30th International Workshop on „Single Molecule Spectroscopy and Super-resolution Microscopy“

www.picoquant.com

30th anniversary workshop!

Berlin

Sept 23 – 26 2025

Program and Abstract Book

▶ hosted by PicoQuant

Media Partners

METHODS IN MICROSCOPY

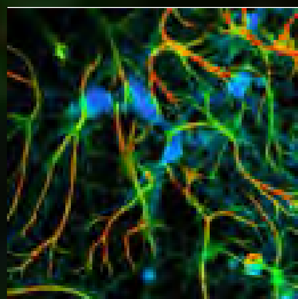
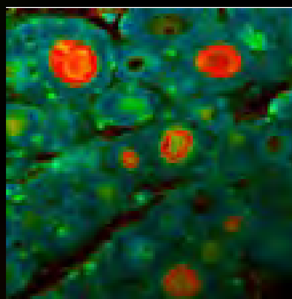
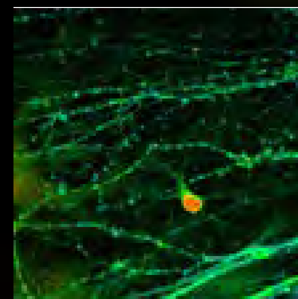
Microscopy and Analysis

PicoQuant

Explore new paths
in confocal microscopy with

LUMINOSA

Remarkably simple. Precisely yours.

Don’t miss the
Luminosa
Demo Sessions

picoquant.com/luminosa

Contents

Program ……………………………………………………………………………5

Abstracts: Oral Presentations ……………………………………………………14

List of Poster Presentations ………………………………………………………56

Abstracts: Poster Presentations ……………………………………………………66

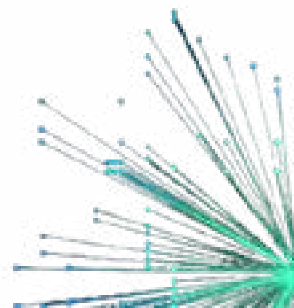
Organized by

PicoQuant GmbH
Rudower Chaussee 29 (IGZ)
12489 Berlin, Germany

Phone: +49-30-1208820-87
workshop@picoquant.com
www.single-molecules.org
www.picoquant.com

Copyright of this document belongs to PicoQuant. No parts of it may be reproduced, translated or transferred to third parties without written permission of PicoQuant.

© PicoQuant, 2025



Welcome to the 30th International Workshop on Single Molecule Spectroscopy and Super-resolution Microscopy!

We are delighted to welcome you to Berlin for this very special anniversary edition of our workshop. For three decades, this event has brought together scientists from all over the world to share insights, exchange ideas, and foster collaborations in the fields of single molecule detection, advanced microscopy, and fluorescence spectroscopy.

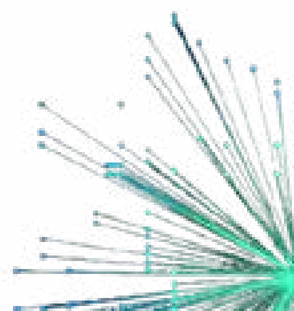
Although Joerg Enderlein and I were very excited about the advances in single-molecule research 30 years ago, none of us could have imagined the fantastic path that this once very small community would take. Thanks to your participation, what was once a one-off insider workshop has developed into one of the world‘s most important conferences.

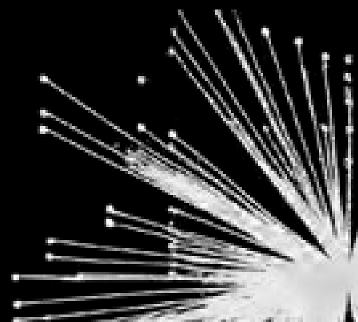
This year’s edition not only marks a milestone in the history of this workshop but also celebrates the ever-evolving scientific progress we have witnessed together. Alongside a program filled with inspiring talks, engaging poster sessions, and opportunities for in-depth discussions with fellow researchers and innovators, we are especially excited to continue our tradition of recognizing young talent through the Student Awards.

We thank all our participants, speakers, and sponsors for being part of this journey. Your curiosity, creativity, and dedication to science make this workshop what it is.

We look forward to a stimulating, enriching, and memorable week together!

Sincerely, yours Rainer Erdmann





Program

as of September 15, 2025

Tuesday, September 23

08.00–09.00	**REGISTRATION**
09.00–09.25	***Rainer Erdmann****, Berlin, Germany* **Opening Remarks**

SESSION 1: SENSOR & LABEL

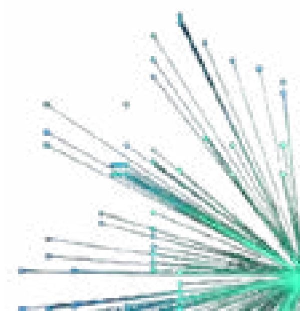
Chair: Jörg Enderlein

09.25–09.50	***Thorben Cordes***, Dortmund, Germany **(Invited Talk)** From accurate FRET studies in proteins to systematic assay design
09.50–10.15	***Mike Heilemann***, Frankfurt am Main, Germany **(Invited Talk)** Imaging membrane receptor biology with single-molecule resolution
10.15–10.30	***Patrick Schüler***, München, Germany **(Student Award)** Beyond strand displacement reactions: DNA computing on the single molecule level
10.30–10.45	***Soohyen Jang***, Frankfurt am Main, Germany **(Student Award)** Quantitative PAINT microscopy of membrane proteins with self-labeling protein tags
10.45–11.20	**COFFEE BREAK & EXHIBITION**

SESSION 2: SINGLE MOLECULE METHODS I

Chair: Petra Schwille

11.20–11.45	***Xiaoliang Sunney Xie,*** Beijing, China **(Invited Talk)** From Single Molecules to Single Cells; From Biology to Medicine
11.45–12.10	***Madhavi Krishnan,*** Oxford, United Kingdom **(Invited Talk)** Measurements of molecular size and shape on a chip
12.10–12.25	***Alexandre Fürstenberg***, Geneva, Switzerland Environment-sensitive fluorescence lifetime probes for single-molecule and super-resolution imaging
12.25–12.40	***Yuval Ebenstein***, Tel Aviv, Israel A Spectral Image Scanning Microscope for Multi-Color Super-Resolution Imaging
12.40–13.00	Flashtalk Session I
13.00–14.15	**LUNCH BREAK**



Tuesday, September 23

SESSION 3: SUPERRESOLUTION I

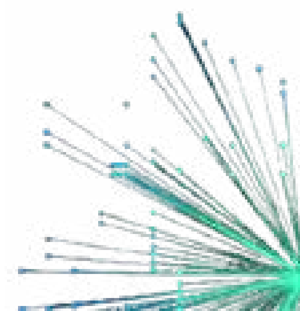
Chair: Mike Heilemann

14.15–14.20	***Rainer Erdmann,*** Berlin, Germany Introduction to Keynote Talk
14.20–14.55	***Stefan W. Hell, Göttingen, Germany*** **(Invited Talk)** Molecule-scale resolution in dynamics and fluorescence microscopy
14.55–15.20	***Aleksandra Radenovic,*** Lausanne, Switzerland **(Invited Talk)** Advancing Single-Molecule Imaging
15.20–15.35	***Rick Seifert,*** Würzburg, Germany **(Student Award)** Correlative confocal and super-resolution imaging of the immunological CAR-T cell synapse
15.35–15.50	***Francisco Matos,*** Orsay, France **(Student Award)** TimeLoc: Integrating Dynamic Excitation and SPAD units for Camera-Free Frequency-Encoded Super-Resolution Imaging and Tracking
15.50–16.10	Flashtalk Session II
16.10–16.40	**COFFEE BREAK & EXHIBITION**

SESSION 4: BIOLOGICAL APPLICATIONS

Chair: Aleksandra Radenovic

16.40–17.05	***Petra Schwille,*** Martinsried, Germany **(Invited Talk)** Understanding biology by building it? The exciting world of synthetic cells
17.05–17.30	***Verena Ruprecht***, Innsbruck, Austria **(Invited Talk)** Mechano-signalling as a regulator of cell behaviour
17.30–17.45	***Abhilash Kulkarni***, Stockholm, Sweden **(Student Award)** Time-gated detection of NIR luminescent nanoparticles in organs using snSPDs
17.45–18.00	***Tom Kache,*** Diepenbeek, Belgium **(Student Award)** Unraveling DNA Ligase Dynamics via Multiparameter Photon-by-Photon smFRET and H2MM Analysis
18.00–18.15	***Marie Reischke,*** Erlangen, Germany **(Student Award)** Chip-based iSCAT microscopy under evanescent illumination
18.15–18.20	**VOTING STUDENT AWARD**
18.20–19.50	**POSTER SESSION I & GET TOGETHER**



Wednesday, September 24

SESSION 5: CORRELATION SPECTROSCOPY & SUPERRESOLUTION MICROSCOPY SESSION

Chair: Paul French

09.00–09.25	***Jörg Enderlein,*** Göttingen, Germany **(Invited Talk)** A journey through 30 years of Single Molecule Science
09.25–09.50	***Paul Wiseman,*** Montréal, Canada **(Invited Talk)** Pollen Tube Growth Dynamics Quantified via Volumetric Spatio-Temporal Image Correlation Spectroscopy
09.50–10.05	***Anders Barth,*** Berlin, Germany Expanding the Horizon of FCS with SPAD Arrays: A Promising Outlook for New Applications
10.05–10.20	***Dominic A. Helmerich, Würzburg, Germany*** Unveiling the invisible: A novel approach illuminates the sub-10 nm cosmos
10.20–10.35	***Tao Chen,*** Göttingen, Germany Measuring membrane and membrane protein structure and dynamics with dynamic metal- and graphene-induced energy transfer spectroscopy (dynaMIET/dynaGIET)
10.35–10.45	**GROUP PICTURE**
10.45–11.20	**COFFEE BREAK & EXHIBITION**

