Program and Abstract Book

29. International Workshop on

Single Molecule Spectroscopy and Super-resolution Microscopy

Berlin, Germany
October 8 - 10, 2024

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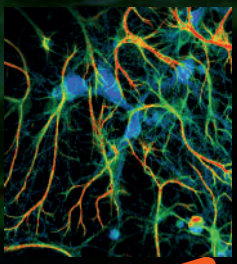
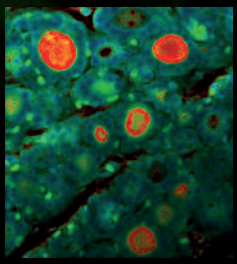
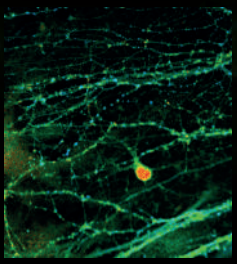
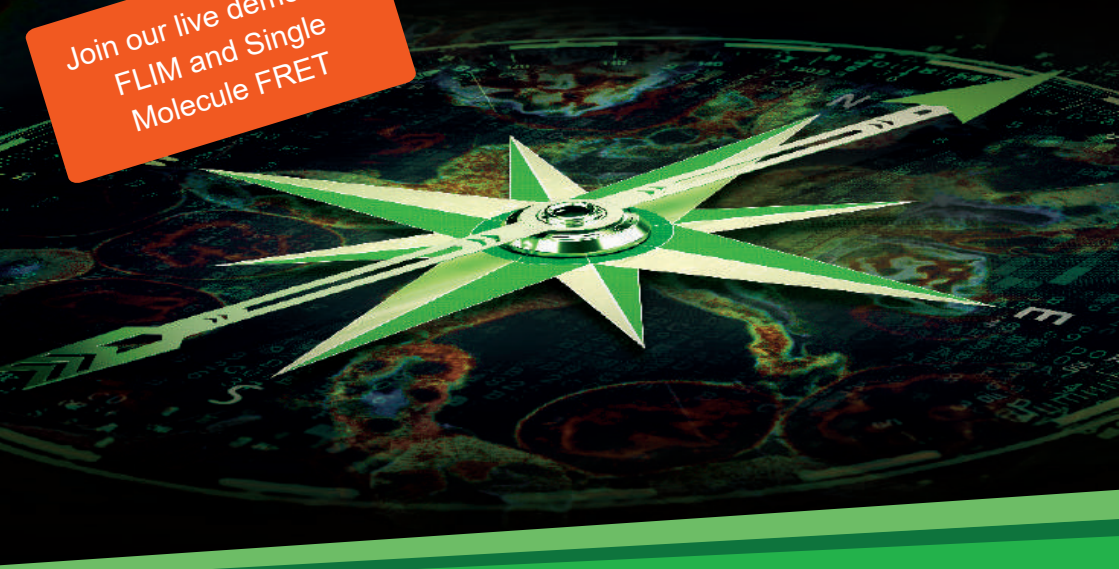






Join our live demos on FLIM and Single Molecule FRET



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Program

as of October 07, 2024

Tuesday, October 8

12.30 - 13.30 REGISTRATION

13.30 - 13.45 ***Rainer Erdmann***, *Berlin, Germany*
Opening Remarks

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13.45 - 14.15 ***Lars Hubatsch***, *Dresden, Germany (Invited Talk)*
Capturing Molecular Transport across Phase Boundaries by Fluorescence Microscopy

14.15 - 14.35 ***Gabriel Moya***, *Munich, Germany (Student Award)*
Single-molecule spectroscopy & super-resolution microscopy at the biochemistry lab bench.

14.35 - 14.55 ***Chenyuan Yan***, *München, Germany (Student Award)*
Exploring the Interactions between DNA and Graphene via Graphene Energy Transfer (GET)

14.55 - 15.15 ***Sigrid Milles***, *Berlin, Germany*
Intrinsically disordered regulators of endocytosis - an integrated NMR/single molecule fluorescence approach

15.15 - 15.50 COFFEE BREAK & EXHIBITION

Session: FRET I Chair: Lars Hubatsch

15.50 - 16.20 ***Quan Wang***, *Bethesda, United States (Invited Talk)*
High-precision single-molecule spectroscopy and charge sensing with an anti-Brownian trap

16.20 - 16.40 ***Thorben Cordes***, *Dortmund, Germany*
Dissecting Mechanisms of Ligand Binding and Conformational Changes in Substrate Binding Proteins

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Confocal microscopy in a controlled atmosphere for nano-scale nuclear magnetic resonance spectroscopy

17.00 - 17.20 ***Noah Salama***, *Düsseldorf, Germany (Student Award)*
Performing and Analyzing FRET Nanoscopy Measurements on DNA-Origami Platforms with sub-Nanometer Precision

17.20 - 18.50 POSTER SESSION 1 & GET TOGETHER

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Studying interaction in biological samples by applying spectroscopic methods

09.35 - 09.55 ***Or Eivgi***, *Heidelberg, Germany*
Subtle Polymer Dynamics Revealed by Fluorescence Lifetime Imaging Microscopy

09.55 - 10.15 ***Koushik Sreenivasa***, *Delft, Netherlands (Student Award)*
Measuring the sequence dependence of DNA looping at high-throughput using single-molecule FRET

10.15 - 10.35 ***Abhilash Kulkarni***, *Stockholm, Sweden (Student Award)*
Multiplexed NIR FCCS using a single superconducting nanowire single photon detector

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Visualizing the coupling between Gag proteolysis and Env clustering in native HIV-1 viruses

11.50 - 12.10 ***Christian Eggeling****, Jena, Germany*
Pitfalls and workarounds of photoblueing effects in advanced fluorescence microscopy

12.10 - 12.30 ***Tao Chen, Göttingen****, Germany*
Super-resolved axial imaging of piconewton cellular traction forces with metal-induced energy transfer spectroscopy/imaging

12.30 - 12.50 ***Dominic A. Helmerich****, Würzburg, Germany*
Beyond Resolution Limits: Sub-10nm Insights via Photoswitching Fingerprint analysis

12.50 - 13.10 ***Roman Tsukanov****, Göttingen, Germany*
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15.10 - 15.30	***Moritz Burmeister***, *Aarhus, Denmark (Student Award)* Extracting Rate Constants Using Single Molecule Localization Microscopy
15.30 - 15.50	***Samrat Basak***, *Göttingen, Germany* Three-dimensional multi-target super-resolution microscopy of cells using Metal-Induced Energy Transfer and DNA-PAINT
15.50 - 16.10	***Asima Nayak***, *Berlin, Germany (Student Award)* Characterization of Lewy Body-like Structures in Cellular System and Patient Samples
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09.35 - 09.55	***Nicola Galvanetto**, Zurich, Switzerland* Observing protein dynamics in biomolecular condensates with single-molecule spectroscopy
09.55 - 10.15	***Daniel Nettels**, Zürich, Switzerland* Disordered linkers enable complex motion of two-domain proteins on double-stranded RNA
10.15 - 10.35	***Rana Mhanna**, Saarbrücken, Germany* Unexpected effect of excitation wavelength in single-molecule photochemistry of terrylene
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Session: Biological applications — Chair: Anindya Datta

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11.50 - 12.10	***Melissa Birol**, Berlin, Germany* Early deviations in cell-to-cell communication in neurodegenerative disease trajectories
12.10 - 12.30	***Johannes Broichhagen**, Berlin, Germany* Heavier fluorophores with boosted brightness, lifetime and stability
12.30 - 12.50	***Michael Börsch**, Jena, Germany* Beyond smFRET - NV qubit in nanodiamond for monitoring subunit rotation in single FoF1-ATP synthase
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Session: Correlation Spectroscopy Chair: Emmanuel Margeat

14.20 - 14.50	***Anindya Datta**, Mumbai, India (Invited Talk)* Elucidation of exciton dynamics in semiconductor nanocrystals using Fluorescence Lifetime Correlation Spectroscopy
14.50 - 15.10	***Tanuja Kistwal**, Bochum, Germany* Fluorescence correlation spectroscopy of Single wall carbon nanotubes
15.10 - 15.30	***Sebastian Kruss**, Bochum, Germany* Near infrared fluorescent nanosensors for biomedical imaging
15.30 - 15.50	***Tjaart Krüger**, Pretoria, South Africa* Comparison of real-time feedback-driven single-particle tracking techniques
15.50 - 16.00	Concluding Remarks
16.00	End of 29th International Workshop on Single Molecule Spectroscopy and Super-resolution Microscopy

Abstracts:
Oral Presentations

according to schedule

Capturing Molecular Transport across Phase Boundaries by Fluorescence Microscopy

Lars Hubatsch

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Cells compartmentalize biochemical processes within organelles. Compartmentalisation can be achieved by selectively admitting biomolecules, either through a membrane or, in the case of biomolecular condensates, via the condensate-bulk interface. While membrane transport is well-studied, the mechanisms regulating transport across condensate interfaces remain unclear, hindering our understanding of dynamic condensate functions—a key focus in our lab. To address this, we employ various optical techniques, combined with data analysis and theory. I will present an example of this interdisciplinary approach, demonstrating how quantitative live microscopy, coupled with mean-field and single-molecule theory, reveals insights into molecular transport across phase boundaries. Our findings show that in binary mixtures, the condensate interface is near local equilibrium, allowing molecules to enter and exit without delay. However, theory predicts complex behavior in multi-component mixtures. I will explore these cases, their experimental predictions, and their intracellular functional implications.

Single-molecule spectroscopy & super-resolution microscopy at the biochemistry lab bench.

Gabriel Moya[1,2], Oliver Brix[1], Philipp Klocke[1], Paul Harris[3], Nicolas Wendler[1,2], Jorge Luna[1], Eitan Lerner[3,4], Niels Zijlstra[1], Thorben Cordes[1,2]

[1]Physical and Synthetic Biology, Faculty of Biology, Ludwig-Maximilians Universität München, Planegg-Martinsried, Germany
[2]Biophysical Chemistry, Faculty of Chemistry and Chemical Biology, Technische Universität, Dortmund, Dortmund, Germany
[3]Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, Faculty of Mathematics Science, The Edmond J. Safra Campus, The Hebrew University of, Jerusalem, Jerusalem, Israel
[4]The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem, Israel

Over the past decades, single-molecule and super-resolution microscopy have advanced and become essential tools for life science research. However, there is a growing gap between the state-of-the-art and what is accessible to biologists, biochemists, medical researchers, or labs with financial constraints[1,2,3]. To bridge this gap, we introduce Brick-MIC, a versatile and affordable 3D-printed micro-spectroscopy and imaging platform. Brick-MIC enables the integration of various fluorescence imaging techniques with single-molecule resolution within a single platform and allows for quick switching between different modalities. We present variants of Brick-MIC that support single-molecule fluorescence detection[4], fluorescence correlation spectroscopy[5], and super-resolution imaging (STORM[6] and PAINT[7]). We foresee that this affordable, flexible platform will be a valuable tool for many laboratories worldwide.

