation backbone-degradable diblock N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer carriers and evaluated sequential combination therapy of HPMA copolymer-paclitaxel and HPMA copolymer-gemcitabine conjugates against A2780 human ovarian carcinoma xenografts. First, extensive in vitro assessment of administration sequence impact on cell cycle, viability, apoptosis, migration, and invasion revealed that treatment with paclitaxel conjugate followed by gemcitabine conjugate was the most effective scheduling strategy. Second, in an in vivo comparison with first-generation (nondegradable, molecular weight below the renal threshold) conjugates and free drugs, the second-generation degradable high-molecular weight conjugates showed distinct advantages, such as favorable pharmacokinetics (three- to five-times half-life compared with the first generation), dramatically enhanced inhibition of tumor growth (complete tumor regression) by paclitaxel and gemcitabine conjugate combination, and absence of adverse effects. In addition, multimodality imaging studies of dual-labeled model conjugates confirmed the efficacy of second-generation conjugates by visualizing more than five-times enhanced tumor accumulation, rapid conjugate internalization, and effective intracellular release of payload. Taken together, the results indicate that the second-generation degradable HPMA copolymer carrier can provide an ideal platform for the delivery of a range of antitumor compounds, which makes it one of the most attractive candidates for potential clinical application.	Diseases; Nucleic Acids, Nucleotides, and Nucleosides; Eukaryota; Chemicals and Drugs
Watanabe S, Lehmann M, Hujber E, Fetter RD, Richards J, Sohl-Kielczynski B, Felies A, Rosenmund C, Schmoranzler J, and Jorgensen EM	Nanometer-resolution fluorescence electron microscopy (nano-EM) in cultured cells.	Methods Mol Biol	2014	Nano-resolution fluorescence electron microscopy (nano-fEM) pinpoints the location of individual proteins in electron micrographs. Plastic sections are first imaged using a super-resolution fluorescence microscope and then imaged on an electron microscope. The two images are superimposed to correlate the position of labeled proteins relative to subcellular structures. Here, we describe the method in detail and present five technical advancements: the use of uranyl acetate during the freeze-substitution to enhance the contrast of tissues and reduce the loss of fluorescence, the use of ground-state depletion instead of photoactivation for temporal control of fluorescence, the use of organic fluorophores instead of fluorescent proteins to obtain brighter fluorescence signals, the use of tissue culture cells to broaden the utility of the method, and the use of a transmission electron microscope to achieve sharper images of ultrastructure.	Cells; Microscopy; Eukaryota

Bruker Super-Resolution Publications

Long BR, Robinson DC, and Zhong H	Subdiffractive microscopy: techniques, applications, and challenges.	Wiley Interdiscip Rev Syst Biol Med	2014	Cellular processes rely on the precise orchestration of signaling and effector molecules in space and time, yet it remains challenging to gain a comprehensive picture of the molecular organization underlying most basic biological functions. This organization often takes place at length scales below the resolving power of conventional microscopy. In recent years, several 'superresolution' fluorescence microscopic techniques have emerged that can surpass the diffraction limit of conventional microscopy by a factor of 2-20. These methods have been used to reveal previously unknown organization of macromolecular complexes and cytoskeletal structures. The resulting high-resolution view of molecular organization and dynamics is already changing our understanding of cellular processes at the systems level. However, current subdiffractive microscopic techniques are not without limitations; challenges remain to be overcome before these techniques achieve their full potential. Here, we introduce three primary types of subdiffractive microscopic techniques, consider their current limitations and challenges, and discuss recent biological applications.	Bacteria; Microscopy; Eukaryota; Chemicals and Drugs
Lichter JG, Carruth E, Mitchell C, Barth AS, Aiba T, Kass DA, Tomaselli GF, Bridge JH, and Sachse FB	Remodeling of the sarcomeric cytoskeleton in cardiac ventricular myocytes during heart failure and after cardiac resynchronization therapy.	J Mol Cell Cardiol	2014	Sarcomeres are the basic contractile units of cardiac myocytes. Recent studies demonstrated remodeling of sarcomeric proteins in several diseases, including genetic defects and heart failure. Here we investigated remodeling of sarcomeric alpha actinin in two models of heart failure, synchronous (SHF) and dyssynchronous heart failure (DHF), as well as a model of cardiac resynchronization therapy (CRT). We applied three-dimensional confocal microscopy and quantitative methods of image analysis to study isolated cells from our animal models. 