SESSION 6: SUPERRESOLUTION II

Chair: Michel Orrit

11.20–11.25	***Rainer Erdmann,*** Berlin, Germany Introduction to Keynote Talk
11.25–12.00	***W.E. Moerner,*** Stanford, United States **(Invited Talk)** A Brief Survey of Single-Molecule Optical Microscopy: From Early Spectroscopy in Solids, to Super-Resolution Nanoscopy in Cells, to a Wealth of New Applications
12.00–12.25	***Jörg Enderlein,*** Göttingen, Germany **(Invited Talk)** Advancing Super-Resolution Imaging: Integrating Fluorescence Lifetime, Scanning Microscopy, and Energy Transfer Techniques for Isotropic Nanoscale Bioimaging
12.25–12.50	***Guillermo Acuna,*** Fribourg, Switzerland **(Invited Talk)** Direct single-molecule detection and super-resolution imaging with a low-cost portable smartphone-based microscope
12.50–13.05	***Christian Franke,*** Jena, Germany Nanotexture – a universal approach of AI-based computational multiplexing and phenotyping of super-resolution data

Wednesday, September 24

SESSION 7: INSTRUMENTATION & SOFTWARE Chair: Jörg Enderlein

13.05–13.20 ***Steffen J. Sahl,*** Göttingen, Germany
Ångström-level, intra-molecular MINFLUX analyses of protein conformation and chemical architecture

13.20–14.35 **LUNCH BREAK**

SESSION 7: INSTRUMENTATION & SOFTWARE Chair: Jörg Enderlein

14.35–15.00 ***Michel Orrit,*** Leiden, Netherlands **(Invited Talk)**
Looking back at 35 years of single-molecule optics

15.00– 15.25 ***Vahid Sandoghdar,*** Erlangen, Germany **(Invited Talk)**
Coherent scattering of light by single molecules

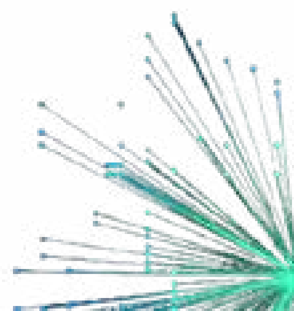
15.25–15.50 ***Paul French,*** London, United Kingdom **(Invited Talk)**
OpenScopes: an open, modular platform to widen access and capabilities in microscopy and high content analysis

15.50–16.05 ***Kunihiko Ishii,*** Wako, Japan
Independent component analysis disentangles fluorescence signals from diffusing single molecules

16.05–16.20 ***Andriy Chmyrov,*** Heidelberg, Germany
Imaging Beyond The Visible: advantages of the Shortwave-Infrared spectral range for confocal microscopy and Raman scattering imaging

16.20–16.35 ***Markus Lippitz***, Bayreuth, Germany
Fluorescence-detected two-dimensional electronic spectroscopy of a single molecule

16.35–23.00 **SOCIAL PROGRAM & DINNER**



Thursday, September 25

SESSION 8: BIOLOGICAL APPLICATIONS II

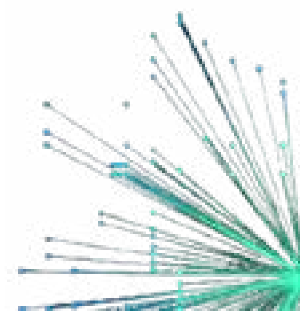
Chair: Claus Seidel

09.00–09.25	***Christian Eggeling,*** Jena, Germany **(Invited Talk)** Advancing super-resolution and single-molecule microscopy for studying molecular interactions in membranes
09.25–09.50	***Jerker Widengren,*** Stockholm, Sweden **(Invited Talk)** Fluorophore blinking in superresolution microscopy, and as a rich source of moleular-scale information
09.50–10.05	***Julius Trautmann,*** Jena, Germany **(Student Award)** Adaptive Optics for Aberration Control in STED and (STED)-FCS: Advancing High-Resolution Single-Molecule Studies
10.05-10.20	***Stijn Dilissen,*** Diepenbeek, Belgium **(Student Award)** Dynamic Burst smFRET in Slow Motion: A Microfluidic Approach for Probing Biocondensates and Liposomes
10.20–10.35	***Robert B. Quast,*** Montpellier, France Dissecting the GPCR conformational landscape using biorthogonal click chemistries and multicolor single molecule FRET
10.35–11.10	**COFFEE BREAK & EXHIBITION**

SESSION 9: FLIM, FRET & FCS I

Chair: Viktorija Glembockyte

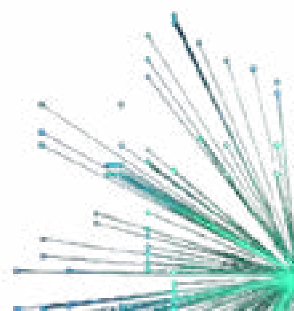
11.10–11.35	***Jessica P. Houston,*** Las Cruces, United States **(Invited Talk)** Applications of fluorescence lifetime measurements in flow cytometry
11.35–12.00	***Claus Seidel,*** Düsseldorf, Germany **(Invited Talk)** FRET nanoscopy maps molecules of life
12.00–12.15	***Léa Brito,*** Orsay, France **(Student Award)** Instant FLIM in SMLM via SPAD Array Imaging
12.15–12.30	***Philipp Gebauer, Konstanz,*** Germany **(Student Award)** Investigating Spectral Fluctuations in the Emission of Halide-Perovskite Nanoparticles using Heralded Spectroscopy
12.30–12.45	**Chi-Jui Feng, Bethesda, United States** Characterizing Barrier Crossing Dynamics of Protein Folding Through Transition Paths Using Single-molecule FRET in Zero-mode Waveguides
12.45–13.05	Flashtalk Session III
13.05–14.20	**LUNCH BREAK**



Thursday, September 25

SESSION 10: FLIM, FRET & FCS II Chair: Jessica Houston

14.20–14.45	***Viktorija Glembockyte,*** Heidelberg, Germany **(Invited Talk)** Leveraging DNA Nanotechnology for Single-Molecule Optical Sensing
14.45–15.10	***Ben Schuler,*** Zurich, Switzerland ***(Invited Talk)*** Probing rapid biomolecular dynamics with single-molecule spectroscopy
15.10–15.25	***Jakob Hartmann,*** München, Germany **(Student Award)** Investigation of DNA and DNA-Protein-Interaction on the Nanometer Scale using Graphene Energy Transfer
15.25–15.40	***Vy Pham,*** Irvine, United States **(Student Award)** Pinpointing Polymer–Active-Catalyst Speciation in Solution
15.40–15.55	***Noah Salama,*** *Düsseldorf, Germany* **(Student Award)** Performing and Analyzing FRET Nanoscopy Measurements on DNA-Origami Platforms with sub-Nanometer Precision
15.55–16.15	Flashtalk Session IV
16.15–16.20	**VOTING STUDENT AWARD**
16.20–16.35	***Thorsten Hugel,*** Freiburg, Germany Single-Molecule FRET in Living Cells
16.35–16.50	***Nicola Galvanetto,*** Zurich, Switzerland Material properties of biomolecular condensates emerge from nanoscale dynamics
16.50–17.05	**Eitan Lerner,** Jerusalem, Israel Single-cell time-resolved multiparameter fluorescence spectroscopy of phytoplankton
17.05–18.35	**POSTER SESSION II & GET TOGETHER**



Friday, September 26

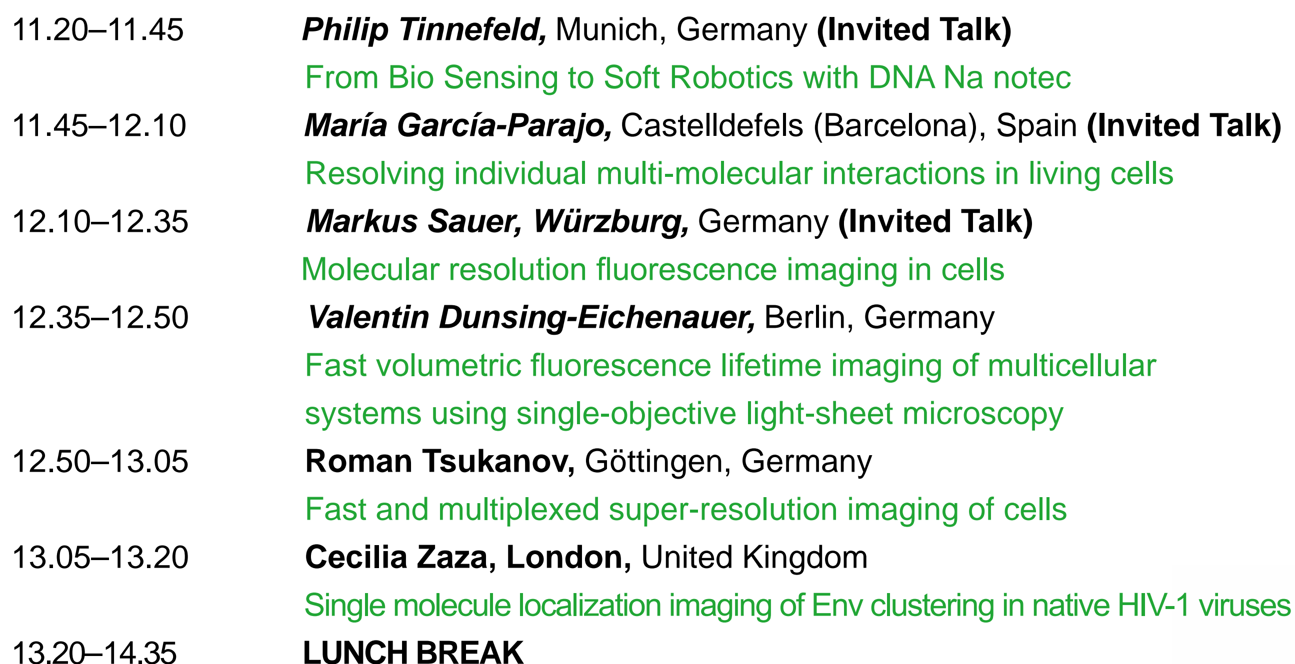
SESSION 11: MOLECULAR SIZING/TRAPPING & SUPERRESOLUTION MICROSCOPY

Chair: Philip Tinnefeld

09.00–09.25	***Allison Squires,*** Chicago, United States **(Invited Talk)** Pulsed Interleaved Excitation for enhanced FRET sensing in an Anti-Brownian ELectrokinetic (ABEL) Trap: ABEL-PIE
09.25–09.50	***Sobhan Sen,*** New Delhi, India **(Invited Talk)** Ligand-Binding Kinetics to G-Quadruplex DNA: Insights from FCS and Molecular Simulations
09.50–10.05	***Jan C. Behrends,*** Freiburg, Germany Simultaneous high-resolution fluorescence and voltage clamp measurements on free-standing membranes on a chip
10.05–10.20	***Eli Slenders,*** Genoa, Italy SPAD array detector enables a large localization range in MINFLUX
10.20–10.35	***Abhishek Sau,*** College Station, United States Uncovering Shared Routes of Nuclear Import and Export Using Dual-Color MINFLUX
10.35–10.45	**STUDENT AWARD CEREMONY**
10.45–11.20	**COFFEE BREAK & EXHIBITION**