[1] G. Marqués, T. Pengo, M. A. Sanders, eLife 9, e55133 (2020)
[2] R. M. Power, J. Huisken, Nat Methods 16, 1069–1073 (2019)
[3] J. Hohlbein, B. Diederich, B. Marsikova, E. G. Reynaud, S. Holden, W. Jahr, R. Haase, K. Prakash, Nat Methods 19, 1020–1025 (2022).
[4] J. Hohlbein, T. D. Craggs, T. Cordes. Chem. Soc. Rev. 43, 1156–1171 (2014).
[5] L. Yu, Y. Lei, Y. Ma, M. Liu, J. Zheng, D. Dan, P. Gao, Frontiers in Physics 9 (2021).
[6] M. J. Rust, M. Bates, X. Zhuang, Nat Methods 3, 793–796 (2006).
[7] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld, F. C. Simmel, Nano Lett. 10, 4756–4761 (2010).

Exploring the Interactions between DNA and Graphene via Graphene Energy Transfer (GET)

Chenyuan Yan[1], Alan M. Szalai[1], Lars Richter[1], Giovanni Ferrari[1], Jakob Hartmann[1], Merve-Zeynep Kesici[1], Bosong Ji[1], Andrés M. Vera[1], Philip Tinnefeld[1], Izabela Kamińska[1,2]

[1]Department of Chemistry and Center for NanoScience, Ludwig Maximilian University of Munich, Butenandtstr. 11, 81377 Munich, Germany

[2]Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

Graphene is well known as an efficient fluorescence quencher, and has been well-studied as a broadband and unbleachable energy acceptor.[1,2] The energy transfer efficiency from an emitter to graphene scales with d-4, where d is a distance between both, and the characteristic d0 = 18 nm represents the distance with the 50% Graphene Energy Transfer (GET) efficiency.[1,2] Taking advantage of the GET, the height of the single dye molecule on graphene can be sensitively determined from the fluorescence intensity and lifetime measurements.[2,3] In our previous work, we successfully utilized DNA origami as nanopositioners to precisely control the height of dye molecules on graphene.[2,3] More recently, we developed a new ssDNA-dsDNA-graphene system, where the dsDNA segment stands vertical on graphene.[4] We use this system to visualize structural properties of DNA or to further visualize the direct interactions between DNA with protein.[4] In this work, we study the interactions between ssDNA/dsDNA with graphene to better understand how long the ssDNA anchor is needed to immobilize the construct on graphene, and what happens if the length of ssDNA anchor is shortened in order to get more information about the interaction mechanism between DNA and graphene.

[1] Gaudreau, L. et al., Nano Lett. 13, 2030 (2013).
[2] Kamińska, I. et al., Nano Lett. 19, 4257 (2019).
[3] Kamińska, I. et al., Adv. Mat. 33, 2101099 (2021).
[4] Szalai, A. M. et al., bioRxiv. /doi.org/10.1101/2023.11.21.567962.

Intrinsically disordered regulators of endocytosis - an integrated NMR/single molecule fluorescence approach

Sigrid Milles

FMP Berlin, Robert-Roessle-Str. 10, 13125 Berlin, Germany

Intrinsically disordered proteins (IDPs) lack clearly defined structure and are therefore highly flexible and easily adaptable to different binding partners. This makes them important players in many biological processes, often with vital regulatory functions. Their dynamic features and broad range of interaction modes, however, render them difficult to study and analyzing their complexes often requires integrated approaches. Integrating complementary parameters from of nuclear magnetic resonance (NMR) and single molecule fluorescence approaches allowed us to describe the conformational landscape of IDPs at molecular resolution and promises to shed new light onto various biological processes.
Among those counts clathrin mediated endocytosis. The early phases of clathrin mediated endocytosis are organized through a highly complex interaction network mediated by clathrin associated sorting proteins (CLASPs) that comprise long intrinsically disordered regions (IDRs). We characterize the IDRs of those CLASPs in their entirety and at molecular resolution, uncovering a plethora of interactions of various strengths and dynamic features with their endocytic interaction partners, proposing a rationale for how first interactions and dynamic rearrangement of partners take place during the uptake of a coated vesicle.

Quantitative Description of Intrinsically Disordered Proteins Using Single-Molecule FRET, NMR, and SAXS.
Naudi-Fabra S, Tengo M, Jensen MR, Blackledge M, Milles S.
J Am Chem Soc. 2021 Dec 8;143(48):20109-20121.
An extended interaction site determines binding between AP180 and AP2 in clathrin mediated endocytosis.
Naudi-Fabra S, Elena-Real CA, Vedel IM, Tengo M, Motzny K, Jiang PL, Schmieder P, Liu F, Milles S.
Nat Commun. 2024 Jul 13;15(1):5884.

High-precision single-molecule spectroscopy and charge sensing with an anti-Brownian trap

Quan Wang

National Institutes of Health, NIDDK, Bethesda, USA

By counteracting random diffusion in aqueous solution, the Anti-Brownian Electrokinetic (ABEL) trap has evolved to be a versatile and powerful platform for single-molecule spectroscopy and sensing. I describe two recently developed modalities, each of which enables new capabilities for single-molecule biophysics. First, ABEL-FRET achieves ultrahigh, shot-noise limited resolution of smFRET efficiency in solution without surface tethering. Second, we demonstrate real-time monitoring of single-molecule phosphorylation cycles by direct sensing of the charge state of individual molecules in solution.

Dissecting Mechanisms of Ligand Binding and Conformational Changes in Substrate Binding Proteins

Thorben Cordes

Biophysical Chemistry, Faculty of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany

Ligand binding and conformational changes of biomacromolecules play a central role in the regulation of cellular processes. It is important to understand how both are coupled and what their role is in biological function. The biochemical properties, conformational states, and structural dynamics of periplasmic substrate-binding proteins (abbreviated SBPs or PBPs), which are associated with a wide range of membrane proteins, have been extensively studied over the past decades. Their ligand-binding mechanism, i.e., the temporal order of ligand-protein interactions and conformational changes, however, remains a subject of controversial discussion. I here summarize our past and ongoing efforts to clarify how ligand binding and conformational changes are coupled in SBPs using a variety of different biophysical techniques including single-molecule FRET.

https://www.nature.com/articles/nsmb.2929
https://elifesciences.org/articles/44652
https://www.pnas.org/doi/abs/10.1073/pnas.2026165118
https://www.biorxiv.org/content/10.1101/2023.08.02.551720v1.abstract

Confocal microscopy in a controlled atmosphere for nano-scale nuclear magnetic resonance spectroscopy

Kseniia Volkova[1], Abhijeet Kumar[2], Karolina Schüle[3], Jens Fuhrmann[3], Fedor Jelezko[3], Kirill Bolotin[2], Boris Naydenov[1]

[1]Department Spins in Energy Conversion and Quantum Information Science (ASPIN), Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Kekuléstraße, Berlin, Germany
[2]Department of Physics, Freie Universität Berlin, Arnimallee, Berlin, Germany
[3]Institut für Quantenoptik, Freie Universität Ulm, Albert-Einstein-Allee, Ulm, Germany

Different studies performed on nitrogen-vacancy (NV) centers in diamond proved them to be efficient quantum sensors of materials deposited on a diamond surface. The properties of the NV centers can be used for detecting nanoscale magnetic fields, allowing nuclear magnetic resonance (NMR) spectroscopy on samples at the level of a single molecule [1] or for sensing single nuclear spins [2]. Among the materials studied or proposed for NV center-based NMR spectroscopy, many are sensitive to oxygen or humidity and can alter their properties upon air exposure. One such material is black phosphorus. Phosphorus isotope 31P is a good candidate for the NV center-based spin detection thanks to its 100% natural abundance and a high gyromagnetic ratio (γ = 1.725 kHz/G). Transferring a thin black phosphorus flake on a diamond with NV centers implanted a few nanometers below the surface could be one of the approaches to fabricate the hardware of a quantum simulator [3]. The main disadvantage of black phosphorus is that it degrades under ambient conditions. Therefore, we present a confocal microscope with a glovebox enclosure for performing NV-based NMR spectroscopy on multi-layered black phosphorus.

[1] I. Lovchinsky, A. O. Sushkov, E. Urbach, N. P. de Leon, S. Choi, K. De Greve, R. Evans, R. Gertner, E. Bersin, C. Müller, L. McGuinness, F. Jelezko, R. L. Walsworth, H. Park and M. D. Lukin, Science, 351, 836-841 (2016).
[2] C. Müller, X. Kong, J. M. Cai, K. Melentijević, A. Stacey, M. Markham, D. Twitchen, J. Isoya, S. Pezzagna, J. Meijer, J. F. Du, M. B. Plenio, B. Naydenov, L. P. McGuinness and F. Jelezko, Nature communications, 5, 4703 (2014).
[3] J. Cai, A. Retzker, F. Jelezko and M.B. Plenio, Nature Physics, 9, 168-173 (2013).