3D Fourier analysis revealed a decrease of the spatial regularity of the alpha-actinin distribution in both SHF and DHF versus control cells. The spatial regularity of alpha-actinin in DHF cells was reduced when compared with SHF cells. The spatial regularity of alpha-actinin was partially restored after CRT. We found longitudinal depositions of alpha-actinin in SHF, DHF and CRT cells. These depositions spanned adjacent Z-disks and exhibited a lower density of alpha-actinin than in the Z-disk. Differences in the occurrence of depositions between the SHF, CRT and DHF models versus control were significant. Also, CRT cells exhibited a higher occurrence of depositions versus SHF, but not DHF cells. Other sarcomeric proteins did not accumulate in the depositions to the same extent as alpha-actinin. We did not find differences in the expression of alpha-actinin protein and its encoding gene in our animal models. In summary, our studies indicate that HF is associated with two different types of remodeling of alpha-actinin and only one of those was reversed after CRT. We suggest that these results can guide us to an understanding of remodeling of structures and function associated with sarcomeres.	Cells; Organelles; Cellular Structures; Diseases
Kiss G, Holl JM, Williams GM, Alonas E, Vanover D, Lifland AW, Gudheti M, Guerrero-Ferreira RC, Nair V, Yi H, Graham BS, Santangelo PJ, and Wright ER	Structural analysis of respiratory syncytial virus reveals the position of M2-1 between the matrix protein and the ribonucleoprotein complex.	J Virol	2014	Respiratory syncytial virus (RSV), a member of the Paramyxoviridae family of nonsegmented, negative-sense, single-stranded RNA genome viruses, is a leading cause of lower respiratory tract infections in infants, young children, and the elderly or immunocompromised. There are many open questions regarding the processes that regulate human RSV (hRSV) assembly and budding. Here, using cryo-electron tomography, we identified virus particles that were spherical, filamentous, and asymmetric in structure, all within the same virus preparation. The three particle morphologies maintained a similar organization of the surface glycoproteins, matrix protein (M), M2-1, and the ribonucleoprotein (RNP). RNP filaments were traced in three dimensions (3D), and their total length was calculated. The measurements revealed the inclusion of multiple full-length genome copies per particle. RNP was associated with the membrane whenever the M layer was present. The amount of M coverage ranged from 24% to 86% in the different morphologies. Using fluorescence light microscopy (FLM), direct stochastic optical reconstruction microscopy (dSTORM), and a proximity ligation assay (PLA), we provide evidence illustrating that M2-1 is located between RNP and M in isolated viral particles. In addition, regular spacing of the M2-1 densities was resolved when hRSV viruses were imaged using Zernike phase contrast (ZPC) cryo-electron tomography. Our studies provide a more complete characterization of the hRSV virion structure and substantiation that M and M2-1 regulate virus organization. IMPORTANCE: hRSV is a leading cause of lower respiratory tract infections in infants and young children as well as elderly or immunocompromised individuals. We used cryo-electron tomography and Zernike phase contrast cryo-electron tomography to visualize populations of purified hRSV in 3D. We observed the three distinct morphologies, spherical, filamentous, and asymmetric, which maintained comparable organizational profiles. Depending on the virus morphology examined, the amount of M ranged from 24% to 86%. We complemented the cryo-imaging studies with fluorescence microscopy, dSTORM, and a proximity ligation assay to provide additional evidence that M2-1 is incorporated into viral particles and is positioned between M and RNP. The results highlight the impact of M and M2-1 on the regulation of hRSV organization.	Viruses; Microscopy; Diseases; Nucleic Acids, Nucleotides, and Nucleosides

Bruker Super-Resolution Publications

Keller D, Orpinell M, Olivier N, Wachsmuth M, Mahen R, Wyss R, Hachet V, Ellenberg J, Manley S, and Gonczy P	Mechanisms of HsSAS-6 assembly promoting centriole formation in human cells.	J Cell Biol	2014	SAS-6 proteins are thought to impart the ninefold symmetry of centrioles, but the mechanisms by which their assembly occurs within cells remain elusive. In this paper, we provide evidence that the N-terminal, coiled-coil, and C-terminal domains of HsSAS-6 are each required for procentriole formation in human cells. Moreover, the coiled coil is necessary and sufficient to mediate HsSAS-6 centrosomal targeting. High-resolution imaging reveals that GFP-tagged HsSAS-6 variants localize in a torus around the base of the parental centriole before S phase, perhaps indicative of an initial loading platform. Moreover, fluorescence recovery after photobleaching analysis demonstrates that HsSAS-6 is immobilized progressively at centrosomes during cell cycle progression. Using fluorescence correlation spectroscopy and three-dimensional stochastic optical reconstruction microscopy, we uncover that HsSAS-6 is present in the cytoplasm primarily as a homodimer and that its oligomerization into a ninefold symmetrical ring occurs at centrioles. Together, our findings lead us to propose a mechanism whereby HsSAS-6 homodimers are targeted to centrosomes where the local environment and high concentration of HsSAS-6 promote oligomerization, thus initiating procentriole formation.	Cells; Cellular Structures; Eukaryota; Chemicals and Drugs