SESSION 12: BIOLOGICAL APPLICATIONS II

Chair: Allison Squires

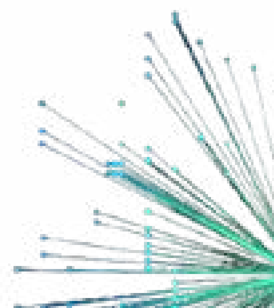


11.20–11.45	***Philip Tinnefeld,*** Munich, Germany **(Invited Talk)** From Bio Sensing to Soft Robotics with DNA Na notec
11.45–12.10	***María García-Parajo,*** Castelldefels (Barcelona), Spain **(Invited Talk)** Resolving individual multi-molecular interactions in living cells
12.10–12.35	***Markus Sauer, Würzburg,*** Germany **(Invited Talk)** Molecular resolution fluorescence imaging in cells
12.35–12.50	***Valentin Dunsing-Eichenauer,*** Berlin, Germany Fast volumetric fluorescence lifetime imaging of multicellular systems using single-objective light-sheet microscopy
12.50–13.05	**Roman Tsukanov,** Göttingen, Germany Fast and multiplexed super-resolution imaging of cells
13.05–13.20	**Cecilia Zaza, London,** United Kingdom Single molecule localization imaging of Env clustering in native HIV-1 viruses
13.20–14.35	**LUNCH BREAK**

Friday, September 26

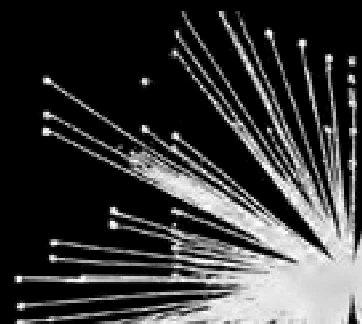
SESSION 13: FRET & SUPERRESOLUTION SESSION Chair: Markus Sauer

14.35-15.00	***Taekjip Ha, Boston,*** United States **(Invited Talk)** Single molecule tracking of mismatch repair in vivo and in vitro
15.00–15.25	***Don C. Lamb,*** München, Germany **(Invited Talk)** PIE and CAKE: How Sweet!;)
15.25–15.50	***Johan Hofkens,*** Leuven, Belgium **(Invited Talk)** From Single Molecule Insights to Real-World Impact: Optical Mapping for Life Sciences
15.50–16.05	***Mikayel AZNAURYAN,*** Pessac, France Molecular behavior of disordered translation factor eIF4B: from monomers to oligomers and condensates
16.05–16.20	**Y*uhan Wang,*** Zurich, Switzerland Single-Molecule Sensors for Mapping Crowding and Ionic Strength in Live Cells
16.20–16.35	**Sandrine LEVEQUE-FORT**, ORSAY, France Brightness demixing for simultaneous multi-target imaging in 3D single-molecule localization microscopy
16.35–16.50	**Hisham Mazal,** Erlangen, Germany Ångström Super-resolution in Structural Biology: Cryogenic Light Microscopy of Proteins in Their Native Environment
16.50–17.00	Concluding Remarks
17.00	**END OF WORKSHOP**



Abstracts:
Oral Presentations

according to schedule



From accurate FRET studies in proteins to systematic assay design

Thorben Cordes

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

Single-molecule FRET (smFRET) has emerged as a powerful tool for studying biomolecular structure and dynamics at the nanoscale. Recent research has focused on improving its reliability and practical applications. Through international blind studies, our community established high measurement precision of quantitative interprobe distances with ≤0.2 nm precision and ≤0.5 nm accuracy [1-2]. While this provided confidence in the use of smFRET for both mechanistic biochemical studies and structural biology, selecting optimal labeling positions for fluorescent dyes remains challenging, particularly in proteins. Empirical guidelines exist for identifying fluorophore labeling sites in proteins, yet, there is no systematic way to predict these site until now. Through literature screening and bioinformatics analysis, we have identified four key parameters that can be combined into a label score system to quantitatively rank residues based on their suitability for fluorophore labelling[3]. We show the predictive power of the score with literature data and new experiments. Available both as a Python script and through a public webserver (https://labelizer.bio.lmu.de/), the Labelizer analyzes protein structures and structural models to predict optimal labeling sites, significantly improving experimental design success rates.

[1] Hellenkamp et al., Nature Methods 15 (2018) 669-676
[3] Agam et al., Nature Methods 20 (2023) 523-535[3] Gebhardt et al., Nature Communications in press (2025): https://www.biorxiv.org/content/10.1101/2023.06.12.544586.abstract

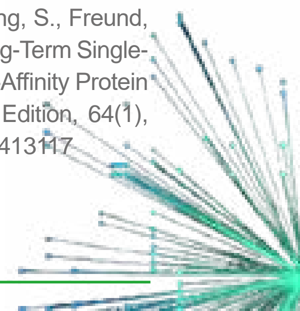
Imaging membrane receptor biology with single-molecule resolution

Mike Heilemann

Institute of Physical and Theoretical Chemistry, Goethe-Universität Frankfurt, Germany

Membrane receptors convert extracellular signals into intracellular responses through highly regulated structural and biochemical mechanisms. Achieving a detailed structural and mechanistic understanding of these signaling processes necessitates observations in living cells. Due to the inherent heterogeneity and lack of synchronization within biological systems, single-molecule experiments are essential.We employ various single-molecule imaging modalities – including quantitative single-molecule localization microscopy (SMLM), single-molecule FRET (smFRET) and single-particle tracking (SPT) – to systematically investigate three key aspects of membrane receptor activation: (i) the molecular stoichiometry and supramolecular assembly patterns of receptor complexes, (ii) the association and dissociation kinetics of receptor complexes, and (iii) the lateral diffusion dynamics of single receptors as a proxy for the microenvironment and activity states. Using these tools and integrating molecular dynamics simulations, we determined the conformation of the receptor-ligand complex (MET:InlB)2 in situ [1] and its dissociation kinetics and lateral mobility in living cells [1, 2]. Furthermore, we established long-term single-particle tracking experiments in living cells and measured ligand-specific activation dynamics of membrane receptor tyrosine kinases [3,4].

[1] Li, Y., Dietz, M. S., Barth, H. D., Niemann, H. H., Heilemann, M. (2025). Single-molecule FRET-tracking of InlB-activated MET receptors in living cells. bioRxiv, 2025.05.06.652421. https://www.biorxiv.org/content/10.1101/2025.05.06.652421v1
[2] Li, Y., Arghittu, S. M., Dietz, M. S., Hella, G. J., Haße, D., Ferraris, D. M., Freund, P., Barth, H. D., Iamele, L., de Jonge, H., Niemann, H. H., Covino, R., & Heilemann, M. (2024). Single-molecule imaging and molecular dynamics simulations reveal early activation of the MET receptor in cells. Nature Communications, 15(1), 9486. https://doi.org/10.1038/s41467-024-53772-7
[3] Catapano, C., Rahm, J. V., Omer, M., Teodori, L., Kjems, J., Dietz, M. S., & Heilemann, M. (2023). Biased activation of the receptor tyrosine kinase HER2. Cellular and molecular life sciences: CMLS, 80(6), 158. https://doi.org/10.1007/s00018-023-04806-8
[4] Catapano, C., Dietz, M. S., Kompa, J., Jang, S., Freund, P., Johnsson, K., & Heilemann, M. (2025). Long-Term Single-Molecule Tracking in Living Cells using Weak-Affinity Protein Labeling. Angewandte Chemie International Edition, 64(1), e202413117. https://doi.org/10.1002/anie.202413117



Beyond strand displacement reactions: DNA computing on the single molecule level

Patrick Schüler, Tim Schröder, Julian Bauer, Philip Tinnefeld

Ludwig-Maximilians-University Munich, Department of Chemistry , Butenandtstr. 5 – 13 (Gerhard-Ertl-Building), D–81377 Munich

DNA computing is an emerging field offering paralleled operation in unconventional media for applications beyond silicon-based computing.[1] Current DNA computing approaches utilize the concept of toehold-mediated strand displacement reactions to create integrated circuits and perform Boolean logic operations. Often these approaches rely on multiple orthogonal diffusive DNA strands, setting limits to sequence space and prolong computation time due to diffusion limited reactions.[2] Recently spatially localized architectures based on the DNA Origami technique could increase calculation time and signal propagation but still require diffusive “fuel” strands for every computational step and was only used in ensemble experiments.[3]

We present a novel DNA computing approach, free of strand displacement reactions. Our confined molecular processing unit (MPU) can perform all essential one- and two-input logic operations. We characterize the MPU performance on the single molecule level as well as read out the calculations of the computation. The single molecule fluorescence readout additionally offers multi-valued logic as readout offering a broad multiplexing potential. Additionally, our MPU is not limited to DNA-inputs but can also be combined with Antibody-Antigen and Protein-Aptamer signal-input for a broad application potential.

[1] M. Adleman, Science 266, 1021-1024 (1994).
[2] G. Seelig, D. Soloveichik, D. Y. Zhang, E. Winfree, Science 314, 1585-1588 (2006).
[3] G. Chatterjee, N. Dalchau, R. A. Muscat, A. Phillips, G. Seelig, Nat Nanotechnol 12, 920-927 (2017).