Performing and Analyzing FRET Nanoscopy Measurements on DNA-Origami Platforms with sub-Nanometer Precision

Noah Salama[1], Jan-Hendrik Budde[1], Nicolaas van der Voort[1], Suren Felekyan[1], Julian Folz[1], Ralf Kühnemuth[1], Paul Lauterjung[1,2], Michelle Rademacher[1,3], Markus Köhler[4], Andreas Schönle[4], Julian Sindram[5], Marius Otten[5], Anders Barth[1,6], Christian Herrmann[2], Matthias Karg[5], Claus A. M. Seidel[1]

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[3]Institute for Physical Chemistry II, Heinrich-Heine-Universität Düsseldorf, Germany
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Super-resolution microscopies provide an invaluable tool for studies of larger cellular structures and molecular assemblies with nanometer precision, while being minimally invasive and highly selective to the molecule of interest. However, the currently achieved spatiotemporal resolution cannot resolve distances on the size of individual molecules, thus conformational fine structure and dynamics on the scale of single molecules remain concealed.
We overcome this resolution limit through the combination of multiparameter FRET-spectroscopy and colocalization stimulated emission depletion (cSTED) microscopy, giving a versatile and readily available tool for investigation of structure and dynamics on a single-molecule level, with Ångström precision.[1]
The analysis of FRET parameters yields the Euclidean distance while colocalization provides the distance projected onto the image plane. Consequently, the combined information allows for the determination of 3D-orientations via Pythagoras‘ theorem. We established an easy-to-follow workflow for performing and analyzing FRET nanoscopy measurements and obtain inter-dye distances with sub-nanometer precision.
We demonstrate the feasibility and accuracy of our approach by using standardized DNA origami platforms with two dye pairs as a benchmark sample. We simultaneously localize donor and acceptor dyes of single FRET pairs with nanometer resolution and quantitatively measure intramolecular distances with sub-nanometer precision over a large dynamic range.

[1] Budde, J.-H. et al., arXiv preprint (2022)

Studying interaction in biological samples by applying spectroscopic methods

Stefanie Weidtkamp-Peters

Heinrich-Heine University Duesseldorf, Germany

A main goal of molecular cell biology is to identify and monitor the interacting network of proteins and other molecules in a cell with respect to different positions and conditions to understand the biological processes on a molecular level. The investigation of protein interactions in living plant tissue has become of increasing importance in recent years. A high spatial and temporal resolution for the observation of in vivo protein interaction is needed, e.g., in order to follow changes of interactions and complex formation over time. In vivo Fluorescence or Förster resonance energy transfer (FRET) measurements allows for detailed analyses of interacting molecules in their natural environment at a subcellular level. Especially FRET-FLIM (Fluorescence lifetime imaging microscopy) measurements provide an extremely powerful and reliable tool meeting the demands for investigating in vivo protein interaction also quantitatively and with high precision. On the other hand, the most commonly used fluorescent reporters used in this context, the fluorescent proteins, show a weak quantum yield and a complex decay behavior and are affected by changing environmental conditions, like changes in the pH. In addition strong autofluorescence of the tissue under investigation can significantly contribute to the acquired signal, which makes the analysis of the recorded data difficult or only allows insufficient quantitative analysis. In my talk I will show examples of interaction studies in plant tissue and ideas on how to overcome the limitations imposed by the measurement conditions to obtain quantitative results to help understand the network of interacting proteins in plant tissues.

Maika, J.E., Kramer, B., Strotmann, V.I., Wellmer, F., Weidtkamp-Peters, S., Stahl, Y., and Simon, R. (2023). One pattern analysis (OPA) for the quantitative determination of protein interactions in plant cells. Plant Methods 19, 73. 10.1186/s13007-023-01049-3.

Burkart, R.C., Strotmann, V.I., Kirschner, G.K., Akinci, A., Czempik, L., Dolata, A., Maizel, A., Weidtkamp-Peters, S., and Stahl, Y. (2022). PLETHORA-WOX5 interaction and subnuclear localization control Arabidopsis root stem cell maintenance. Embo Rep 23, e54105. 10.15252/embr.202154105.

Stahl, Y., Grabowski, S., Bleckmann, A., Kuhnemuth, R., Weidtkamp-Peters, S., Pinto, K.G., Kirschner, G.K., Schmid, J.B., Wink, R.H., Hulsewede, A., et al. (2013). Moderation of Arabidopsis root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. Curr Biol 23, 362-371. 10.1016/j.cub.2013.01.045.

Subtle Polymer Dynamics Revealed by Fluorescence Lifetime Imaging Microscopy

Or Eivgi, Suzanne A. Blum

Department of Chemistry, University of California, Irvine, Irvine California, 92697-2025 United States

In this work, Fluorescence lifetime imaging microscopy (FLIM) is developed to investigate the dynamics of ring-opening-metathesis-polymerization-(ROMP-) based polymers. By designing ROMP functionalized viscosity-sensitive fluorescent molecular rotors that change their fluorescence lifetime based on the viscosity of their polymeric microenvironment, simultaneous imaging of changing physical parameters and catalytic activity in living polymers was achieved.1 In this system, coupling FLIM with intensity fluorescence microscopy enabled correlation of the decreasing catalytic activity of Grubbs catalysts inside of polydicyclopentadiene particles with increasing microenvironment viscosity. Moreover, it was demonstrated that microenvironment viscosity changes during polymerization are monomer-dependent, accelerated by crosslinking, and variable in the rate of change between different particles and subparticle regions of the same sample. Together, these data provide a physical mechanism for irregular reaction kinetics observed for single Grubbs catalysts. The sensitivity of the viscosity-sensitive rotors also uncovered and quantitatively elucidated undisclosed differential block-selective responses toward solvation changes upon addition of DMSO and THF to self-assembled ROMP-based amphiphilic block copolymers.2 The sensitivity of this method provided unique information on block-selective solvent-triggered assembly and disassembly mechanisms, revealing behaviors invisible to or with superior sensitivity to traditional 1H-NMR spectroscopy. This block-selective information can be further used to fine tune block copolymer assembly and disassembly.

(1) Eivgi, O.; Blum S. A. J. Am. Chem. Soc. , 144, 13574–13585 (2022)
(2) Eivgi, O.; Ravenscroft, A. C.; Blum, S. A., J. Am. Chem. Soc. , 145, 2058–2063 **(2023)**

Measuring the sequence dependence of DNA looping at high-throughput using single-molecule FRET

Koushik Sreenivasa, Chirlmin Joo

Department of BioNanoScience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands

DNA bending/flexibility is shown to be a characteristic in many processes within a cell such as gene regulation and DNA repair. Despite its‘ importance it is not yet completely understood on a molecular scale how the sequence of DNA controls the bending propensity. One of the limitations is the lack of high-resolution, high-throughput technique(s) for analyzing the large breadth of sequence space. Using a new high-throughput, single-molecule fluorescence method labelled SPARXS, we characterise the kinetics of DNA looping of many different sequences in a single-experiment. With conventional technique, we find that differing the sequences by few nucleotides is enough to change the rates of looping and unlooping. We aim to use SPARXS to get a more complete picture of how sequence dictates DNA looping kinetics and thereby setup foundation for further experiments.

Multiplexed NIR FCCS using a single superconducting nanowire single photon detector

Abhilash Kulkarni, Niusha Bagheri, Jerker Widengren

Department of Applied Physics, KTH Royal Institute of Technology, S-10691, Stockholm, Sweden

Fluorescence fluctuation spectroscopy is widely used in biomolecular studies to extract useful information about molecular dynamics and interactions, including also monitoring of photophysical transitions in fluorophores. However, fluorescence correlation spectroscopy (FCS) ultimately relies on single-molecule detection conditions, and its application for cellular and in-vivo studies can be limited, mainly due to autofluorescence and scattering issues, resulting in low signal-to-background ratios (SBRs). In this investigation, we aim to reduce such limitations by exploiting the benefits of Superconducting Nanowire Single Photon Detectors (SNSPDs) which include no after-pulsing (as seen in APDs), better detection efficiency in the near infrared region (NIR) and high time resolution. We have devised a novel setup which utilizes a single SNSPD channel to perform dual-color fluorescence cross correlation spectroscopy (FCCS) measurements by segmenting the signal temporally. The same strategy can also be used for antibunching measurements, at the same time monitoring lifetimes and rotational diffusions on similar shorter time scales. Here, we investigate different emissive species of a NIR cyanine dye (Sulfo-Cy7) by spectrally separating them, thereby providing a specific signature for this dye which can be used as an identifier with a high SBR. Furthermore , we demonstrate the strategy by extracting signals from two spectrally different species of emitters in the same solution. This is demonstrated by binding one type of species to vesicles while the other diffuses freely. Further cross correlations are displayed when both the species are bound to vesicles.

Visualizing the coupling between Gag proteolysis and Env clustering in native HIV-1 viruses

Sergio Padilla-Parra

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Envelope glycoprotein (Env) trimers in HIV-1 serve as molecular machines for viral entry into host cells, including CD4+ T cells and macrophages. Recent advancements in super resolution light microscopy techniques have shed light on the previously unseen organization of Env on the surface of mature HIV-1 virions, revealing distinct protein clusters rather than random distribution. These Env clusters are not only central to viral entry mechanisms but also serve as critical targets for both innate and adaptive immune responses. Despite significant progress in elucidating the structural aspects of Env a crucial gap remains in understanding the collective behavior of multiple units and their impact on viral entry and fusion processes. Notably, a tight coupling between Env clustering and Gag proteolysis, wherein proteolytic processing of the Gag polyprotein by the viral protease facilitates the transition from immature to mature, infectious virions, has been observed. However, the precise timing and kinetics of Gag processing and its relationship with Env clustering dynamics are not fully understood. To address this gap, we introduce a novel approach involving the tagging of the variable region 4 (V4) loop of Env with a synthetic tag called ALFAtag. This innovative tagging strategy, guided by AlphaFold predictions, enables specific labeling using anti-ALFA single-domain antibodies (sdAbs), thus allowing for the study of Env clustering within native viruses while preserving the native Env structure. Due to the small size of both the ALFA-tag and sdAbs, the ALFA system results in minimal linkage error and it is perfectly suitable to ultra-resolved Env clustering via a single-molecule based super-resolution fluorescence microscopy, known as DNA-PAINT. Furthermore, by labeling the Gag polyprotein with mTurquoise2 internally and employing a dark fluorogen (FAST) for Förster resonance energy transfer (FRET), we can monitor proteolytic release of the fluorophore from Gag, which correlates with an increase in fluorescence lifetime due to FRET disruption. Ultimately, this strategy enables us to discern the degree of virus maturation with high precision and dynamic range whilst visualizing Env clustering, offering a detailed depiction of how Env organises before and during virus maturation. Cryo-electron Tomography (CryoET) shows that the maturation biosensors preserve capsid structure in mature viruses. Additionally, we validate and cross-compare our findings using Ångström resolution localization microscopy (RESI) adapted for native viruses, further enhancing the robustness of our observations. Our results highlight the dynamic clustering and cooperative behavior of individual Envs and how they affect intramolecular dynamics as critical determinants of efficient viral cell entry.