Alonas E, Lifland AW, Gudheti M, Vanover D, Jung J, Zurla C, Kirschman J, Fiore VF, Douglas A, Barker TH, Yi H, Wright ER, Crowe JE Jr, and Santangelo PJ	Combining single RNA sensitive probes with subdiffraction-limited and live-cell imaging enables the characterization of virus dynamics in cells.	ACS Nano	2014	The creation of fluorescently labeled viruses is currently limited by the length of imaging observation time (e.g., labeling an envelope protein) and the rescue of viral infectivity (e.g., encoding a GFP protein). Using single molecule sensitive RNA hybridization probes delivered to the cytoplasm of infected cells, we were able to isolate individual, infectious, and fluorescently labeled human respiratory syncytial virus virions. This was achieved without affecting viral mRNA expression, viral protein expression, or infectivity. Measurements included the characterization of viral proteins and genomic RNA in a single virion using dSTORM, the development of a GFP fusion assay, and the development of a pulse-chase assay for viral RNA production that allowed for the detection of both initial viral RNA and nascent RNA production at designated times postinfection. Live-cell measurements included imaging and characterization of filamentous virion fusion and the quantification of virus replication within the same cell over an eight-hour period. Using probe-labeled viruses, individual viral particles can be characterized at subdiffraction-limited resolution, and viral infections can be quantified in single cells over an entire cycle of replication. The implication of this development is that MTRIP labeling of viral RNA during virus assembly has the potential to become a general methodology for the labeling and study of many important RNA viruses.	Cells; Viruses; Nucleic Acids, Nucleotides, and Nucleosides; Eukaryota
Wilfling F, Wang H, Haas JT, Kraemer N, Gould TJ, Uchida A, Cheng JX, Graham M, Christiano R, Frohlich F, Liu X, Buhman KK, Coleman RA, Bewersdorf J, Farese RV Jr, and Walther TC	Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocating from the ER to lipid droplets.	Dev Cell	2013	Lipid droplets (LDs) store metabolic energy and membrane lipid precursors. With excess metabolic energy, cells synthesize triacylglycerol (TG) and form LDs that grow dramatically. It is unclear how TG synthesis relates to LD formation and growth. Here, we identify two LD subpopulations: smaller LDs of relatively constant size, and LDs that grow larger. The latter population contains isoenzymes for each step of TG synthesis. Glycerol-3-phosphate acyltransferase 4 (GPAT4), which catalyzes the first and rate-limiting step, relocates from the endoplasmic reticulum (ER) to a subset of forming LDs, where it becomes stably associated. ER-to-LD targeting of GPAT4 and other LD-localized TG synthesis isozymes is required for LD growth. Key features of GPAT4 ER-to-LD targeting and function in LD growth are conserved between Drosophila and mammalian cells. Our results explain how TG synthesis is coupled with LD growth and identify two distinct LD subpopulations based on their capacity for localized TG synthesis.	Cells; Organelles; Cellular Structures; Lipids
Ritter B, Murphy S, Dokainish H, Girard M, Gudheti MV, Kozlov G, Halin M, Philie J, Jorgensen EM, Gehring K, and McPherson PS	NECAP 1 regulates AP-2 interactions to control vesicle size, number, and cargo during clathrin-mediated endocytosis.	PLOS Biol	2013	AP-2 is the core-organizing element in clathrin-mediated endocytosis. During the formation of clathrin-coated vesicles, clathrin and endocytic accessory proteins interact with AP-2 in a temporally and spatially controlled manner, yet it remains elusive as to how these interactions are regulated. Here, we demonstrate that the endocytic protein NECAP 1, which binds to the alpha-ear of AP-2 through a C-terminal WxxF motif, uses an N-terminal PH-like domain to compete with clathrin for access to the AP-2 beta2-linker, revealing a means to allow AP-2-mediated coordination of accessory protein recruitment and clathrin polymerization at sites of vesicle formation. Knockdown and functional rescue studies demonstrate that through these interactions, NECAP 1 and AP-2 cooperate to increase the probability of clathrin-coated vesicle formation and to control the number, size, and cargo content of the vesicles. Together, our data demonstrate that NECAP 1 modulates the AP-2 interactome and reveal a new layer of organizational control within the endocytic machinery.	Cells; Synapses; Organelles; Protein Binding

Bruker Super-Resolution Publications