Quantitative PAINT microscopy of membrane proteins with self-labeling protein tags

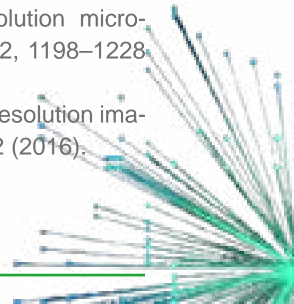
Soohyen Jang[1,2], Julian kompa[3], Claudia Catapano[1], Marina S. Dietz[1], Kai Johnsson[3], Mike Heilemann[1,2]

[1]Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany
[2]Institute of Physical and Theoretical Chemistry, IMPRS on Cellular Biophysics, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany
[3]Department of Chemical Biology, Max Planck Institute for Medical Research, Jahnstr. 29, 69120 Heidelberg, Germany

Single-molecule localization microscopy achieves near-molecular resolution by separating the fluorophores in time and space.1 Point accumulation in nanoscale topography (PAINT) separates the fluorescence signal by employing transient binding of fluorophore labels to the target structure1,2. In DNA-PAINT, short DNA oligonucleotides are used to increase the specificity and the number of labeling3 PAINT is free from photobleaching because the fluorophores are continuously supplemented from the imaging buffer.

Next to providing super-resolved images of biomolecules, quantitative PAINT (qPAINT) extracts molecule numbers in tightly packed nano-clusters from the binding kinetics of fluorophores to the target.1,2,4 However, accurate application of qPAINT demands for stoichiometric labeling of target molecules, which is difficult to achieve with widely used antibody labeling. Here, we introduce quantitative PAINT using a self-labeling protein tag, RhoTag1.0, which is targeted transiently by the fluorophore TMR. RhoTag1.0 is used to endogenously label several membrane proteins with a 1:1 ratio to increase the molecular counting precision. We utilized monomeric and dimeric membrane proteins to calibrate quantitative PAINT with the RhoTag1.0. Furthermore, we measured the dimerization of EGFR in resting and EGF-stimulated cells.

1. Lelek, M. et al. Single-molecule localization microscopy. Nature Reviews Methods Primers 1, 1–27 (2021).
2. Sharonov, A. & Hochstrasser, R. M. Wide-field subdiffraction imaging by accumulated binding of diffusing probes. Proc Natl Acad Sci U S A 103, 18911–18916 (2006).
3. Schnitzbauer, J., Strauss, M. T., Schlichthaerle, T., Schueder, F. & Jungmann, R. Super-resolution microscopy with DNA-PAINT. Nature Protocols 12, 1198–1228 (2017).
4. Jungmann, R. et al. Quantitative super-resolution imaging with qPAINT. Nat Methods 13, 439–442 (2016).



From Single Molecules to Single Cells; From Biology to Medicine

Xiaoliang Sunney Xie

Peking University, China

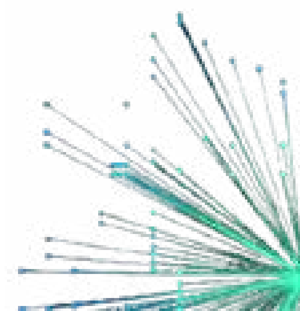
Thanks to contributions from many groups, single-molecule and single-cell techniques have transformed the way biomedical research is conducted. Since DNA exists as a single molecule within a single cell, single-cell genomics can be viewed fundamentally as a single-molecule problem. In this talk, I will highlight genomic medical advances—from preventing genetic disorders in newborns to developing broad-spectrum SARS-CoV-2 neutralizing antibodies, as well as identifying driver mutations in cancer and Alzheimer's disease.

Measurements of molecular size and shape on a chip

Madhavi Krishnan

University of Oxford, United Kingdom

Size and shape are critical discriminators between molecular species and states. I shall describe a microchip-based high-throughput imaging approach offering rapid and precise determination of molecular properties under native solution conditions. Our method detects differences in molecular weightacross at least three orders of magnitude and down to two carbon atoms in small molecules. We quantify the strength of molecular interactions across more than six orders of magnitude in affinity constant and track reactions in real time. Highly parallel measurements on individual molecules serveto characterize sample-state heterogeneity at the highest resolution, offering predictive input to model three-dimensional structure. We further leverage the method's structural sensitivity for diagnostics, exploiting ligand-induced conformational changes in the insulin receptor to sense insulin concentration in serum at the subnanoliter and subzeptomole scale.



Environment-sensitive fluorescence lifetime probes for single-molecule and super-resolution imaging

Alexandre Fürstenberg

Department of Physical Chemistry and Department of Inorganic and Analytical Chemistry, University of Geneva, Geneva, Switzerland

Despite their importance, fluorescence lifetime imaging (FLIM) and single-molecule-based super-resolution microscopy (SMLM) have rarely been combined into a single experiment, mostly for two reasons: (1) the absence of widely applicable experimental implementation until recently; (2) the sparsity of suitable environment-sensitive probes compatible with both FLIM and SMLM. The experimental issue was solved by the introduction of fluorescence-lifetime single-molecule localization microscopy (FL-SMLM) by the Enderlein group which was followed by a commercially available solution. On the probe side however, although the advent of super-resolution microscopy triggered the development of new fluorescent probes, emphasis was put onto making photostable fluorophores which reliably report their position and whose fluorescence is insensitive to their nanoenvironment.

In this contribution, we describe our efforts to develop targetable probes based on red-emitting fluorophores compatible with SMLM and FLIM whose lifetime directly reports on the number of water molecules in their contact sphere, opening the door to sensing hydration in biological environments such as protein surfaces and microgels [1-2]. In addition, we demonstrate how novel mechanosensitive Flipper probes, which act as membrane tension reporters with broad FLIM applications, can be used in SMLM and single-molecule tracking experiments, enabling to discriminate different membrane compositions at the nanoscale [3-4].

J. Maillard, C. A. Rumble, A. Fürstenberg, Red-Emitting Fluorophores as Local Water-Sensing Probes, J. Phys. Chem. B 125, 9727 (2021).

S. Jana, O. Nevskyi, H. Höche, L. Trottenberg, E. Siemes, J. Enderlein, A. Fürstenberg, and D. Wöll, Angew. Chem. Int. Ed. 63, e202318421 (2024).

J. Garcia-Calvo, J. Maillard, I. Fureraj, K. Strakova, A. Colom, V. Mercier, A. Roux, E. Vauthey, N. Sakai, A. Fürstenberg, S. Matile, Fluorescent Membrane Tension Probes for Super-Resolution Microscopy: Combining Mechanosensitive Cascade Switching with Dynamic-Covalent Ketone Chemistry, J. Am. Chem. Soc. 142, 12034 (2020)

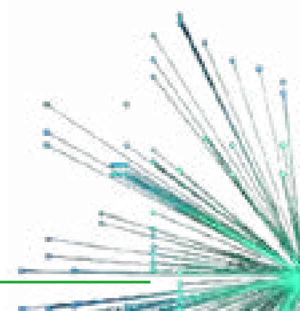
J. Maillard, E. Grassin, E. Bestsennaia, J. Garcia-Calvo, M. Silaghi, N. Sakai, S. Matile, and A. Fürstenberg, J. Phys. Chem. B 128, 7997 (2024)

A Spectral Image Scanning Microscope for Multi-Color Super-Resolution Imaging

Lanna Bram, Yael Roichman, Yuval Ebenstein, Jonathan Jeffe

Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 6997801, Israel

The recent realization of image scanning microscopy (ISM) using confocal spinning disks (CSD) enhanced the spatial resolution of fluorescence microscopy to twice the diffraction limit with minimal sample perturbation and fast acquisition rates. However, capturing multi-color images using ISM is still time-consuming, and different colors are not acquired simultaneously. Here, we present a spectral CSD-ISM system designed for concurrent high-resolution and simultaneous multi-color acquisition. By integrating a custom linear Amici prism into the CSD-ISM optical detection path, we achieve multi-color, super-resolution images at a fraction of the acquisition time and with a flexible color palette selection. A digital signal processor (DSP) is employed as a cost-effective alternative to Field-Programmable Gate Arrays (FPGAs) used in previous studies. A GPU-compatible, python-based image processing pipeline decomposes spectral signatures into multi-color images, preserving the optical resolution. System characterization using fluorescent beads demonstrated 1.73-fold resolution improvement over the diffraction limit and accurate color classification with three times faster acquisition compared to standard CSD-ISM. Application to neuron cells induced with Parkinson's disease showcased improved resolution and contrast of four distinctly labeled cellular components. This spectral CSD-ISM system provides a valuable tool for biological imaging, enabling the simultaneous acquisition of high-resolution spatial information and multi-color spectral data.



Molecule-scale resolution in dynamics and fluorescence microscopy

Stefan W. Hell & co-workers

Max Planck Institute for Multidisciplinary Sciences, Göttingen & Max Planck Institute for Medical Research, Heidelberg

I will discuss MINFLUX [1-4], a recent molecular localization and superresolution method that has reached Angström localization precision and resolution of the size of a fluorophore molecule. MINFLUX and the related MINSTED concept [5,6] are being established for routine applications in cell and molecular biology, structural biology and neuroscience. Relying on much fewer fluorescence photons than the widely used camera-based localization methods, these techniques are poised to characterize dynamic processes of single proteins, as demonstratedby tracking the nanometer conformational changes of the motor proteins kinesin-1 [7] and dynein in living cells [8]. MINFLUX has also been demonstrated to measure intramolecular distances with Angström precision, providing a precise and reliable alternative to FRET [9]. Harnessing confocal detection, MINFLUX also provides nanometer-range resolution deeper down in layers of cells and (mildly) scattering tissue [10]. Finally, I will show an arguably surprising ability of MINFLUX to separate individual identical fluorophores without sequential ON/OFF switching or activation of fluorescence. Thus, the simultaneous, uninterrupted, nanometer-scale tracking and imaging of multiple, identical (same-color) fluorophores becomes possible for the first time [11]. This novel superresolution principle should allow MINFLUX to reveal the conformational changes of individual proteins in their native environment.

[1] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S.W. Nanometer resolutionimaging and tracking of fluorescent molecules with minimal photon fluxes. Science 355, 606-612 (2017).

[2] Eilers, Y., Ta, H., Gwosch, K. C., Balzarotti, F., Hell, S. W. MINFLUX monitors rapid molecular jumps with superiorspatiotemporal resolution. PNAS 115, 6117-6122 (2018).

[3] Gwosch, K. C., Pape, J. K., Balzarotti, F., Hoess, P., Ellenberg, J., Ries, J., Hell, S. W. MINFLUX nanoscopy delivers 3Dmulticolor nanometer resolution in cells. Nat. Methods 17, 217–224 (2020).