Pitfalls and workarounds of photoblueing effects in advanced fluorescence microscopy

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Photobleaching is a major limitation in fluorescence microscopy. A special photobleaching pathway is photoblueing, i.e. the conversion of fluorescent molecules into species of blue-shifted emission properties. We investigated details of this photoblueing effect for confocal and STED microscopy as well as for spectral imaging of environment sensitive membrane dyes and outline induced artefacts as well as strategies to avoid these.

Super-resolved axial imaging of piconewton cellular traction forces with metal-induced energy transfer spectroscopy/imaging

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Cell mechanics play a pivotal role in regulating numerous vital biological processes. To better understand how mechanical forces are coupled to biochemical signaling pathways, methods are needed to map the nanoscale distribution of forces in living cells. Molecular tension probes (MTPs) have been developed to map the magnitude of receptor force with pN sensitivity.1,2 This enables various fluorescence microscopes to map receptor forces for different cellular adhesion structures. However, current fluorescence techniques can only map force along the lateral dimension with a resolution of tens of nanometers and the axial dimension with a resolution of 50-60 nm. Here, we utilized metal-induced energy transfer (MIET) spectroscopy/imaging3 with a widely recognized DNA-based hairpin MTP (MIET-MTP, see Figure) to map the cell force along the axial dimension with a nanometer-level resolution. MIET not only reports the height change in MTP but also presents the corresponding height profile of plasma membrane (PM), revealing a positive and negative correlation between the MTP and PM for focal adhesions and podosomes, respectively. Moreover, MIET-MTP exhibits remarkable versatility, including its potential integration with DNA-PAINT to facilitate 3D force mapping at nanometer precision. MIET-MTP will serve as a potent tool, bridging the gap between structural biology and mechanobiology.

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Beyond Resolution Limits: Sub-10nm Insights via Photoswitching Fingerprint analysis

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The field of super-resolution microscopy has revolutionized biological imaging by providing direct insights into cellular structures and protein arrangements. Spatial resolution in the single-digit nanometer range can be achieved, matching the size of cellular molecules. Recent high-resolution microscopy methods have demonstrated localization accuracies in the angstrom range and spatial resolution in the lower single-digit nanometer range for reference structures. However, achieving this spatial resolution in biological samples remains challenging due to critical parameters that must be met, posing a significant hurdle to attaining molecular resolution. In this work, we demonstrate how to leverage this challenge to our advantage. We present a widely accessible method to extract information well below the lateral resolution using simple microscopy techniques. This approach can reveal insights into biological systems and processes that would typically remain hidden and is applicable to a broad range of subjects, including living systems.

Helmerich, D.A., Beliu, G., Taban, D. et al., Nat Methods, 19, 986–994 (2022)

A versatile microfluidics platform for enhanced multi-target super-resolution microscopy

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Super-resolution microscopy (SRM) [1] has become a standard tool for biomedical imaging, capable of revealing structural information with unprecedented detail. However, achieving localization precision on the nanometer scale requires advanced SRM technologies and optimized sample performance. To achieve this, the most suitable fluorophores for the specific type of microscopy must be selected. Furthermore, the photophysical performance of a fluorophore must be optimized through tight control of the fluorophore environment, achieved by manual injection of an imaging buffer or by using a versatile microfluidics system.
Here, we develop an Air-PREssure-based MIcroFluidics System (APREMIFS) and demonstrate its implementation for multiplexed super-resolution imaging. We used APREMIFS to perform the sequential imaging of cellular targets (cytoskeleton and focal adhesion proteins) via Exchange-PAINT [2], achieving an average localization precision of 10 nm for all imaged targets. Furthermore, we utilized gold nanoparticles as fiducial markers to correct for mechanical drift and to precisely align and overlay images of different targets. APREMIFS is a highly versatile system that can be adjusted for multiple experimental scenarios and combined with different types of microscopes. We anticipate that APREMIFS will significantly enhance multiplexed super-resolution imaging and enable full automation of complex multiplexed SRM imaging protocols.

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Imaging Cellular Forces: From Micro to the Nano Scale

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Understanding the complex interplay between cells and their surrounding matrix is paramount for advancing biomimetic scaffold design. By pushing and pulling on the extracellular matrix (ECM), cells continuously sense the dynamic mechanical cues from their environment and generate mechanical feedback. Mechanical characterization of the matrix surrounding the cells has shown that contractile cells can generate a stiffness gradient in biological gels. Such cell-generated forces can reorganize and deform the natural ECM fibers, causing fiber densification and alignment. Traditional characterization methods like electron microscopy and scanning probe microscopy provide high spatial resolution but fall short in capturing these dynamic processes in situ. This talk highlights the use of fluorescence microscopy to characterize the structure of synthetic hydrogels and quantify the traction forces generated by the cells.We use confocal imaging and bead-free traction force microscopy (TFM) to demonstrate how a fully synthetic biomimetic hydrogel can be used as a platform for exploring the influence of biochemical and mechanical factors on cell-matrix interactions.. This biomimetic hydrogel, formed from oligo(ethyleneglycol)-functionalized polyisocyanate (PIC) polymers, is formed by non-covalent interactions and exhibits a nonlinear mechanical response at low stresses. We further investigate the forces that cells apply at a molecular scale using FRET-based tension sensors. These sensors allow us to measure the molecular-scale forces exerted by the cells, providing insights into how cells interact with and remodel their microenvironment. Our fluorescence microscopy-based approach sheds light on how physical cues regulate cell-matrix interactions, offering insights for the rational design of biomimetic materials.

Extracting Rate Constants Using Single Molecule Localization Microscopy

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Understanding the fundamental principles of biomolecular interactions is crucial for advancements in diagnostics and drug development. Single molecule techniques provide key insights into these interactions by looking beyond ensemble averages and giving access to potentially heterogenous population distributions.
We employ single molecule localization microscopy to investigate dynamic binding interactions between biomolecules, using thrombin and some of its aptamers as a model system. Thrombin, essential in blood coagulation, interacts with its aptamers at distinct binding sites with varying affinities, which we seek to characterize on a single molecule basis.
By tracking binding events of fluorescently labeled thrombin using total internal reflection fluorescence microscopy, we extracted kinetic rate constants of binding and unbinding to different aptamers. For this, we built a robust data analysis procedure, incorporating techniques from PAINT image-processing, machine learning and kinetic modeling to mitigate artifacts from unspecific binding events.
Our comprehensive approach was benchmarked against simulated and experimental data. The insights gained from this study lay the groundwork for future investigations into more complex multivalent binding.

Three-dimensional multi-target super-resolution microscopy of cells using Metal-Induced Energy Transfer and DNA-PAINT

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DNA-points accumulation for imaging in nanoscale topography (DNA-PAINT) is a potent variant of single-molecule localization microscopy (SMLM) which is highly effective for multiplexed super-resolution imaging. It achieves localization precision down to nanometers in the lateral direction. However, its routine axial localization precision is approximately three-fold lower as compared to the lateral localization precision. Recently, a technique known as Metal-Induced Energy Transfer (MIET) has been introduced, offering excellent axial resolution at the nanometer scale up to 200 nm above a surface. MIET is characterized by a low entry barrier, as its sole technical requirement is the availability of a fluorescence lifetime imaging modality. In this study, we harness the synergy between the exceptional axial resolution provided by MIET and the lateral resolution achieved with DNA-PAINT (MIET-PAINT) to accomplish multi-target 3D super-resolution imaging. We implemented MIET-PAINT using a wide-field fluorescence lifetime imaging microscope. We validated our technique by measuring the height of emitters placed on top of spacers of known thicknesses. We then demonstrated multiplexed MIET-PAINT imaging of fixed cells to visualize mechanotransduction proteins in the focal adhesion complex (FAC) and the cytoskeleton. We explored the structural arrangement of paxillin, zyxin, and actin stress fibers in U2OS cells and discovered that MIET-PAINT can reliably address multiple targets, providing lateral and axial nanometer-scale resolution. Furthermore, MIET-PAINT can be implemented using confocal microscope equipped with fast scanner, for example Luminosa from PicoQuant GmbH. Preliminary data will be shown.

Oleksiievets, N., Mougios, N., et al. “Three-dimensional multi-target super-resolution microscopy of cells using Metal-Induced Energy Transfer and DNA-PAINT” BiorXiv, (2024)

Characterization of Lewy Body-like Structures in Cellular System and Patient Samples

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The presence of proteinaceous inclusions known as Lewy bodies (LBs) is a hallmark of Lewy body disorders, characterized by abnormal protein deposits primarily composed of α-synuclein. These deposits disrupt neuronal function, leading to cognitive and motor deficits and ultimately cause cell death. While immunohistochemical studies and mass spectrometry identified nearly three hundred different proteins in LBs, including mitochondria-related proteins and components of the ubiquitin-proteasome system, the biogenesis of LBs remains unclear. Recently, we reverse engineered Lewy body-like inclusions (LBLs) in cellular system. Immunohistochemical studies of patient LBs and LBLs demonstrate that both share a core-shell architecture, where α-synuclein forms a dense shell surrounding a core containing various proteins and membrane-bound organelles. This structural similarity is further supported by soft X-ray tomography and live-cell imaging, which reveal that both LBs and LBLs accumulates mitochondria at their interface. Additionally, the dynamic interactions between α-synuclein and membrane-bound organelles in LBLs mimic those observed in patient LBs, indicating that the processes governing their formation and maturation are likely conserved. These findings highlight the potential of LBLs as cellular models for studying LB architecture and developing novel therapeutic strategies.