Olivier N, Keller D, Gonczy P, and Manley S	Resolution doubling in 3D-STORM imaging through improved buffers.	PLoS One	2013	Super-resolution imaging methods have revolutionized fluorescence microscopy by revealing the nanoscale organization of labeled proteins. In particular, single-molecule methods such as Stochastic Optical Reconstruction Microscopy (STORM) provide resolutions down to a few tens of nanometers by exploiting the cycling of dyes between fluorescent and non-fluorescent states to obtain a sparse population of emitters and precisely localizing them individually. This cycling of dyes is commonly induced by adding different chemicals, which are combined to create a STORM buffer. Despite their importance, the composition of these buffers has scarcely evolved since they were first introduced, fundamentally limiting what can be resolved with STORM. By identifying a new chemical suitable for STORM and optimizing the buffer composition for Alexa-647, we significantly increased the number of photons emitted per cycle by each dye, providing a simple means to enhance the resolution of STORM independently of the optical setup used. Using this buffer to perform 3D-STORM on biological samples, we obtained images with better than 10 nanometer lateral and 30 nanometer axial resolution.	Cells; Microscopy; Eukaryota; Chemicals and Drugs
Metcalf DJ, Edwards R, Kumarswami N, and Knight AE	Test samples for optimizing STORM super-resolution microscopy.	J Vis Exp	2013	STORM is a recently developed super-resolution microscopy technique with up to 10 times better resolution than standard fluorescence microscopy techniques. However, as the image is acquired in a very different way than normal, by building up an image molecule-by-molecule, there are some significant challenges for users in trying to optimize their image acquisition. In order to aid this process and gain more insight into how STORM works we present the preparation of 3 test samples and the methodology of acquiring and processing STORM super-resolution images with typical resolutions of between 30-50 nm. By combining the test samples with the use of the freely available rainSTORM processing software it is possible to obtain a great deal of information about image quality and resolution. Using these metrics it is then possible to optimize the imaging procedure from the optics, to sample preparation, dye choice, buffer conditions, and image acquisition settings. We also show examples of some common problems that result in poor image quality, such as lateral drift, where the sample moves during image acquisition and density related problems resulting in the 'mislocalization' phenomenon.	Cells; Microscopy; Eukaryota; Chemicals and Drugs
Liu S, Kromann EB, Krueger WD, Bewersdorf J, and Lidke KA	Three dimensional single molecule localization using a phase retrieved pupil function.	Opt Express	2013	Localization-based superresolution imaging is dependent on finding the positions of individual fluorophores in a sample by fitting the observed single-molecule intensity pattern to the microscope point spread function (PSF). For three-dimensional imaging, system-specific aberrations of the optical system can lead to inaccurate localizations when the PSF model does not account for these aberrations. Here we describe the use of phase-retrieved pupil functions to generate a more accurate PSF and therefore more accurate 3D localizations. The complex-valued pupil function contains information about the system-specific aberrations and can thus be used to generate the PSF for arbitrary defocus. Further, it can be modified to include depth dependent aberrations. We describe the phase retrieval process, the method for including depth dependent aberrations, and a fast fitting algorithm using graphics processing units. The superior localization accuracy of the pupil function generated PSF is demonstrated with dual focal plane 3D superresolution imaging of biological structures.	Chemicals and Drugs
Kim D, Curthoys NM, Parent MT, and Hess ST	Bleed-through correction for rendering and correlation analysis in multi-colour localization microscopy.	J Opt	2013	Multi-colour localization microscopy has enabled sub-diffraction studies of colocalization between multiple biological species and quantification of their correlation at length scales previously inaccessible with conventional fluorescence microscopy. However, bleed-through, or misidentification of probe species, creates false colocalization and artificially increases certain types of correlation between two imaged species, affecting the reliability of information provided by colocalization and quantified correlation. Despite the potential risk of these artefacts of bleed-through, neither the effect of bleed-through on correlation nor methods of its correction in correlation analyses has been systematically studied at typical rates of bleed-through reported to affect multi-colour imaging. Here, we present a reliable method of bleed-through correction applicable to image rendering and correlation analysis of multi-colour localization microscopy. Application of our bleed-through correction shows our method accurately corrects the artificial increase in both types of correlations studied (Pearson coefficient and pair correlation), at all rates of bleed-through tested, in all types of correlations examined. In particular, anti-correlation could not be quantified without our bleed-through correction, even at rates of bleed-through as low as 2%. Demonstrated with dichroic-based multi-colour FPALM here, our presented method of bleed-through correction can be applied to all types of localization microscopy (PALM, STORM, dSTORM, GSDIM, etc.), including both simultaneous and sequential multi-colour modalities, provided the rate of bleed-through can be reliably determined.	