[4] Schmidt, R., Weihs, T., Wurm, C. A., Jansen, I., Rehman, J., Sahl, S. J., Hell, S. W. (2021) MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. Nat. Commun. 12:1478..

5] Weber, M., Leutenegger, M., Stoldt, S., Jakobs, S., Mihaila, T. S., Butkevich, A. N., Hell, S. W. MINSTED fluorescence localization and nanoscopy. Nat. Photon. 15, 361-366 (2021).

[6] Weber, M., von der Emde, H., Leutenegger, M., Gunkel, P., Sambandan, S., Khan, T. A., Keller-Findeisen, J., Cordes, V. C., Hell, S.W. MINSTED nanoscopy enters the Ångström localization range. Nat. Biotechnol., 41, 569-576 (2023).

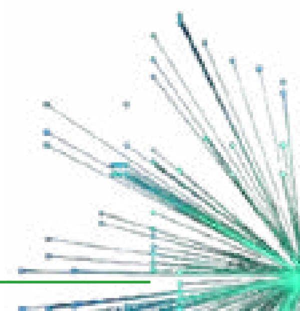
[7] Wolff, J. O., Scheiderer, L., Engelhardt, T., Engelhardt, J., Matthias, J., Hell, S.W. MINFLUX dissects the unimpeded walking of kinesin-1. Science, 379, 1004-1010 (2023).

[8] Schleske, J. M., Hubrich, J., Wirth, J. O., D'Este, E., Engelhardt, J., Hell, S. W. MINFLUX reveals dynein stepping in live neurons. PNAS 121, e2412241121 (2024).

[9] Sahl, S. J., Matthias, J., Inamdar, K., Weber, M., Khan, T. A., Brüser, C., Jakobs, S., Becker, S., Griesinger, C., Broichhagen, J., Hell, S. W. Direct optical measurement of intramolecular distances with angstrom precision. Science 386, 180-187 (2024).

[10] Moosmayer, T., Kiszka, K. A., Pape, J. K., Leutenegger, M., Steffens, H., Grant, S. G. N., Sahl, S. J., Hell, S. W. MINFLUX fluorescence nanoscopy in biological tissue. PNAS 121, e2422020121 (2024).

[11] Hensel, T. A., Wirth, J. O., Schwarz, O. L. Hell, S. W. Diffraction minima resolve point scatterers at few hundredths of the wavelength. Nat. Phys. https://doi.org/10.1038/s41567-024-02760-1 (2025).



Advancing Single-Molecule Imaging

Aleksandra Radenovic

EPFL, Lausanne, Switzerland

In this talk, I will demonstrate how advancements in imaging methods can open new avenues for research at solid-liquid interface. In the first part, I will discuss our efforts to explore nanophotonics at the single-molecule level, with a focus on the optical dynamics and conformations of individual emitters at the solid-liquid interface and in confined environments.In our recent work, we employed fluorescence microscopy to monitor electrochemical reactions at the single-molecule scale, achieving a wide field of view and high temporal resolution. Additionally, we introduced an advanced bifocal polarization single-molecule localization microscopy (pSMLM) to enable real-time, multi-dimensional observations of quantum emissions formed by organic molecules and h-BN native defects. Our findings reveal a strong correlation between the orientation of quantum emitters and the symmetry of the h-BN lattice.I will also discuss the use of SPAD cameras for single-particle tracking applications, as well as in high-throughput single-molecule fluorescence lifetime imaging (smFLIM).

[1] Ronceray, Nathan, Yi You, Evgenii Glushkov, Martina Lihter, Benjamin Rehl, Tzu-Heng Chen, Gwang-Hyeon Nam et al. „Liquid-activated quantum emission from pristine hexagonal boron nitride for nanofluidic sensing.“ Nature Materials 22, no. 10 (2023): 1236-1242.

[2] Mayner, Eveline, Nathan Ronceray, Martina Lihter, Tzu-Heng Chen, Kenji Watanabe, Takashi Taniguchi, and Aleksandra Radenovic. „Monitoring electrochemical dynamics through single-molecule imaging of hBN surface emitters in organic solvents.“ arXiv preprint arXiv:2405.10686 (2024).

[3] Guo, Wei, Tzu-Heng Chen, Nathan Ronceray, Eveline Mayner, Kenji Watanabe, Takashi Taniguchi, and Aleksandra Radenovic. „Dipole orientation reveals single-molecule interactions and dynamics on 2D crystals.“ arXiv preprint arXiv:2408.01207 (2024).

Correlative confocal and super-resolution imaging of the immunological CAR-T cell synapse

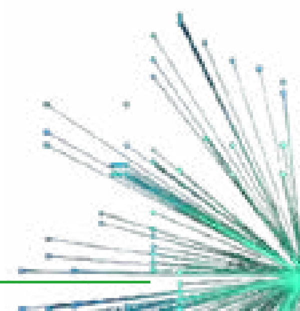
Rick Seifert[1], Leon Gehrke[2], Nicole Seifert[1], Sören Doose[1], Michael Hudecek[2], Thomas Nerreter[2], Markus Sauer1

[1]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany
[2]Chair in Cellular Immunotherapy, Medical Clinic II, University Hospital of Würzburg, Würzburg, Germany

Chimeric antigen receptor (CAR)-T cell therapy uses T cells engineered to express CARs specifically targeting surface antigens on cancer cells. Upon recognition, CAR-T cells form immunological synapses (IS) against cancer cells and eliminate them. Unlike the conventional bull's-eye IS of T cells, CAR-T cells form a multifocal IS. The ultrastructure of this IS can be used for assessing the efficacy of different CAR designs [1].
To investigate and quantify CAR rearrangement during IS formation, we are developing a correlative confocal and super-resolution microscopy approach. For 3D quantification of CARs, we developed a deformable mirror-based 3D-direct stochastic optical reconstruction microscopy (dSTORM) workflow. The deformable mirror allows us to engineer a tetrapod point spread function (PSF). Additionally, we integrated a Rescan confocal microscope (RCM) [2], enabling correlative RCM and 3D-dSTORM imaging. This allows us to correlate CAR distribution at a high spatial resolution with different cellular structures and other membrane receptors.
Our approach provides new insights into CAR-T cell IS architecture and offers a potential framework to evaluate CAR designs based on IS ultrastructure for improved CAR-T cell immunotherapies.

[1] Liu D, Badeti S, Dotti G, et al., Cell Communication and Signaling, 18(1), 134 (2020)

[2] De Luca GMR, Breedijk RMP, Brandt RAJ, et al., Biomed Opt Express, 4(11), 2644 (2013)



TimeLoc: Integrating Dynamic Excitation and SPAD units for Camera-Free Frequency-Encoded Super-Resolution Imaging and Tracking

Francisco Matos1, Maximilian Lengauer1,2, Emmanuel Fort2, Sandrine Lévêque-Fort1

[1]Institut des Sciences Moléculaires d Orsay, 598 Rue André Rivière, 91400 Orsay, France
[2]Institut Langevin,1 Rue Jussieu, 75005 Paris, France

Fluorescence super-resolution microscopy capabilities have progressively advanced by leveraging molecular sparsity to achieve nanometer-scale localization. While conventional methods typically rely on camera-based detection and spatial PSF fitting, recent approaches have turned toward temporal encoding to enhance localization performance [1].
Pursuing this line of development, Time Localization Microscopy (TimeLoc) employs dynamic structured excitation, encoding each position in the field of view with a distinct temporal modulation frequency [2]. This frequency-based strategy enables spatial localization through analysis of the emitted signal, removing the need for pixelated camera detection by using a Single Photon Avalanche Diode (SPAD).
Initial experiments with a single SPAD have demonstrated the feasibility of wide-field localization based solely on temporal modulation, highlighting the potential of frequency-based encoding for spatial reconstruction. Building on these results, a 23-element SPAD array is now being incorporated to enable parallel detection and expand spatial sampling capacity.
Current efforts focus on evaluating array-based performance and assessing two-dimensional excitation schemes, including sequential and simultaneous modulation modes. These studies aim to improve the robustness of frequency encoding and support scalable imaging and tracking. Preliminary results using dSTORM and DNA-PAINT will be presented as part of the continued assessment of the technique.

[1] Jouchet, P; Cabriel, C.; Bourg, N.; et al.: Nanometric axial localization of single fluorescent molecules with modulated excitation. Nature Photonics, Vol. 15, 2021, pp. 297–304. DOI: 10.1038/s41566-020-00749-9.

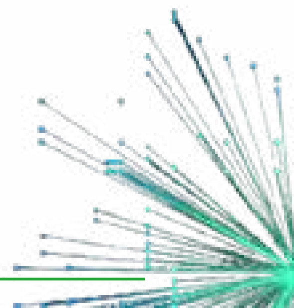
[2] Lengauer, M: Wide-field super-resolution imaging from a time-modulated fluorescence signal. Dissertation, Université Paris-Saclay and École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 2023.

Understanding biology by building it? The exciting world of synthetic cells.

Petra Schwille

Max-Planck-Institute of Biochemistry, Martinsried, Germany

tba



Mechano-signalling as a regulator of cell behaviour

Verena Ruprecht

University of Innsbruck, Austria

tba

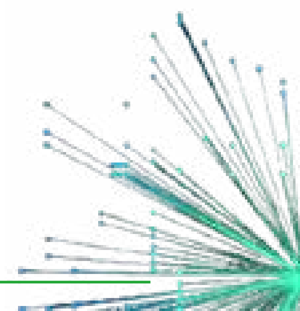
Time-gated detection of NIR luminescent nanoparticles in organs using snSPDs

Abhilash Kulkarni[1], Olof Eskilson[2,3], Georgios A. Sotiriou[2,3], Jerker Widengren[1]

[1]Experimental Biomolecular Physics, Dept. of Applied Physics, Albanova University Center, Royal Institute of Technology (KTH), 106 91 Stockholm, Sweden.
[2]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 17, Stockholm, Sweden.
[3]Department of Materials and Environmental Chemistry, Stockholm University, 106 91, Stockholm, Sweden.