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Dissecting the structural dynamics of a GPCR using smFRET

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Metabotropic glutamate receptors (mGluR) regulate neuronal excitability and synaptic transmission by sensing L-glutamate – the major excitatory neurotransmitter in the central nervous system. Their crucial role for synaptic function makes them attractive targets for the treatment of numerous neurological and psychiatric diseases including for instance anxiety, depression, schizophrenia and addiction.To explore mGluR activation, we used single molecule FRET, as it allows to screen the conformations explored by single protein complexes, with high temporal and spatial resolution. However, smFRET is generally limited to one dimensional observations, ie a single distance per protein. 3 color single-molecule FRET extends the conformational analysis to the measurements of 3 distances simultaneously, and therefore to the observation of correlated movements. However, the site-specific labeling of a biomolecular complex with 3 single molecule-compatible fluorophores remains challenging.Here, we established a series of 2-color and 3-color smFRET sensors, through incorporation of two orthogonally reactive non-canonical amino-acids (ncAA) in response to two different stop codons, together with the addition of a SNAP self-labeling tag. These sensors report on the initial steps of mGluR2 activation, including the reorientation of the upper and the lower lobes of the venus flytrap domain (VFT) in an intersubunit fashion, its closure in an intrasubunit fashion [1,2], and the correlation between these 2 movements. We then used 2- and 3-color FRET on single diffusing molecules with time-resolved detection to explore ligand induced conformational changes on mGlu2 receptors. We show that agonist-binding efficiently depopulates the inactive state, leading to an equilibrium of receptors switching between the active and a newly identified intermediate state. Only the addition of a synthetic allosteric modulator, or of the G-protein, leads to a full stabilization of the activated receptor.Our study highlights the power of minimally invasive, ncAA-based, bioorthogonal labeling to dissect domain-specific conformational rearrangements of single, multidomain, multimeric proteins using smFRET.

[1] Quast et al., Nature Communications, 2021, doi.org/10.1038/s41467-021-25620-5
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Observing protein dynamics in biomolecular condensates with single-molecule spectroscopy

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A wide range of biomolecules in solution can phase-separate and form membraneless organelles in the cell. These assemblies often have liquid-like properties, and the corresponding dynamics and exchange of molecules with the environment are important for biological function. The dynamics and materials properties of these systems are commonly assessed based on translational diffusion or rheological properties, typically covering timescales of milliseconds and longer. However, information on the structure and dynamics at the molecular level is lacking. Using coacervates of two disordered highly oppositely charged proteins as a prototypical example of phase separation similar to that observed in the cell nucleus, we show with single-molecule fluorescence spectroscopy that the proteins in the condensates remain disordered, and that their chain dynamics occur on a sub-microsecond timescale, remarkably close to the dynamics in dilute solution [1]. This is despite the condensate being 1000-times more concentrated and 250-times more viscous than the dilute solution. The experimental results are in good agreement with large-scale all-atom molecular dynamics simulations, which reveal the molecular origin of the rapid dynamics.

[1] Galvanetto et al., Nature, 619, 876–883 (2023)

Disordered linkers enable complex motion of two-domain proteins on double-stranded RNA

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Double-stranded RNA-binding proteins (dsRBPs) specifically recognize double-stranded RNA in various contexts of post-transcriptional gene regulation [1]. They typically consist of multiple folded dsRNA-binding domains separated by long disordered linkers. In this study, we used single-molecule fluorescence spectroscopy to unravel the complex dynamic interaction of the dsRBP TRBP with dsRNA. TRBP plays a critical role in the RNA interference pathway, where it interacts with Dicer to facilitate the processing of pre-microRNA.[2-4]

Using two- and three-color single-molecule FRET experiments, we resolved and characterized the kinetics of processes occurring over a wide range of timescales from microseconds to hours; including the diffusive motion of TRBP along the RNA on the second timescale; the motion of its domains relative to each other on the millisecond timescale; the flipping of orientations on the RNA on the millisecond timescale; and the full dissociation of the protein from the RNA on the seconds-to-hours timescale.

By combining our single-molecule data with structural information from nuclear magnetic resonance (NMR) spectroscopy [2], maximum entropy ensemble reweighting, and extensive kinetic modeling, we were able to reduce these various complex behaviors of TRBP on dsRNA to a basic mechanism of single-domain association and dissociation on the microsecond to millisecond timescale.

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UNEXPECTED EFFECT OF EXCITATION WAVELENGTH IN SINGLE-MOLECULE PHOTOCHEMISTRY OF TERRYLENE

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Single-molecule chemistry (SMC) by means of fluorescence microscopy allows us to study a reactive system at the molecular scale, providing deep and unique insights that cannot be revealed in bulk. Among the imaging techniques, especially Total Internal Reflection Fluorescence (TIRF) microscopy is nowadays most commonly used, as it permits the analysis of molecular processes at or near the surface of the sample in a parallelized way, including a high signal-to-noise ratio. Our approach in SMC is to monitor changes of fluorescence properties during the reaction[1], and in the current case, we study the fluorescence colour change during the photooxidation of terrylene[2] using TIRF microscopy. Actually, terrylene is the ideal compound for single-molecule fluorescence chemistry owing to its luminescent properties, photostability and its ability to be embedded in solid matrices[3]. We report herein a comparative study between two excitation wavelengths on a population of approximately 100 reacting terrylene molecules for each condition. This study not only reveals the quantum yield for the photooxidation and the lifetime of the intermediate but also an unexpected effect of the excitation wavelength on promoting one reaction pathway over another. Our finding will be discussed with respect to findings in ultra-fast spectroscopy.

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Chemical Reaction Insights through FLIM

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My laboratory is fascinated by understanding how chemical reactions work. Without the right tools to investigate chemical reactions, critical details are missed. Thus, my laboratory is also fascinated by tool development. Here the development of FLIM application methods provides the ability to simultaneously image physical and chemical changes inside of polymers in real time during catalytic polymerization. FLIM enables not only an understanding of the dynamic chemical and physical changes inside polymers as they are growing with high spatiotemporal resolution, but also how these two features impact each other and catalytic turnover. Case studies in ring-opening metathesis polymerization (ROMP) include development of spectroscopic (fluorescence-lifetime-imaging) methods for the determination of the molecular weights of growing polymers during ongoing reactions, identification of the causes of assembly–disassembly processes in block-copolymers at the individual-block level, and pinpointing the impact of real-time changing physical parameters inside growing polymers on the catalytic chemical reactivity of monomer insertion and polymer growth. The impact of these microscopic behaviors on macroscopic material properties leads to opportunities for improvement in catalyst efficiency and in tailoring bulk polymer properties.

Early deviations in cell-to-cell communication in neurodegenerative disease trajectories

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Aberrant lipid metabolism is emerging as a central component, interfacing with all major facets of neurodegenerative diseases (NDs)[1]. These include pathological hallmarks of intracellular deposits of intrinsically disordered proteins (IDPs) and their high spatiotemporal pattern of progression through the brain[2,3]. It is unclear when and how protein propagation initiates and what defines cellular vulnerability to spread IDPs throughout the brain. In our group we question how the redistributed lipidome in Alzheimer's and Parkinson's disease brains affect IDP functions and influence their spreading kinetics both in neurons and in surrounding glia. We monitor changes in lipid metabolism and IDP functions to enable the discovery of common nodes and targetable pathways applicable for early intervention. We probe quantitative cell biology by single-molecule imaging technologies to detect and monitor the evolution of early-stage disease phenotypes. We combine systems level -omics approaches with functional calcium and metabolic imaging to elucidate how misregulation of cellular crosswalks within the brain insult neuronal function. Questions are addressed on different biological scales - from molecular studies to patient derived induced pluripotent stem cell 2D co-/cultures and 3D brain organoids, to map early events that initiate propagation trajectories and connect modulation of specific lipid metabolic pathways to IDP-induced proteinopathies.

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Heavier fluorophores with boosted brightness, lifetime and stability

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Chromophores are setting benchmarks in the life sciences to visualize biomolecules and to optically control biological function. Organic chemistry is at the forefront in designing, synthesizing and applying small molecule fluorophores for better image quality and higher sensitivity. We present the introduction of deuterium into fluorescent dyes to boost brightness, photostability and lifetime. With a color palette in hand from red to the near-infrared spectrum, we showcase super-resolution imaging and single molecule detection. Hand in hand with different tagging strategies, we aim to explore the localization and behaviour of cell surface proteins that play crucial roles in neuro-degenerative disease.

Beyond smFRET - NV qubit in nanodiamond for monitoring subunit rotation in single FoF1-ATP synthase

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Nitrogen-vacancy (NV) centers in nanodiamonds (10 to 100 nm diameter) can be applied as single fluorescent quantum sensors. The surface of nanodiamonds can be tailored for specific binding to biological targets. The extraordinary photo-physical properties such as very high photo-stability and non-blinking behaviour allow for optical detection of magnetic resonance of the NV triplet spin states [1] and nanoscale distance measurements. Taking advantage of the extended observation times using our version of a confocal anti-Brownian electrokinetic trap (ABEL trap [2]), we determined molecular brightness, spectral ratio, diffusion coefficient, surface charge and multiexponential fluorescence lifetimes for each nanodiamond one by one in solution [3].
The goal of our quantum-sensing project (with A. Krueger, J. Wrachtrup, F. Jelezko) is to exploit the spin properties of the luminescent NV- center to reveal the dynamics of biological systems. For 25 years we have studied subunit rotation of the membrane enzyme FoF1-ATP synthase in solution by intramolecular single-molecule FRET, with increased observation times for about a second in the ABELtrap [4]. Now, monitoring fluorescence lifetime changes of the NV- center due to the Zeeman effect of local magnetic fields enables us to record conformational changes of a diffusing single protein for tens of seconds.