Bruker Super-Resolution Publications

Jung J, Liffand AW, Alonas EJ, Zurla C, and Santangelo PJ	Characterization of mRNA-cytoskeleton interactions in situ using FMTRIP and proximity ligation.	PloS One	2013	Many studies have demonstrated an association between the cytoskeleton and mRNA, as well as the asymmetric distribution of mRNA granules within the cell in response to various signaling events. It is likely that the extensive cytoskeletal network directs mRNA transport and localization, with different cytoskeletal elements having their own specific roles. In order to understand the spatiotemporal changes in the interactions between the mRNA and the cytoskeleton as a response to a stimulus, a technique that can visualize and quantify these changes across a population of cells while capturing cell-to-cell variations is required. Here, we demonstrate a method for imaging and quantifying mRNA-cytoskeleton interactions on a per cell basis with single-interaction sensitivity. Using a proximity ligation assay with flag-tagged multiply-labeled tetraivalent RNA imaging probes (FMTRIP), we quantified interactions between mRNAs and beta-tubulin, vimentin, or filamentous actin (F-actin) for two different mRNAs, poly(A) + and beta-actin mRNA, in two different cell types, A549 cells and human dermal fibroblasts (HDF). We found that the mRNAs interacted predominantly with F-actin (>50% in HDF, >20% in A549 cells), compared to beta-tubulin (<5%) and vimentin (11-13%). This likely reflects differences in mRNA management by the two cell types. We then quantified changes in these interactions in response to two perturbations, F-actin depolymerization and arsenite-induced oxidative stress, both of which alter either the cytoskeleton itself and mRNA localization. Both perturbations led to a decrease in poly(A) + mRNA interactions with F-actin and an increase in the interactions with microtubules, in a time dependent manner.	Cells; Cellular Structures; Microscopy; Nucleic Acids, Nucleotides, and Nucleosides
Huang F, Hartwich TM, Rivera-Molina FE, Lin Y, Duim WC, Long JJ, Uchil PD, Myers JR, Baird MA, Mothes W, Davidson MW, Toomre D, and Bewersdorf J	Video-rate nanoscopy using sCMOS camera-specific single-molecule localization algorithms.	Nat Methods	2013	Newly developed scientific complementary metal-oxide semiconductor (sCMOS) cameras have the potential to dramatically accelerate data acquisition, enlarge the field of view and increase the effective quantum efficiency in single-molecule switching nanoscopy. However, sCMOS-intrinsic pixel-dependent readout noise substantially lowers the localization precision and introduces localization artifacts. We present algorithms that overcome these limitations and that provide unbiased, precise localization of single molecules at the theoretical limit. Using these in combination with a multi-emitter fitting algorithm, we demonstrate single-molecule localization super-resolution imaging at rates of up to 32 reconstructed images per second in fixed and living cells.	Microscopy
Hodges J, Tang X, Landesman MB, Ruedas JB, Ghimire A, Gudheti MV, Perrault J, Jorgensen EM, Gerton JM, and Saffarian S	Asymmetric packaging of polymerases within vesicular stomatitis virus.	Biochem Biophys Res Commun	2013	Vesicular stomatitis virus (VSV) is a prototypic negative sense single-stranded RNA virus. The bullet-shape appearance of the virion results from tightly wound helical turns of the nucleoprotein encapsidated RNA template (N-RNA) around a central cavity. Transcription and replication require polymerase complexes, which include a catalytic subunit L and a template-binding subunit P. L and P are inferred to be in the cavity, however lacking direct observation, their exact position has remained unclear. Using super-resolution fluorescence imaging and atomic force microscopy (AFM) on single VSV virions, we show that L and P are packaged asymmetrically towards the blunt end of the virus. The number of L and P proteins varies between individual virions and they occupy 57 +/- 12 nm of the 150 nm central cavity of the virus. Our finding positions the polymerases at the opposite end of the genome with respect to the only transcriptional promoter.	Viruses; Microscopy; Nucleic Acids, Nucleotides, and Nucleosides; Chemicals and Drugs
Hartwich TM, Subach FV, Cooley L, Verkhusha VV, and Bewersdorf J	Determination of two-photon photoactivation rates of fluorescent proteins.	Phys Chem Chem Phys	2013	The application of two-photon activation of photoactivatable fluorescent proteins is limited by a lack of information about two-photon activation rates. Here we present rates for the commonly used photoactivatable proteins PAmCherry, PAmKate and PA-GFP at different wavelengths using a novel method that allows us to determine the two-photon activation rates directly, independent of any reference data, with microscopic sample volumes. We show that PAmCherry features the highest rates of the tested proteins at 700 nm activation wavelength followed by PAmKate. Towards longer wavelengths, two photon activation rates decrease for all three proteins. For PAmCherry, our data contradicts an activation model relying solely on two-photon activation and suggests additional activation pathways requiring at least two absorption steps. Our method is readily expandable to other photoactivatable fluorescent molecules. The presented results allow optimization of experimental conditions in spectroscopic and imaging techniques such as super-resolution fluorescence microscopy.	Cells; Bacteria; Chemicals and Drugs