Luminescent nanoparticles (NPs) are promising candidates for near-infrared (NIR) in-vivo and biomedical imaging due to their high brightness, photostability, and low photobleaching. Their surface can be functionalized with proteins to target specific tissues. This study investigates the biodistribution of Neodymium (Nd)-doped NPs in vital mouse organs, exploring their potential as drug carriers for both diagnostic and therapeutic applications.
We utilize Superconducting Nanowire Single Photon Detectors (snSPDs), previously shown to offer high time resolution, low dark counts and no afterpulsing in NIR single-molecule studies(1). The current snSPD is optimized for detecting sharp NP emission lines in the 900–1100 nm range, improving penetration depth and reducing autofluorescence and scattering in biological media.
We exploit techniques pioneered in single molecule spectroscopy to enhance the quantitative information gained to accurately estimate the biodistribution. We take advantage of long excited state lifetime of the NPs to perform time gated detection with a high repetition rate laser combined with confocal laser scanning microscopy at various depths. Burst analysis is performed to differentiate actual emission from noise. Furthermore, we implement a coincidence count-based detection to improve SBR by employing a Hanbury-Brown-Twiss arrangement. Results are benchmarked against ICP-MS and compared with an in-house camera-based lock-in detection method.(2)

(2) Bagheri, N.; Wang, C.; Guo, D.; Lakshmanan, A.; Zhu, Q.; Ghazyani, N.; Zhan, Q.; Sotiriou, G. A.; Liu, H.; Widengren, J. Lanthanide upconversion nonlinearity: a key probe feature for background-free deep-tissue imaging. 2025; p arXiv:2503.05325.



Unraveling DNA Ligase Dynamics via Multiparameter Photon-by-Photon smFRET and H2MM Analysis

Tom Kache[1], David M Wilson III[2], Jelle Hendrix[1]

[1]Dynamic Bioimaging Lab, BIOMED, Hasselt University, 3590 Diepenbeek, Belgium
[2]The GRAND Team, BIOMED, Hasselt University, 3590 Diepenbeek, Belgium

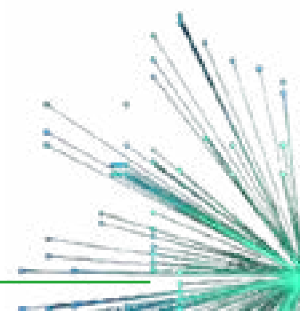
Multiparameter, photon-by-photon, single-molecule FRET (smFRET) allows for integrating several aspects of fluorescence detection to aid the structural study of biomolecules. Here, we employed anisotropy-resolved smFRET and mpH2MM to characterize the interplay between FRET-labeled nicked DNA (nDNA) and purified human DNA ligase 3 (LIG3). LIG3 stands out among human DNA ligases as the sole mitochondrial DNA ligase and the only one possessing an additional DNA-binding zinc-finger domain capable of sensing DNA nicks. smFRET allowed us to detect discrete LIG3-induced bending states of the nDNA substrate and an increase in anisotropy, indicative of tight protein-DNA interactions. By maintaining constant ionic strength, we observed that varying magnesium concentrations modulated the nDNA FRET and anisotropy signatures, suggesting different binding modes for LIG3. Employing anisotropy-resolved multiparameter H2MM analysis, we identified several structural states that dynamically interconvert at rates of hundreds of times per second. The rate and nature of these conformational changes were found to depend on the presence of magnesium ions. Our results align with previous structural and kinetic studies that have suggested a dynamic hand-off mechanism between the zinc-finger and the catalytic part of the protein, which may regulate LIG3's function.

Chip-based iSCAT microscopy under evanescent illumination

Marie Reischke[1,2,3], Daniel Böning[4], Vahid Sandoghdar[1,2,3], Pierre Türschmann4

[1]Max Planck Institute for the Science of Light, 91058 Erlangen, Germany
[2]Max-Planck-Zentrum für Physik und Medizin, 91058 Erlangen, Germany
[3]Department of Physics, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany
[4]Interherence GmbH, Henkestraße 91, 91052 Erlangen, Germany

Characterization of biological nanoparticles (BNPs) such as viral vectors and proteins is in high demand in modern molecular biology and biotechnology. Interferometric scattering (iSCAT) microscopy has shown that not only individual BNPs can be detected, but also their mass can be assessed, providing a quantitative method that can operate under physiological conditions. Here, we report on a new experimental arrangement, where a chip platform for total internal reflection microscopy (QuScite, Interherence GmbH) is modified to allow for the analysis of BNPs via light scattering. We show that chip-based iSCAT can detect BNPs down to single proteins over a large field of view using evanescent illumination and a common-path reference. We discuss the working principles of chip-based iSCAT and present measurements on various BNPs such as adeno-associated viruses (AAVs). The compact nature of our new device lends itself to robust, high throughput, and easy-to-use measurements.



Pollen Tube Growth Dynamics Quantified via Volumetric Spatio-Temporal Image Correlation Spectroscopy

Paul Wiseman

McGill University, Montreal, Canada

Rapid pollen tube growth is key for fertilization from pollen to the ovary in flowering plants. Tube growth necessitates the expansion of adjoining cell walls and plasma membranes, both requiring local delivery of the new material via cargo vesicles which contain precursor molecules within a lipid bilayer. Due to the three-dimensional planar form of a cell wall, cellular expansion must be precisely controlled in space with targeted deposition of new material to specific surface areas of the growing cell front. Biophysical characterization of pollen tube growth in space and time is challenging due to the high density of transport vesicles and requires a combination of high-temporal-frequency 3D imaging and advanced image analysis methods.3D imaging time series of growing pollen tubes were collected via field-synthesis lattice light-sheet microscopy to minimize photobleaching and increase sample viability for long-term imaging. To quantify vesicle trafficking, the volumetric extension of the established 2D spatio-temporal image correlation spectroscopy (STICS) was used to provide the first 3D mapping of vectorial transport of the cargo vesicles in a living plant pollen tube. We also performed lifetime-filtering FLIM, to separate fluorescence signal localized to vesicles from mitochondrial-localized emission to simultaneously measure cargo vesicle and mitochondrial transport dynamics via STICS, and the local redox microenvironment in growing pollen tubes from camellia japonica pollen grains

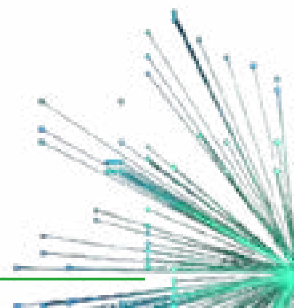
Expanding the Horizon of FCS with SPAD Arrays: A Promising Outlook for New Applications

Anders Barth[1] Marcelle Koenig[1], Evangelos Sisamakis[1], Fabian Barachati[1,] Johan Hummert[1], Felix Koberling[1,] Ivan Michel Antolovic[2], Rainer Erdmann[1]

[1]PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
[2]Pi Imaging Technology SA, EPFL Innovation Park, 1015 Lausanne, Switzerland

Fluorescence Correlation Spectroscopy (FCS) is a well-established tool for studying molecular interactions and dynamics at the single-molecule level. The recent integration of single-photon avalanche diode (SPAD) arrays combined with time-resolved instrumentation in confocal microscopy provides new possibilities for FCS that provide new insights for live cell investigations.
Here, we evaluate enhanced FCS applications which are enabled by the integration of a cooled high-performance 23-pixel SPAD-array that was developed jointly with Pi Imaging Technologies as an add-on to the confocal microscope Luminosa. The SPAD array allows for the simultaneous detection of multiple fluorescence signals based on single photon counting with high temporal resolution. Any pixel combination within the SPAD array can selected for advanced FCS analyses. Thus, compared to point detectors, spatially resolved information about molecular diffusion and dynamics becomes available. This enables e.g. spot-variation FCS for the identification of potentially hindered diffusion in live cell investigations. Spatial pixel cross-correlations can be used to uncover directional diffusion. The integration of Time-Correlated Single-Photon Counting (TCSPC) provides further information about the fluorescence lifetimes. These can be utilized for an even more comprehensive understanding of complex biological mechanisms.

The integration of SPAD-arrays with time-resolved detection represents a significant advancement for confocal microscopes. Apart from the improved optical resolution for imaging purposes via image scanning microscopy (ISM), SPAD array based detection allows for a multitude of new FCS modalities for studying complex biological processes in both temporal and spatial domains.



Unveiling the invisible: A novel approach illuminates the sub-10 nm cosmos

Dominic A. Helmerich[1], Made Budiarta[2], Danush Taban[1], Mara Meub[1], Marcel Streit[2], Alexander Kuhlemann[1], Sören Doose1, Gerti Beliu[2], Markus Sauer[1,2]

[1]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, 97074, Würzburg, Germany
[2]Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, 97080, Würzburg, Germany

Super-resolution microscopy has transformed our understanding of cellular structures, achieving spatial resolution down to single-digit nanometers. Despite advancements in angstrom-level accuracy on reference structures, applying this precision to biological samples remains difficult. Our work presents a novel approach that turns this challenge into an opportunity for discovery.
Here we utilize photoswitching fingerprint analysis, which leverages the unique temporal behavior of fluorescent dyes to extract information in the sub-10 nm range. By analyzing the blinking patterns of fluorophore systems, we can reveal molecular arrangements and interactions that were previously hidden.
This method combines advanced analysis with specially designed protein-based reference structures, enabling precise calibration of super-resolution techniques. The stability of these structures in cellular environments makes them ideal for benchmarking high-resolution imaging methods and avoiding misinterpretations during live cell studies.
This innovative methodology opens new avenues for exploring the nanoscale world of biology, providing unprecedented insights into cellular processes and molecular interactions.