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Elucidation of exciton dynamics in semiconductor nanocrystals using Fluorescence Lifetime Correlation Spectroscopy

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Fluorescence correlation spectroscopy has been used to investigate the photoprocesses in two kinds of nanocrystals, namely 3-mercaptopropionic acid (3-MPA) capped Cu(I)-doped CdS (Cu:CdS)[1] and Copper Indium Sulphide (CIS)[2] quantum dots (QDs). Photoactivation of the Cu:CdS QDs via dim/dark to bright particle conversion is observed at higher excitation powers. Dispersive blinking[1] kinetics in undoped QDs reflects the involvement of a broad distribution of trap states. A lesser extent of dispersity is observed for doped QDs, in which the hole-capture by the Cu-defect states predominates. Excitation fluence dependence of blinking rate highlights the role of Auger recombination in undoped QDs, which is suppressed significantly upon doping, due to disruption of electron-hole correlation. On the other hand, for the CIS QDs, an unusual excitation wavelength-dependence of photoactivation / photocorrosion is manifested in the increase in the initial correlation amplitude G(0) for λ_{ex}= 532 nm, but decrease for λ_{ex} = 405 nm. This has been rationalized in terms of different contributions from surface-assisted recombination in the two cases. Blinking times obtained from the Autocorrelation Functions (ACF) of the 100-200 ns lifetime component (core Cu-mediated recombination) are almost unaffected by shelling, but those from the ACF for the 10-30 ns lifetime (surface states) increases significantly. Absence of cross-correlation between the two recombinative states of bare CIS QDs and the emergence of an anticorrelation with the introduction of ZnS shell is observed, indicating the diffusive nature of the two states for CIS-ZnS. The diffusion is inhibited in base CIS QDs due to the preponderance of surface states. On a different note, blinking dynamics in FAPbBr3 perovskite nanocrystals have been analysed using Change Point Analysis (CPA), to throw light upon the involvement of a cluster of states.[3]

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Fluorescence correlation spectroscopy of Single wall carbon nanotubes

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Fluorescence Correlation Spectroscopy (FCS) is a non-invasive method that analyzes temporal fluctuations in fluorescence intensity and provides access to molecular information at the single molecule level [1]. Here, we implement near-infrared (NIR) FCS to understand the dynamics of single-walled carbon nanotubes (SWCNTs) based fluorescent biosensors [2]. We explore the intricate interactions between individual analyte molecules and DNA-functionalized SWCNTs, which act as model biosensors for biomolecules like the neurotransmitter dopamine. By tracking the diffusion of individual SWCNTs, we uncover new insights into molecular recognition. In this study, we employed FCS measurement using a modified confocal microscope capable of time correlated single photon counting (TCSPC) to determine cross-correlation functions of SWCNTs and their modulation in response to analyte molecules. The SWCNTs were excited at a wavelength of 480 nm and emission was detected in the near-infrared (NIR) region above 900 nm using single-photon avalanche detectors (SPAD). We present diffusion constants using power variation and illustrate their alteration due to molecular binding events. These findings not only advance our fundamental understanding of single-walled carbon nanotube (SWCNT) photophysics and their associated dynamics but also have profound implications for biophysics and materials science.

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Near infrared fluorescent nanosensors for biomedical imaging

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Near infrared (NIR, 800 nm -1700 nm) fluorescence imaging promises ultra-low background and scattering (tissue transparency window). We use nanomaterials such as NIR fluorescent single-walled carbon nanotubes (SWCNTs) as building blocks for advanced sensors/probes.
Here, I will present novel approaches to extract more information from their fluorescent signals and applications in bioimaging. Fluorescence lifetime imaging microscopy (FLIM) of SWCNT-based sensors is introduced as absolute and calibration-free NIR imaging method for biomolecules. This technique is enabled by laser scanning confocal microscopy (LSCM) optimized for NIR signals (>800 nm) and time correlated single photon counting (TCSPC). Moreover, the potential of spectral phasor approaches is discussed, and how it enables fast multispectral NIR imaging and multiplexing. Such sensors are shown to detect (bio)molecules over multiple length and time scales: From single proteins and single signaling molecules (neurotransmitters) released from networks of cells to molecular profiles of pathogens or stress (reactive oxygen species) in plants.[1-4]

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Comparison of real-time feedback-driven single-particle tracking techniques

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One of the main challenges in studying single biomolecules in a native or near-native environment is their diffusive motion. Real-time feedback-driven single-particle tracking (RT-FD-SPT) overcomes this limitation by using feedback control to keep a particle of interest in the detection volume. RT-FD-SPT offers a marked improvement in the 3D spatiotemporal resolution compared to image-based SPT and additionally enables an extended tracking time and region and the ability to perform concurrent spectroscopic measurements on the tracked molecules. Selection of the most appropriate RT-FD-SPT method for a particular application has thus far been hindered by a lack of objective, systematic comparisons. We have developed a theoretical approach, based on statistical calculations and dynamical simulations, to objectively compare three commonly used RT-FD-SPT methods, viz., the orbital, knight's tour, and MINFLUX methods [1]. We also compared the performance of two photon sources – fluorescence and interferometric scattering (iSCAT) – to labelled and autofluorescent biological samples. Our results indicate a fundamental trade-off between precision and speed [1,2]. Finally, we demonstrate our experimental implementation of RT-FD-SPT on aggregates of the main light-harvesting pigment-protein complex of plants to correlate their size, spectra, and fluorescence lifetimes, revealing new size–function relationships in the context of photoprotection.

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List of Poster Presentations

ordered by assigned poster number

Poster presentations

(in alphabetical order, as of October 01, 2024)

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Bodescu, Mihai Adrian	TUE	P3	Studying intrinsically disordered proteins from the endocytic uptake machineryby integrated NMR and single molecule FRET
Gimber, Niclas	TUE	P5	Simultaneous multicolor DNA-PAINT without sequential fluid exchange using spectral demixing
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Hummert, Johan	TUE	P9	Widefield and lightsheet lifetime imaging with a novel SPAD camera
Kühnemuth, Ralf	TUE	P11	An optofluidic antenna for enhancing the sensitivity of single-emitter measurements
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Brøchner, Bo Volf	WED	P4	Direct measurements of α-synuclein oligomers display pore-like activity modulated by lipid membrane charge and curvature
Gligonov, Ivan	WED	P6	Variational calculus approach to Zernike polynomials with application to fluorescence correlation spectroscopy.
Hanada, Erin M.	WED	P8	Fluorescence Lifetime Imaging Spectroscopy Reveals Potential Reaction Activation Method of Trimethylsilyl Chloride Additives in Zinc Oxidative Addition Reactions
Krüger, Tjaart	WED	P10	Multipurpose GUI-based software package for single-molecule spectroscopic data analysis
Lu, Siyu	WED	P12	Using supramolecular chemistry to establish functional dyes
Sadiq, Abdul Rahman	WED	P14	smFRET insights: How a small-molecule disrupts fungal ribozyme activity
Sisamakis, Evangelos	WED	P16	Adding the fluorescence lifetime dimension to Single-Molecule Localization Microscopy with the confocal microscope Luminosa
Sykora, Jan	WED	P18	Modified graphene-based surfaces for the preparation of the cushioned lipid bilayers and their utilization in the biomembrane research
Zundel, Fabian	WED	P20	New ways to study life at the nanoscale: the NEOtrap, DyeCycling, & more.

Abstracts:
Poster Presentations

ordered by assigned poster number

P1

Single-Molecule Spectroscopy of the Excited-State Proton Transfer

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Single-molecule spectroscopy applied to chemical reactions can reveal competing reaction pathways or micro-heterogeneities in the sample that remain hidden in ensemble studies.[1] Excited-state proton transfer (ESPT) is the only photochemical reaction which is compatible with fluorescence and therefore appears particularly suitable for single-molecule studies: Electronic excitation of a so-called photoacid leads to the release of a proton in the excited state and after emission, reprotonation takes place in the ground state.[2,3] This photocycle allows for studying one individual molecule and its proton transfer reaction repeatably. By embedding highly fluorescent and photostable photoacid molecules[4] in non-fluorescent solid phosphine oxide matrices, intermediates in the ESPT can be studied using total internal reflection fluorescence microscopy (TIRFM). Spectra of the single photoacid/phosphine oxide complexes can be recorded by adding a transmission grating in front of the CMOS-camera.[5] Deconvolution of the obtained single-molecule spectra then allows us to derive the population of the different intermediates[6] providing information about the heterogeneity of the surrounding polarity in a solid, polar, aprotic matrix.

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Water-sensing fluorophores targeting organelles in living cells

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Red-emitting fluorophores are widely used for imaging living systems as the red part of the spectrum is less photodamaging and better transmitted in biological samples. At the same time, these fluorophores are known to be quenched by surrounding water molecules [1]. Quenching by water reduces both the fluorescence quantum yield and lifetime of such dyes so that the dye‘s direct hydration can be assessed by monitoring changes in its lifetime [2]. In this work, we develop and use derivatives of a water-sensing red-emitting fluorophore specifically targeted to different organelles in living cells. We perform fluorescence lifetime imaging (FLIM) to visualize variations in the local hydration of the probe within different organelles. Characterizing the label‘s hydration properties opens perspectives for using these labels as water-sensing probes in functional imaging of cellular processes. Upcoming use of these probes in fluorescence lifetime single-molecule localization microscopy (FL-SMLM) [3] will enable the detection of local hydration heterogeneities at the nanoscale.

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P3

Studying intrinsically disordered proteins from the endocytic uptake machinery by integrated NMR and single molecule FRET

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Intrinsically disordered proteins (IDPs) lack clearly defined structure and are therefore highly flexible and easily adaptable to different binding partners. However, their dynamic features and broad range of interaction modes render them difficult to study and analyzing their complexes often requires integrated approaches. We have recently developed an approach to calculate conformational ensembles of IDPs using Förster resonance energy transfer (FRET) efficiencies acquired on the single molecule level together with NMR parameters (chemical shifts, paramagnetic relaxation enhancements - PREs). We have bench-marked this approach with a large set of in silico FRET efficiencies and NMR PREs, which we then validated with experimental FRET, fluorescence lifetime and NMR data [1].
We now apply this strategy to study the conformational landscape of intrinsically disordered proteins involved in the pathway of clathrin mediated endocytosis. The early phases of clathrin mediated endocytosis are organized through a highly complex interaction network mediated by clathrin associated sorting proteins (CLASPs) that comprise long intrinsically disordered regions (IDRs), which interact with various partners. Using single molecule FRET, we characterize the long-range inter- and intra-molecular interactions within this IDR-network and aim at integrating those data using NMR parameters with the goal to generate a molecular picture of endocytosis onset.

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P4

Direct measurements of α-synuclein oligomers display pore-like activity modulated by lipid membrane charge and curvature

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Parkinson‘s disease (PD) is a progressive neurodegenerative disorder marked by the loss of dopaminergic neurons and the accumulation of alpha-synuclein protein, forming toxic oligomers and fibrils. These cytotoxic alpha-synuclein oligomers (αSO) possess a pore-like structure, can disrupt cell membranes and is able to release small molecules, contributing to PD‘s cytotoxic effects [1].
To address the unclear mechanisms of αSO interactions with lipid membranes, we employed a novel single-liposome assay to model these interactions at the single vesicle level [2]. Using single-particle localization, we deconvoluted the contributions of charge and curvature in these interactions and their impact on the release of membrane-encapsulated small molecules. Our findings reveal that αSO membrane association depends on curvature and lipid composition, with density increasing exponentially with negative membrane charge.
We propose a two-step model for αSO membrane interactions: an initial membrane recruitment process followed by a charge-dependent reorientation process. Real-time imaging shows that oligomers fully incorporate into membranes, acting as pores and translocating small molecules. Understanding these interactions is crucial for preventing cellular dysfunction, potentially leading to new therapeutic approaches to stabilize αSO and avoid toxicity.