Gudheti MV, Curthoys NM, Gould TJ, Kim D, Gunewardene MS, Gabor KA, Gosse JA, Kim CH, Zimmerberg J, and Hess ST	Actin mediates the nanoscale membrane organization of the clustered membrane protein influenza hemagglutinin.	Biophys J	2013	The influenza viral membrane protein hemagglutinin (HA) is required at high concentrations on virion and host-cell membranes for infectivity. Because the role of actin in membrane organization is not completely understood, we quantified the relationship between HA and host-cell actin at the nanoscale. Results obtained using superresolution fluorescence photoactivation localization microscopy (FPALM) in nonpolarized cells show that HA clusters colocalize with actin-rich membrane regions (ARMRs). Individual molecular trajectories in live cells indicate restricted HA mobility in ARMRs, and actin disruption caused specific changes to HA clustering. Surprisingly, the actin-binding protein cofilin was excluded from some regions within several hundred nanometers of HA clusters, suggesting that HA clusters or adjacent proteins within the same clusters influence local actin structure. Thus, with the use of imaging, we demonstrate a dynamic relationship between glycoprotein membrane organization and the actin cytoskeleton at the nanoscale.	Cells; Membrane; Viruses; Cellular Structures

Bruker Super-Resolution Publications

Gould TJ, Kromann EB, Burke D, Booth MJ, and Bewersdorf J	Auto-aligning stimulated emission depletion microscope using adaptive optics.	Opt Lett	2013	Stimulated emission depletion (STED) microscopy provides diffraction-unlimited resolution in fluorescence microscopy. Imaging at the nanoscale, however, requires precise alignment of the depletion and excitation laser foci of the STED microscope. We demonstrate here that adaptive optics can be implemented to automatically align STED and confocal images with a precision of 4.3 +/- 2.3 nm.	Microscopy
Curthoys NM, Mlodzianoski MJ, Kim D, and Hess ST	Simultaneous multicolor imaging of biological structures with fluorescence photoactivation localization microscopy.	J Vis Exp	2013	Localization-based super resolution microscopy can be applied to obtain a spatial map (image) of the distribution of individual fluorescently labeled single molecules within a sample with a spatial resolution of tens of nanometers. Using either photoactivatable (PAFP) or photoswitchable (PSFP) fluorescent proteins fused to proteins of interest, or organic dyes conjugated to antibodies or other molecules of interest, fluorescence photoactivation localization microscopy (FPALM) can simultaneously image multiple species of molecules within single cells. By using the following approach, populations of large numbers (thousands to hundreds of thousands) of individual molecules are imaged in single cells and localized with a precision of ~10-30 nm. Data obtained can be applied to understanding the nanoscale spatial distributions of multiple protein types within a cell. One primary advantage of this technique is the dramatic increase in spatial resolution: while diffraction limits resolution to ~200-250 nm in conventional light microscopy, FPALM can image length scales more than an order of magnitude smaller. As many biological hypotheses concern the spatial relationships among different biomolecules, the improved resolution of FPALM can provide insight into questions of cellular organization which have previously been inaccessible to conventional fluorescence microscopy. In addition to detailing the methods for sample preparation and data acquisition, we here describe the optical setup for FPALM. One additional consideration for researchers wishing to do super-resolution microscopy is cost: in-house setups are significantly cheaper than most commercially available imaging machines. Limitations of this technique include the need for optimizing the labeling of molecules of interest within cell samples, and the need for post-processing software to visualize results. We here describe the use of PAFP and PSFP expression to image two protein species in fixed cells. Extension of the technique to living cells is also described.	Cells; Microscopy; Eukaryota; Chemicals and Drugs
Allen JR, Ross ST, and Davidson MW	Sample preparation for single molecule localization microscopy.	Phys Chem Chem Phys	2013	Single molecule localization-based optical nanoscopy was introduced in 2006, surpassing traditional diffraction-limited resolutions by an order of magnitude. Seven years later, this superresolution technique is continuing to follow a trend of increasing popularity and pervasiveness, with the proof-of-concept work long finished and commercial implementations now available. However one important aspect that tends to become lost in translation is the importance of proper sample preparation, with very few resources addressing the considerations that must be made when preparing samples for imaging with single molecule level sensitivity. Presented here is an in-depth analysis of all aspects of sample preparation for single molecule superresolution, including both live and fixed cell preparation, choice of fluorophore, fixation and staining techniques, and imaging buffer considerations.	Microscopy; Eukaryota; Chemicals and Drugs