D. A. Helmerich, G. Beliu, D. Taban, et al., Nat Methods, 19, 986–994 (2022)
D. A. Helmerich, M. Budiarta, et al., Adv. Mater., 36, 2310104 (2024)

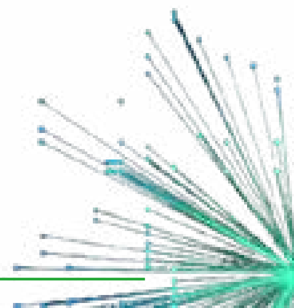
Measuring membrane and membrane protein structure and dynamics with dynamic metal- and graphene-induced energy transfer spectroscopy (dynaMIET/dynaGIET)

Narain Karedla, Jörg Enderlein, Tao Chen

Georg-August-Universitat Gottingen, Drittes Physikalisches Institut-Biophysik, Friedrich-Hund-Platz 1, 37077 Göttingen, GERMANY

Cell membranes are dynamic, fluid structures composed of a phospholipid bilayer with embedded proteins, exhibiting fluidity and viscoelastic properties essential for various biological functions like cell signaling, membrane trafficking, and cell division. However, accurately measuring the dynamics of intricate membrane systems, like mitochondria, characterized by rapid and subtle fluctuations, poses significant challenges. In this study, we introduce a novel methodology (dynaMIET/dynaGIET) that combines metal/graphene-induced energy transfer (MIET/GIET) (1) with various fluorescence correlation spectroscopy (FCS)- based techniques to precisely quantify the structure and dynamics of different membrane systems. With these combinations, we measured the membrane fluctuations (2), leaflet-specific structure and diffusions (3, 4), and membrane protein conformation dynamics (5). Moreover, we showcase the versatility and applicability of dynaMIET/dynaGIET in studying various membrane systems.

1. A. I. Chizhik, J. Rother, I. Gregor, A. Janshoff, J. Enderlein, Metal-induced energy transfer for live cell nanoscopy. Nat. Photonics 8, 124–127 (2014).
2. T. Chen, N. Karedla, J. Enderlein, Measuring sub-nanometer undulations at microsecond temporal resolution with metal- and graphene-induced energy transfer spectroscopy. Nat. Commun. 15, 1789 (2024).
3. N. Karedla, F. Schneider, J. Enderlein, T. Chen, Leaflet-specific Structure and Dynamics of Solid and Polymer Supported Lipid Bilayers. Angew. Chem. Int. Ed. n/a, e202423784.
4. T. Chen, A. Ghosh, J. Enderlein, Cholesterol-Induced Nanoscale Variations in the Thickness of Phospholipid Membranes. Nano Lett. 23, 2421–2426 (2023).
5. T. Chen, N. Karedla, J. Enderlein, Observation of E-cadherin adherens junction dynamics with metal-induced energy transfer imaging and spectroscopy. Commun. Biol. 7, 1–10 (2024).



A Brief Survey of Single-Molecule Optical Microscopy: From Early Spectroscopy in Solids, to Super-Resolution Nanoscopy in Cells, to a Wealth of New Applications

W. E. (William E.) Moerner

Departments of Chemistry and Applied Physics (courtesy) Stanford University, Stanford, CA USA 94305

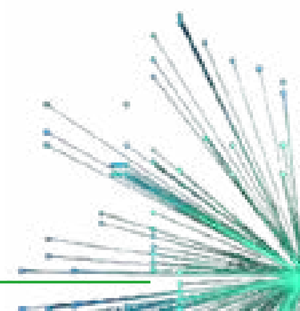
First observed optically 36 years ago in my laboratory at IBM Research, single molecules have enabled much interesting science spanning physics, chemistry, materials science, medicine, and biology. In this new field of optical microscopy of the nanoscale, ensemble averaging is removed, so each single molecule can act as a reporter of not only its position, but also about local information about the nearby environment. Combined with blinking and photoswitching (first observed at low temperatures in 1992 and then for single GFP proteins at room temperature in 1997) to ensure sparsity, in the mid-2000's, super-resolution fluorescence microscopy based on single molecules has opened up a frontier in which structures and behavior can be observed in materials and in fixed and live cells with resolutions down to the one and two digit nm scale. Cellular studies of single molecules have been enhanced by PSF engineering to extract 3D position and orientation, deep learning to estimate molecular variables and structured backgrounds, light sheet illumination, and much more. A recent study shows fascinating intracellular structures formed by SARS-CoV-2 viral RNA and proteins in infected mammalian cells. Three-dimensional single-molecule tracking in live cells provides time-dependent information about biological regulation and condensed complexes, as well as about anomalous diffusion of DNA loci in nuclei and more. Acronyms abound (PAINT, ODMR, ABEL,…) - the future is bright, indeed!

Advancing Super-Resolution Imaging: Integrating Fluorescence Lifetime, Scanning Microscopy, and Energy Transfer Techniques for Isotropic Nanoscale Bioimaging

Jörg Enderlein

Georg August University Göttingen, Germany

Recent advancements in super-resolution microscopy have enabled unprecedented insights into the spatial organization of cellular structures. In this talk, I will present a series of methodological innovations that synergistically integrate fluorescence-lifetime single-molecule localization microscopy (FL-SMLM) [1,2], image scanning microscopy (ISM) [3,4], and metal- or graphene-induced energy transfer (MIET/GIET) imaging [5–7]. These approaches collectively offer isotropic three-dimensional resolution at the nanometer scale, multiplexed imaging capabilities, and robustness against chromatic aberrations.First, I will discuss our work on MIET and GIET microscopy, which exploit distance-dependent quenching phenomena near metallic or graphene interfaces to determine the axial position of single emitters with sub-10 nm accuracy. The combination of MIET with direct Stochastic Optical Reconstruction Microscopy (dSTORM) or DNA-based Points Accumulation for Imaging in Nanoscale Topography (DNA-PAINT) provides truly isotropic 3D resolution, extending the reach of localization microscopy into the axial dimension without interferometric complexity.Second, I will highlight the development of fluorescence lifetime DNA-PAINT (FL-PAINT), a technique that enables multi-target super-resolution imaging through fluorescence lifetime multiplexing without fluid exchange. By utilizing orthogonally designed imager strands conjugated to fluorophores with distinct lifetimes, we achieve simultaneous imaging of multiple targets in the dense intracellular environment. Lastly, I will introduce our latest development of fluorescence-lifetime image scanning microscopy SMLM (FL-iSMLM), which achieves a near twofold enhancement in lateral resolution by integrating a single-photon detector array into a confocal laser scanning microscope (CLSM). This method combines the localization precision of ISM with the multiplexing power of fluorescence-lifetime detection, enabling sub-5 nm resolution in fixed cells while simultaneously allowing discrimination of targets based solely on their fluorescence lifetimes.



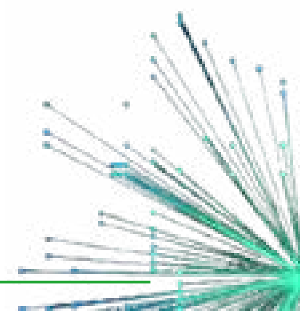
[1] J. C. Thiele, D. A. Helmerich, N. Oleksiievets, R. Tsukanov, E. Butkevich, M. Sauer, O. Nevskyi, and J. Enderlein, Confocal Fluorescence-Lifetime Single-Molecule Localization Microscopy, ACS Nano 14, 14190 (2020).
[2] J. C. Thiele, O. Nevskyi, D. A. Helmerich, M. Sauer, and J. Enderlein, Advanced Data Analysis for Fluorescence-Lifetime Single-Molecule Localization Microscopy, Front. Bioinform. 1, 740281 (2021).
[3] C. B. Müller and J. Enderlein, Image Scanning Microscopy, Phys. Rev. Lett. 104, 198101 (2010).
[4] N. Radmacher, O. Nevskyi, J. I. Gallea, J. C. Thiele, I. Gregor, S. O. Rizzoli, and J. Enderlein, Doubling the resolution of fluorescence-lifetime single-molecule localization microscopy with image scanning microscopy, Nat. Photon. 18, 1059 (2024).
[5] A. I. Chizhik, J. Rother, I. Gregor, A. Janshoff, and J. Enderlein, Metal-induced energy transfer for live cell nanoscopy, Nature Photon 8, 124 (2014).
[6] A. Ghosh, A. Sharma, A. I. Chizhik, S. Isbaner, D. Ruhlandt, R. Tsukanov, I. Gregor, N. Karedla, and J. Enderlein, Graphene-based metal-induced energy transfer for subnanometre optical localization, Nat. Photonics 13, 860 (2019).
[7] J. C. Thiele, M. Jungblut, D. A. Helmerich, R. Tsukanov, A. Chizhik, A. I. Chizhik, M. Schnermann, M. Sauer, O. Nevskyi, and J. Enderlein, Isotropic Three-Dimensional Dual-Color Super-Resolution Microscopy with Metal-Induced Energy Transfer, Science Advances 8, 14190 (2021).

Direct single-molecule detection and super-resolution imaging with a low-cost portable smartphone-based microscope

Guillermo Acuna

Department of Physics, University of Fribourg, Switzerland

We present a novel, low-cost, portable smartphone-based fluorescence microscope capable of detecting single-molecule fluorescence directly, i.e., without the need for any signal amplification. The setup leverages the image sensors and data handling capacity of mass-produced smartphones, making it adaptable to different smartphones and capable of detecting single molecules across the visible spectral range. We showcase this capability through single-molecule measurements on DNA origami models and super-resolution microscopy of biological cells by single-molecule localization microscopy. Last, we illustrate its potential as a point-of-care (POC) device by implementing a single-molecule bioassay for RNA detection. This development paves the way for biotechnology innovations, making use of massively distributed or personalized assays with single-molecule sensitivity, with the potential to revolutionize digital bioassays, POC diagnostics, field expeditions, STEM outreach, and life science education.



Nanotexture – a universal approach of AI-based computational multiplexing and phenotyping of super-resolution data.

Bela Vogler[1,2], Gregor Gentsch1, Pablo Caravilla[1,2,3,] Dominic Helmerich[4,] Teresa Klein4, Katharina Reglinski[1,2,] Markus Sauer[4,5,] Christian Eggeling[1,2], Christian Franke[1]

[1]Faculty of Physics and Astronomy, Institute of Applied Optics and Biophysics, Friedrich Schiller University Jena, Jena, Germany
[2]Leibniz Institute of Photonic Technology e.V., Jena, Germany, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
[3]Present address: Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, Solna, Sweden
[4]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, Würzburg, Germany
[5]Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, Germany

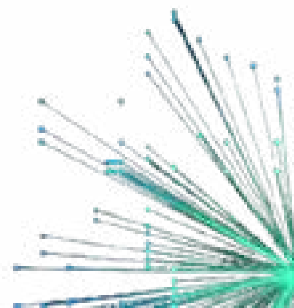
Fluorescence-based super-resolution microscopy (SRM) enables nanometer-scale visualization of cellular organelles. Traditional multicolor SRM relies on spectral multiplexing, but leading methods—STED, SMLM, MINFLUX—require specialized dyes with delicate photophysical properties, limiting multi-color imaging fidelity and live-cell compatibility. Fast acquisition techniques like SIM and Airy-Scan also face speed-resolution trade-offs and lack synchronicity in multi-color applications.