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P5

Simultaneous multicolor DNA-PAINT without sequential fluid exchange using spectral demixing

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Several variants of multicolor single-molecule localization microscopy (SMLM) have been developed to resolve the spatial relationship of nanoscale structures in biological samples. The oligonucleotide-based SMLM approach 'DNA-PAINT' robustly achieves nanometer localization precision and can be used to count binding sites within nanostructures. However, multicolor DNA-PAINT has primarily been realized by 'Exchange-PAINT' that requires sequential exchange of the imaging solution and thus leads to extended acquisition times. To alleviate the need for fluid exchange and to speed up the acquisition of current multichannel DNA-PAINT, we here present a novel approach that combines DNA-PAINT with simultaneous multicolor acquisition using spectral demixing (SD). By using newly designed probes and a novel multichannel registration procedure we achieve simultaneous multicolor SD-DNA-PAINT[1] with minimal cross-talk. We demonstrate high localization precision (3 – 6 nm) and multicolor registration of dual and triple-color SD-DNA-PAINT by resolving patterns on DNA origami nanostructures and cellular structures.

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P6

Variational calculus approach to Zernike polynomials with application to fluorescence correlation spectroscopy

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Zernike polynomials, introduced in 1934, are crucial in optics for modeling microscopy systems and describing wavefront aberrations. Despite their importance, their theoretical foundations are often overlooked. This research aims to develop a new derivation approach using variational calculus, establishing a connection between Zernike polynomials and Bessel functions.
The study applies these polynomials to analyze aberrations in fluorescence microscopy, focusing on their effects on Point Spread Function (PSF) shape and Fluorescence Correlation Spectroscopy (FCS) curves. Using electrodynamic principles, experiments were modeled with fluorophores as oscillating dipoles and microscopes as low-pass filters. MATLAB was used for numerical calculations.
Results reveal the impact of aberrations on estimated diffusion time, with a curious finding that two-photon excitation is more affected by aberrations than one-photon excitation. This challenges the common belief that two-photon techniques are inherently more precise.
This research contributes to a deeper understanding of optical system modeling and highlights the importance of considering aberrations in interpreting microscopy results, potentially influencing future experimental designs and data analysis in the field.

Fluorescence Intensity and time domain analysis by Time Correlated Multi-Photon Counting (TCMPC).

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Fluorescence behavior of new materials differ from traditional fluorochromes. Developed Time Correlated Multi-Photon Counting (TCMPC) system provides a powerful tool to study emission intensity, time response and dynamics at the individual quantum molecular response level.
The aim is to present experiments on photon spectroscopy, fluorescence intensity and lifetime decay using the TCMPC and Successive Molecular Decay (SMD) techniques. Fluorescence time domain experiments were conducted using fluorochromes and quantum dots. We analyzed the quantum dynamic response of samples after the analog-recording of the photons detected with our developed TCMPC system.
The photon counting rate of our TCMPC system ranges up to 1Gcps with minimized death time. Due its high counting rate, it is possible to capture data not only for the first detected photon but also for consecutive photons in the same laser excitation period. This allows to observe the dynamic behavior and quantum efficiency during the ground, excitation, stabilization and decay states of the samples.
Findings shown most of the organic fluorochromes exhibited shorter lifetimes and faster stabilization after excitation. However, quantum multidots showed longer lifetimes and delayed emission. Both TCMPC and SMD techniques provide simultaneous intensity and decay information, which could enhance analysis of excitation-electron interactions at molecular level.

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Fluorescence Lifetime Imaging Spectroscopy Reveals Potential Reaction Activation Method of Trimethylsilyl Chloride Additives in Zinc Oxidative Addition Reactions

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Trimethylsilyl chloride (TMSCl) is commonly used to “activate” metal(0) powders toward oxidative addition of organohalides, but knowledge of its mechanism remains limited by the inability to characterize chemical intermediates under reaction conditions. Here, fluorescence lifetime imaging microscopy (FLIM) overcomes these prior limitations and shows that TMSCl aids in solubilization of the organozinc intermediate from zinc(0) metal after oxidative addition, a previously unknown mechanistic role. This mechanistic role is in contrast to previously known roles for TMSCl before the oxidative addition step. To achieve this understanding, experiments develop FLIM, a tool traditionally used in biology, to characterize intermediates during a chemical reaction—revealing mechanistic steps that are unobservable without fluorescence lifetime data. These findings impact organometallic reagent synthesis and catalysis by providing a previously uncharacterized mechanistic role for a widely used activating agent, an understanding of which is suitable for revising activation models and for developing strategies to activate currently unreactive metals.

Widefield and lightsheet lifetime imaging with a novel SPAD camera

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Fluorescence lifetime imaging microscopy (FLIM) offers unique advantages, especially for imaging of living cells and organisms. Lifetime based sensors enable functional imaging of, for instance, pH, membrane tension or fluidity, or ion concentration. However, FLIM is typically implemented in confocal microscopy, limiting applicability to living samples due to high local laser power densities and slow frame rates. Faster and gentler imaging would be possible with widefield FLIM, but available FLIM cameras often impose other limitations.
Here we evaluate a novel 512x512 pixel SPAD camera in combination with PicoQuant pulsed lasers. We demonstrate widefield lifetime imaging of commercial cell samples at up to 30 fps. We further demonstrate lightsheet FLIM on a single objective lightsheet microscope for even gentler imaging. This approach enables 3D FLIM on live embryonic organoids including lifetime-based multiplexing, and time-lapse 3D FLIM of mechanosensitive tension probes. The selected applications highlight the potential of the novel hardware for FLIM at unprecedented speed and throughput, providing a powerful tool for functional imaging of dynamic multicellular systems.

P10

Multipurpose GUI-based software package for single-molecule spectroscopic data analysis

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We have developed Full SMS [1], an open-source, multipurpose graphical user interface (GUI)-based software package for analysing single-molecule spectroscopy (SMS) data. SMS typically delivers multiparameter data — such as fluorescence brightness, lifetime, and spectra — of molecular- or nanometre-scale particles such as single dye molecules, quantum dots, or fluorescently labelled biological macromolecules. Full SMS allows an unbiased statistical analysis of fluorescence brightness through level resolution and clustering, analysis of fluorescence lifetimes through decay fitting, as well as the calculation of second-order correlation functions and the display of fluorescence spectra and raster-scan images. Additional features include extensive data filtering options, a custom HDF5-based file format, and flexible data export options. The software is open source and written in Python but GUI-based so it may be used without any programming knowledge. A multi-process architecture was employed for computational efficiency. The software is also designed to be easily extendable to include additional import data types and analysis capabilities.

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P11

An optofluidic antenna for enhancing the sensitivity of single-emitter measurements

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Many single-molecule optical studies are performed in fluidic environments, e.g., to avoid unwanted consequences of contact with surfaces. The inherent diffusion of molecules in this arrangement limits the observation time and the number of collected photons, thus, compromising studies of processes with both fast and slow dynamics. Here, we introduce a planar optofluidic antenna (OFA), which enhances the fluorescence signal from molecules by up to a factor of 5 per passage, leads to about 7-fold more frequent returns to the observation volume, and significantly lengthens the diffusion time within one passage. We use single-molecule multi-parameter fluorescence detection (sm-MFD), fluorescence correlation spectroscopy (FCS) and Förster resonance energy transfer (FRET) measurements to characterize our OFAs. We then showcase the advantages of the antenna by examining both slow (ms) and fast (50 µs) dynamical behavior of Holliday junctions with real-time resolution. The ease of implementation and compatibility with various microscopy modalities make OFAs broadly applicable to a large variety of studies.

P12

Using supramolecular chemistry to establish functional dyes

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Many imaging techniques and biochemical assays rely on selective labeling of target structures in vitro and in vivo with commercial fluorophores that have specific yet invariant properties. Consequently, a fluorophore (or dye) is only useful for a limited range of applications, e.g., as a label for cellular compartments, super-resolution imaging, DNA sequencing or for a specific biomedical assay. Modifications of fluorophores with the goal to alter their bioconjugation chemistry, photophysical or functional properties typically require complex synthesis schemes. We recently introduced a general strategy that allows to customize these properties during the labelling process with the goal to introduce the fluorophore in the last step of bio-labeling[1] or to obtain multifunctional dyes via the same scaffold[2]. We here describe unpublished work on the use of supramolecular chemistry to transiently modify fluorophore properties. We characterize different pairs of commercially available hosts and dyes, frequently used for single-molecule detection and super-resolution microscopy[3-4], in light of their interaction, photophysical properties, biophysical and imaging applications.

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P13

Single molecule FRET analysis of the role of chromatin-associated protein LEDGF in nucleosome remodelling

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Transcription is controlled by epigenetic regulators that modify histones via deposition of epigenetic marks, which are in turn 'read' by epigenetic readers, leading to appropriate cellular responses. Lens epithelium-derived growth factor (LEDGF) is an essential epigenetic reader and a transcriptional co-activator. LEDGF is therapeutically important as a host cell cofactor in HIV-1 integration and a key factor in MLL (Mixed lineage leukemia) transformation. It forms complexes with HIV-1 Integrase and chimeric MLL/menin, respectively, via its C-terminal protein-binding domain. It then 'reads' the mark of active genes (di-/trimethylated H3K36) and tethers the complexes to active chromatin via its N-terminal PWWP domain. These two domains are linked by the central intrinsically disordered region (IDR). Due to its disorder and flexibility, the protein is difficult to study using traditional structural biology methods. Nucleosome core particle is a good fit for smFRET analyses as its dimensions correspond with FRET sensitivity range. Two independent smFRET studies presented here show that binding of LEDGF/p75, the longer LEDGF splice variant, lead to a looser, more unwound nucleosome, confirming the previously reported chromatin remodeller role of LEDGF/p75. Analyses performed validated smFRET as an excellent tool to study the LEDGF-nucleosome interaction, the details of which are still poorly understood.