Watanabe S, Richards J, Holloper G, Hobson RJ, Davis WM, and Jorgensen EM	Nano-fEM: protein localization using photo-activated localization microscopy and electron microscopy.	J Vis Exp	2012	Mapping the distribution of proteins is essential for understanding the function of proteins in a cell. Fluorescence microscopy is extensively used for protein localization, but subcellular context is often absent in fluorescence images. Immuno-electron microscopy, on the other hand, can localize proteins, but the technique is limited by a lack of compatible antibodies, poor preservation of morphology and because most antigens are not exposed to the specimen surface. Correlative approaches can acquire the fluorescence image from a whole cell first, either from immuno-fluorescence or genetically tagged proteins. The sample is then fixed and embedded for electron microscopy, and the images are correlated (1-3). However, the low-resolution fluorescence image and the lack of fiducial markers preclude the precise localization of proteins. Alternatively, fluorescence imaging can be done after preserving the specimen in plastic. In this approach, the block is sectioned, and fluorescence images and electron micrographs of the same section are correlated (4-7). However, the diffraction limit of light in the correlated image obscures the locations of individual molecules, and the fluorescence often extends beyond the boundary of the cell. Nano-resolution fluorescence electron microscopy (nano-fEM) is designed to localize proteins at nano-scale by imaging the same sections using photo-activated localization microscopy (PALM) and electron microscopy. PALM overcomes the diffraction limit by imaging individual fluorescent proteins and subsequently mapping the centroid of each fluorescent spot (8-10). We outline the nano-fEM technique in five steps. First, the sample is fixed and embedded using conditions that preserve the fluorescence of tagged proteins. Second, the resin blocks are sectioned into ultrathin segments (70-80 nm) that are mounted on a cover glass. Third, fluorescence is imaged in these sections using the Zeiss PALM microscope. Fourth, electron dense structures are imaged in these same sections using a scanning electron microscope. Fifth, the fluorescence and electron micrographs are aligned using gold particles as fiducial markers. In summary, the subcellular localization of fluorescently tagged proteins can be determined at nanometer resolution in approximately one week.	Microscopy; Eukaryota; Chemicals and Drugs

Bruker Super-Resolution Publications

Lv C, Gould TJ, Bewersdorf J, and Zenisek D	High-resolution optical imaging of zebrafish larval ribbon synapse protein RIBEYE, RIM2, and CaV 1.4 by stimulation emission depletion microscopy.	Microsc Microanal	2012	The synaptic ribbon is a unique presynaptic structure with an intricate morphology in photoreceptors. Because of the resolution limit in conventional fluorescence microscopy, investigating ribbon protein locations has been challenging, especially in the early development stages of model animals. Here, we used stimulated emission depletion microscopy, a super-resolution imaging technique, to look at retina sections in 4 days post-fertilization (dpf) zebrafish. We observed that in photoreceptor cells, RIBEYE and RIM2 are expressed along the synaptic ribbon, with RIM2 consistently located inside of the horseshoe-shaped synaptic ribbon structure with RIBEYE located on the outside. The L-type calcium channel subunit, CACNA1F, exhibited small spot-like staining beneath the RIM2 and RIBEYE structures. Using morpholino antisense oligonucleotides to knock down RIBEYE expression, we observed fewer and shorter ribbons in the photoreceptor outer plexiform layers of 4 dpf fish retina as well as a reduction in RIM2 expression. The clustering of CACNA1F in these blind fish was no longer observed, but instead showed a diffuse expression in the photoreceptor terminal.	Cells; Synapses; Membrane; Nervous System
Gould TJ, and Bewersdorf J	Nanoscopy at low light intensities shows its potential.	Elife	2012	A new form of green fluorescent protein allows super-resolution imaging to be performed faster on living cells with low radiation doses.	Cells; Microscopy; Eukaryota; Chemicals and Drugs
Gould TJ, Hess ST, and Bewersdorf J	Optical nanoscopy: from acquisition to analysis.	Annu Rev Biomed Eng	2012	Recent advances in far-field microscopy have demonstrated that fluorescence imaging is possible at resolutions well below the long-standing diffraction limit. By exploiting photophysical properties of fluorescent probe molecules, this new class of methods yields a resolving power that is fundamentally diffraction unlimited. Although these methods are becoming more widely used in biological imaging, they must be complemented by suitable data analysis approaches if their potential is to be fully realized. Here we review the basic principles of diffraction-unlimited microscopy and how these principles influence the selection of available algorithms for data analysis. Furthermore, we provide an overview of existing analysis strategies and discuss their application.	Microscopy; Eukaryota; Chemicals and Drugs
Gould TJ, Burke D, Bewersdorf J, and Booth MJ	Adaptive optics enables 3D STED microscopy in aberrating specimens.	Opt Express	2012	Stimulated emission depletion (STED) microscopy allows fluorescence far-field imaging with diffraction-unlimited resolution. Unfortunately, extending this technique to three-dimensional (3D) imaging of thick specimens has been inhibited by sample-induced aberrations. Here we present the first implementation of adaptive optics in STED microscopy to allow 3D super-resolution imaging in strongly aberrated imaging conditions, such as those introduced by thick biological tissue.	Microscopy; Eukaryota