P14

smFRET insights: How a small-molecule disrupts fungal ribozyme activity

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Human pathogenic fungi cause significant mortality, with current antifungals limited by their human-cell toxicity[1]. The self-splicing of group II introns in housekeeping genes is essential for pathogenic yeasts[2]. Targeting these ribozymes promises safer antifungals due to their absence in humans. Intronistat B, a small-molecule inhibitor, targets the active-site of group II introns, inhibiting their splicing[3]. While ribozyme-inhibitor crystal structures provide snapshots of inhibition, they fail to capture the inhibitor's impact on structural rearrangements during splicing[4]. To address this, we investigated the impact of Intronistat B on the folding and splicing of a group II intron Sc.ai5γ.
To track individual ribozymes in real-time, we fluorescently labeled them and used TIRF (Total Internal Reflection Fluorescence) and confocal smFRET (single-molecule Förster Resonance Energy Transfer) techniques. Interestingly, the result revealed stabilization of ribozyme structural conformations upon the addition of the inhibitor. It suggests the inhibitor halts the folding process by confining the ribozyme to certain structural intermediates critical for splicing. These findings shed light on the inhibitor-induced variation in the conformational dynamics of the ribozyme providing further details to the mechanism of Intronistat B action, aiding in rational development of splicing inhibitors. Additionally, the project validates smFRET techniques as powerful tools for probing RNA-inhibitor interactions, bridging single-molecule biophysics and drug development.

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P15

Polarized FL-SMLM for Sub-Nanometer Single Molecule Colocalization

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Precise biomolecular structure analysis is a cornerstone of modern biology, as these structures offer insights into the functions, interactions, and mechanisms of biomolecules. Over the last three decades, FRET has been widely adopted for this purpose. However, despite its vast utility, FRET studies require complex data analysis and calibrations and suffer from the generally unknown relative orientation of the fluorescent dyes used. Therefore, the development of angstrom-scale single molecule colocalization methods for non-invasive and precise inter- or intramolecular distance estimation emerges as a pivotal advancement in this quest. In the present work, we explore the potential of fluorescence lifetime single molecule localization microscopy (FL-SMLM) for single molecule colocalization applications, focusing on the inter- or intramolecular distance distributions in biomolecular structures with sub-nanometer spatial accuracy. Fluorescence lifetime adds another dimension to the fluorescence intensity and color acquired by conventional fluorescence microscopy, allowing multiplexing of fluorescent labels even with similar spectral properties. Combined with polarization filtering, this approach further eliminates the need for fluorescence blinking or chromatic correction for single molecule colocalization while achieving molecular-scale spatial resolution. This is particularly useful in single molecule colocalization under cryogenic conditions, opening new avenues for understanding the molecular structures of otherwise highly dynamic molecules.

P16

Adding the fluorescence lifetime dimension to Single-Molecule Localization Microscopy with the confocal microscope Luminosa

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Confocal fluorescence microscopy is an essential tool in many research disciplines, particularly in the life sciences. Its axial sectioning capability is particularly useful for imaging thick samples. Additionally, it can be upgraded to detect fluorescence lifetimes, which can facilitate e.g. multiplexing, environmental sensing, or FLIM-FRET imaging. However, all images have a limited spatial resolution due to diffraction.
Single-molecule localization microscopy (SMLM) approaches such as PAINT or STORM enable fluorescence imaging with spatial super-resolution. But they are usually implemented on camera-based widefield microscopes, which do not support photon counting-based lifetime imaging.
The aim of this research was to add fluorescence lifetime contrast to super-resolution imaging and provide confocal sectioning ability. The fluorescence lifetime information should be exploited either for multiplexing, complementary to spectral approaches, or for providing enhanced axial resolution via MIET-SMLM, or for FRET imaging.
To this end, DNA-PAINT and dSTORM image acquisition were implemented on our time-resolved confocal microscope Luminosa.
For PAINT, the acquisition time per image frame needed to match the binding kinetics of the DNA-PAINT imager strands, such that it was significantly smaller than the average binding time. For dSTORM, the acquisition time should be tuned according to the blinking kinetics of the fluorophore.
Furthermore, an autofocus proved necessary for the lengthy acquisition of a sufficient number of frames, and stable temperature to minimize lateral drift.
The resulting confocal FLIM movies were analyzed similarly as for SMLM measurements to obtain a final super-resolved image. Lastly, the photon arrival times of each single-molecule event were retrieved and analyzed to get the lifetime contrast.
For 3D super-rsolution imaging with MIET-PAINT, the lifetime values were converted to axial positions using the MIET lifetime-distance dependency calculated based on the particular fluorophore properties. For FRET-PAINT, the lifetime was used to detect FRET events.
In conclusion, we demonstrated super-resolved FLIM with PAINT and dSTORM, MIET-PAINT to enable 3D super-resolution as well as super-resolved FRET-PAINT. Importantly, these cutting-edge imaging approaches were realized on a commercially available microscope. Luminosa's combination of stability, imaging speed, sensitivity and precision in lifetime determination facilitates the easy adaptation of state-of-the-art super-resolution imaging methodologies by other research groups.

Small SPAD Arrays for Confocal Fluorescence Lifetime Imaging

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Confocal microscopy is an essential tool in many academic disciplines due to its intrinsic sectioning capability. It combines naturally with time-resolved single photon detection and time-correlated single photon counting (TCSPC) approaches. This has established it as the leading platform for time-resolved investigation methods such as fluorescence lifetime imaging (FLIM) and fluorescence correlation spectroscopy (FCS). Recently, high-performance SPAD-arrays featuring few tens of pixels have become available. Combining these with suitable multi-channel TCSPC devices opens up new possibilities in confocal time-resolved sensing.
In this work we present the two central hardware building blocks which are incorporated into PicoQuant`s new add-on for our confocal microscope Luminosa: a multi-channel TCSPC device and a cooled high-performance 23-pixel SPAD-array developed jointly with Pi Imaging Technologies. We show how the combination of these devices can bring super-resolution imaging modalities such as image scanning microscopy (ISM) to the realm of lifetime imaging. The main benefit of ISM is an increase in SNR as well as lateral and axial resolution, a gain that is fully compatible with lifetime information for species separation. We discuss how advanced data processing can be applied to FLIM-ISM for additional performance gains.
We see the application of small arrays to super-resolution imaging as just one example how this technology can shape the future of confocal time-resolved microscopy. Many more applications are and will potentially be discovered in the coming years as the hardware becomes more readily available.

P18

Modified graphene-based surfaces for the preparation of the cushioned lipid bilayers and their utilization in the biomembrane research

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Classical fluorescence microscopy is a widely used technique in biological research. However, it inherently suffers from limited spatial resolution, with lateral resolution in the order of hundreds of nanometers and axial resolution of several microns. Recent advancements in novel techniques have achieved nanometric resolution in the lateral dimension. Despite this progress, there remains a high demand for robust methods to achieve nanometer-scale axial resolution. One of the most promising approaches to address this need is Graphene Induced Energy Transfer (GIET).1 GIET relies on the distance-dependent quenching of fluorescence by an atomically thin graphene sheet. Since GIET can operate up to approximately 30 nm, it allows for detailed resolution of lipid bilayers, which are about 5 nm thick.
In this study, we aim to fabricate novel graphene-based supports suitable for GIET. Specifically, graphene deposited on a glass support is cushioned with Pyrene-PEG polymer. Pyrene promotes interaction with graphene, while PEG minimizes the effect of graphene on bilayer behavior. Our experiments focused on forming supported lipid bilayers (SLBs) containing negatively charged lipids on these substrates. The properties of the SLBs were monitored using time-resolved fluorescence microscopy and fluorescence correlation spectroscopy (FCS).

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P19

Direct interfacing of single organic molecules with an optical nanofiber

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In quantum technologies, organic molecules have emerged as promising candidates for solid state-based quantum emitters. Owing to their wide range of emission wavelengths, photostability, defined transition dipole moments, and strong zero-phonon lines, organic molecules can be used as bright and versatile single photon sources. Single-photon characteristics have been observed for different molecules, such as dibenzoterrylene (DBT), even at room temperature [1].
For the integration of these photon sources into quantum photonic devices, efficient optical interfaces are crucial. A considerable overlap of the molecule's emission pattern with a propagating light field can be achieved using optical nanofibers. These subwavelength-diameter waveguides provide a pronounced evanescent field component of the guided light [2]. We fabricate self-assembled organic nanocrystals (NCs) of anthracene doped with single DBT molecules (DBT:Ac) or a cluster of DBT. The DBT:Ac NCs are then suspended in polyvinyl alcohol (PVA) for protection against oxidation and spin-coated onto a magnesium fluoride (MgF2) substrate. A home-built epi-fluorescence scanning confocal microscope is used to determine the position of the NCs on the substrate and to optically characterize their fluorescence signal. A nanofiber mounted on a nanopositioning stage can then be placed with respect to the NCs. In this configuration, we can excite and collect the fluorescence emission either through the nanofiber or through the microscope. This allows decoupling of the excitation and detection path or a fully fiber-based approach without additional optical access. Our approach provides a robust and versatile platform for a range of photonic quantum applications [3].

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New ways to study life at the nanoscale: the NEOtrap, DyeCycling, & more.

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Proteins are the molecular makers in our body. Researchers successfully identified a vast proteome, a dense web of metabolic interactions, and many thousands of static 3D structures. But the essential dynamic processes causing protein function are still challenging to detect – yet they are the key to the energetics controlling life at the nanoscale. In our group we focus on acquiring time-resolved information to reveal the nano-dynamics of biomolecular systems, such as cancer-assisting chaperone and kinase proteins, and CRISPR-associated proteins. To approach this matter, we develop new electrical and optical single-molecule methods based on nanopores [1,2] and FRET [3,4,5]. The NEOtrap [2] can trap and electrically sense single proteins using nanopores, and with DyeCycling [5], we aim to break the photobleaching limit of smFRET. Most recently, we reported the label-free counting and identification of single secondary messenger molecules produced by type III CRISPR/Cas, viz. cyclic oligo-adenylates [1]. They allosterically activate downstream CARF proteins which act as non-specific ribonucleases in prokaryotic immune systems causing collateral damage by attacking invading but also host RNA [6]. We aim to elucidate this potentially deadly function using smFRET, to directly observe how the underlying conformational dynamics regulate this process allosterically.

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