Aaron JS, Carson BD, and Timlin JA	Characterization of differential Toll-like receptor responses below the optical diffraction limit.	Small	2012	Many membrane receptors are recruited to specific cell surface domains to form nanoscale clusters upon ligand activation. This step appears to be necessary to initiate cell signaling, including pathways in innate immune system activation. However, virulent pathogens such as <i>Yersinia pestis</i> (the causative agent of plague) are known to evade innate immune detection, in contrast to similar microbes (such as <i>Escherichia coli</i>) that elicit a robust response. This disparity has been partly attributed to the structure of lipopolysaccharides (LPS) on the bacterial cell wall, which are recognized by the innate immune receptor TLR4. It is hypothesized that nanoscale differences exist between the spatial clustering of TLR4 upon binding of LPS derived from <i>Y. pestis</i> and <i>E. coli</i> . Although optical imaging can provide exquisite details of the spatial organization of biomolecules, there is a mismatch between the scale at which receptor clustering occurs (<300 nm) and the optical diffraction limit (>400 nm). The last decade has seen the emergence of super-resolution imaging methods that effectively break the optical diffraction barrier to yield truly nanoscale information in intact biological samples. This study reports the first visualizations of TLR4 distributions on intact cells at image resolutions of <30 nm using a novel, dual-color stochastic optical reconstruction microscopy (STORM) technique. This methodology permits distinction between receptors containing bound LPS from those without at the nanoscale. Importantly, it is also shown that LPS derived from immunostimulatory bacteria result in significantly higher LPS-TLR4 cluster sizes and a nearly twofold greater ligand/receptor colocalization as compared to immunoevading LPS.	Cells; Membrane; Bacteria; Cellular Structures
Pellett PA, Sun X, Gould TJ, Rothman JE, Xu MQ, Correa IR Jr, and Bewersdorf J	Two-color STED microscopy in living cells.	Biomed Opt Express	2011	Diffraction-unlimited resolution provided by Stimulated Emission Depletion (STED) microscopy allows for imaging cellular processes in living cells that are not visible by conventional microscopy. However, it has so far not been possible to study dynamic nanoscale interactions because multicolor live cell STED microscopy has yet to be demonstrated and suitable labeling technologies and protocols are lacking. Here we report the first realization of two-color STED imaging in living cells. Using improved SNAP(f) and CLIP(f) technologies to label epidermal growth factor (EGF) and EGF receptor (EGFR), we report resolutions of 78 nm and 82 nm for 22 sequential two-color scans in living cells.	

Bruker Super-Resolution Publications

Mlodzianoski MJ, Schreiner JM, Callahan SP, Smolkova K, Dlaskova A, Santorova J, Jezek P, and Bewersdorf J	Sample drift correction in 3D fluorescence photoactivation localization microscopy.	Opt Express	2011	The recent development of diffraction-unlimited far-field fluorescence microscopy has overcome the classical resolution limit of ~250 nm of conventional light microscopy by about a factor of ten. The improved resolution, however, reveals not only biological structures at an unprecedented resolution, but is also susceptible to sample drift on a much finer scale than previously relevant. Without correction, sample drift leads to smeared images with decreased resolution, and in the worst case to misinterpretation of the imaged structures. This poses a problem especially for techniques such as Fluorescence Photoactivation Localization Microscopy (FPALM/PALM) or Stochastic Optical Reconstruction Microscopy (STORM), which often require minutes recording time. Here we discuss an approach that corrects for three-dimensional (3D) drift in images of fixed samples without the requirement for fiduciary markers or instrument modifications. Drift is determined by calculating the spatial cross-correlation function between subsets of localized particles imaged at different times. Correction down to ~5 nm precision is achieved despite the fact that different molecules are imaged in each frame. We demonstrate the performance of our drift correction algorithm with different simulated structures and analyze its dependence on particle density and localization precision. By imaging mitochondria with Biplane FPALM we show our algorithm's feasibility in a practical application.	Cells; Organelles; Cellular Structures; Microscopy
Gould TJ, Myers JR, and Bewersdorf J	Total internal reflection STED microscopy.	Opt Express	2011	Stimulated emission depletion (STED) microscopy achieves diffraction-unlimited resolution in far-field fluorescence microscopy well below 100 nm. As common for (single-lens) far-field microscopy techniques, the lateral resolution is better than the axial sectioning capabilities. Here we present the first implementation of total internal reflection (TIR) illumination into STED microscopy which limits fluorophore excitation to ~70 nm in the vicinity of the cover slip while simultaneously providing ~50 nm lateral resolution. We demonstrate the performance of this new microscope technique with fluorescent bead test samples as well as immuno-stained microtubules. Total internal reflection STED microscopy provides superior axial sectioning capabilities with the potential to reduce photo-bleaching and photo-damage in live cell imaging.	Microscopy
Baker M	Microscopy: Bright light, better labels.	Nature	2011	?	Microscopy; Chemicals and Drugs