We introduce NanTex, a ML-based context-agnostic multiplexing approach leveraging organelle-specific nanotextures, applicable to SMLM, MINFLUX, STED, SIM, and Airy scan microscopy. NanTex demixes overlapping organelles from single-channel images without spectral separation, using AI-enabled textural demixing via U-Net learning [1].

NanTex trained on SMLM is directly applicable to MINFLUX without retraining, facilitating multiplexing at MINFLUX resolution without the extreme task to gather sensible amounts of training data. We demonstrate multiplexing in artificial overlays and real experimental datasets with almost all major cellular organelles (actin, microtubules, clathrin, endosomes, lysosomes, ER, mitochondria, golgi, etc.), including live-cell SIM and airyscan multiplexing. Furthermore, we present NanTex on our novel high-speed SIM, based on random pattern structured illumination (speckle SIM) with 100 nm resolution at framerates of >100 Hz [2].

NanTex also enables computational phenotyping, exemplified by quantifying microtubule depolymerization upon nocodazole treatment. NanTex advances super-resolution multi-organellar imaging in diverse SRM techniques.

[1] B. Vogler, G.J. Gentsch et al. in preparation (will be on biorxiv in February 2025)
[2] A. Platz, G.J. Gentsch et al. in preparation (will be on biorxiv in April 2025)



Ångström-level, intra-molecular MINFLUX analyses of protein conformation and chemical architecture

Steffen J. Sahl

Max Planck Institute for Multidisciplinary Sciences, Department of NanoBiophotonics, Am Fassberg 11, 37077 Göttingen, Germany

The recent demonstration of intra-molecular MINFLUX resolution capabilities [1] has extended single-molecule fluorescence analysis to the direct position-based probing of macromolecular conformation. Photon-efficient 2D or 3D MINFLUX localization protocols [2], together with photoactivatable fluorescent dyes, enable the independent detection and Ångström-precise relative coordinate registration of dye-labeled protein sites even in the 1-to-5-nanometer distance regime [1]. Here, I will describe further progress in quantifying intra-molecular details such as equilibria of distinct conformations in an enzymatic system, or the ability to measure small protein conformational changes. Initial demonstrations of directly visualized chemical architecture uncover a further new frontier of what can be probed with MINFLUX today, and will be probed in the future. They are a strong testament to the continued vigorous development in the field of single-molecule analysis over the last decade [3].

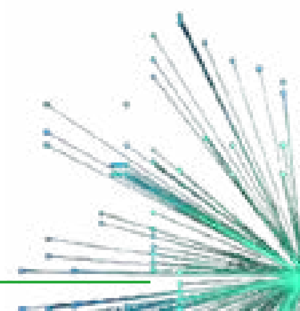
[1] Sahl et al. Science 386, 180 (2024).
[2] Schmidt et al. Nat. Commun. 12, 1478 (2021).
[3] Sahl & Moerner, Curr. Opin. Struct. Biol. 23, 778 (2013).

Looking back at 35 years of single-molecule optics

Michel Orrit

Leiden University, Netherlands

Beyond scanning probe microscopies, a variety of optical signals give direct access to single molecules and single nanoparticles. For more than 30 years, fluorescence has been the workhorse of single-molecule optics, and has spawned breakthroughs in optical superresolution. A large variety of photochemical and biochemical processes influence fluorescence and thereby give access to the ultimate single-molecule level, free from ensemble averaging. More recently, under the lead of several groups, other optical techniques have reached single-molecule sensitivity. Photothermal microscopy proved sensitive enough to detect single photostable dye molecules or single organic conjugated polymers. The differential absorption of circularly polarized light provides quantitative circular dichroism data of single absorbing chiral or magnetic nanoparticles. Non-absorbing nanoparticles and large molecules can now be detected individually through their optical polarizability only, without need for fluorescent or absorbing labels. Their selectivity and signal-to-noise ratio are enhanced considerably in the near-field of plasmonic gold nanoparticles or even in wide field, thanks to resonant optical microcavities. The arrival and departure of single protein molecules from a solution cause sudden steps in the optical signal, opening micro-analytical applications and in-situ sensing. The capacity to detect and characterize single unlabeled diffusing protein molecules on-the-fly opens fascinating perspectives in bio-medical science.



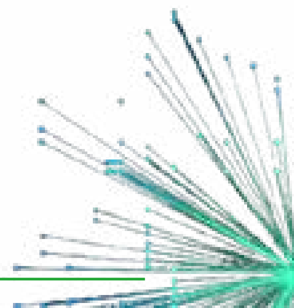
Coherent scattering of light by single molecules

Vahid Sandoghdar

[1]Max Planck Institute for the Science of Light, 91058 Erlangen, Germany
[2]Max-Planck-Zentrum für Physik und Medizin, 91054 Erlangen, Germany3Department of Physics, Friedrich-Alexander University of Erlangen-Nürnberg, 91058 Erlangen, Germany

The very first report of single-molecule detection in 1989 involved cryogenic extinction measurements assisted by double-FM modulation [1]. This achievement was followed by experiments, which were conducted based on the detection of the Stokes-shifted fluorescence. However, lack of coherence in spontaneously emitted fluorescence hampered access to the phase of the molecular wavefunction and of the laser light. In 2007, we revisited direct extinction measurements on single molecules with a simple realization that the intrinsic extinction cross section is large enough to be detected directly if the illumination laser field is confined strongly [2]. A series of near-field [3] and far-field [4,5] experiments demonstrated extinction dips as large as 20%. Such a large efficiency in the interaction between light and a single quantum system opened the door to nonlinear effects at the few-photon level [6]. Furthermore, combination with high-finesse microcavities opened the door to near-unity extinction, strong coupling and single-photon nonlinearity [7,8]. More recently, these developments were used to show that coherent cooperative coupling of several individual molecules via a common mode of a microcavity [9]. Interestingly, coherent Rayleigh scattering has also become a powerful method for label-free detection and tracking of biological nanoparticles and nanostructures such as cellular organelles and viruses, down to single proteins at ambient conditions [10]. In this lecture, I will give an overview of these developments and discuss some exciting new perspectives.

[1] W. E. Moerner and L. Kador, PRL 62, 2535 (1989).
[2] G. Zumofen, N. Mojarad, V. Sandoghdar, M. Agio, PRL 101, 180404 (2008).
[3] I. Gerhardt, G. Wrigge, P. Bushev, G. Zumofen, R. Pfab, V. Sandoghdar, PRL 98, 033601 (2007)
[4] G. Wrigge, I. Gerhardt, J. Hwang, G. Zumofen, V. Sandoghdar, Nat. Phys. 4, 60 (2008).
[5] M. Potoschnig, Y. Chassagneux, J. Hwang, G. Zumofen, A. Renn, V. Sandoghdar, PRL 107, 063001 (2011).
[6] A. Maser, B. Gmeiner, T. Utikal, S. Götzinger, V. Sandoghdar, Nat. Photonics 10, 450 (2016).
[7] D. Wang, H. Kelkar, D.-M. Cano, D. Rattenbacher, A. Shkarin, T. Utikal, S. Götzinger, V. Sandoghdar, Nat. Physics 15, 483 (2019).
8] A. Pscherer, M. Meierhofer, D. Wang, H. Kelkar, D.-M. Cano, T. Utikal, S. Götzinger, V. Sandoghdar, PRL 127, 133603 (2021).
[9] J. Nobakht, A. Pscherer, J. Renger, S. Götzinger, V. Sandoghdar, PNAS, June 2025.
[10] N. Ginsberg, C-L. Hsieh, P. Kukura, M. Piliarik, V. Sandoghdar, Nat. Rev. Meth. Prim. 5, 23 (2025).



openScopes: an open, modular platform to widen access and capabilities in microscopy and high content analysis

Paul French

Imperial College London, United Kingdom

I will review recent development and applications of our multidimensional fluorescence and phase imaging instrumentation and outline how we are working to widen access to such advanced imaging modalities, including for high content analysis (HCA) through our "openScopes" open instrumentation platform (www.openscopes.com).openScopes includes research-grade instruments for multidimensional fluorescence and quantitative phase imaging, super-resolved microscopy, and optical projection tomography. To widen access, we have developed an open-source modular, microscope stand, "openFrame" that can be used flexibly for affordable and sustainable instruments in lower resource (e.g., LMIC) settings or for rapidly prototyping of advanced microscopy concepts. A basic openFrame-based brightfield/fluorescence microscope (from ~£10,000) can be upgraded to almost any other microscopy modality. For HCA and slide scanning (e.g., for histopathology), we have developed a novel open-source optical autofocus module.Advanced openFrame-based instruments include automated SMLM based on easySTORM, and a modular multiphoton multiwell plate microscope (M3M) incorporating multiplexed TCSPC FLIM and quantitative phase imaging using our single-shot polarisation differential phase contrast (pDPC) technique that enables label-free single cell segmentation and tracking for long time-lapse fluorescence (FRET) assays.

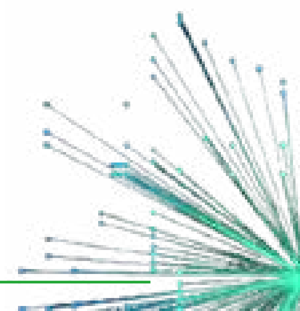
Independent component analysis disentangles fluorescence signals from diffusing single molecules

Kunihiko Ishii[1,2], Miyuki Sakaguchi[1], Tahei Tahara[1,2]

[1]Molecular Spectroscopy Laboratory, RIKEN, 2-1 Hirosawa, Wako 351-0198, Japan
[2]Ultrafast Spectroscopy Research Team, RIKEN Center for Advanced Photonics (RAP), RIKEN, 2-1 Hirosawa, Wako 351-0198, Japan

Single-molecule fluorescence (SMF) measurement, including smFRET, has become an essential tool in current biophysical research, serving as a unique probe for molecular interactions and structural heterogeneities. By applying SMF to freely diffusing molecules, one can resolve and characterize subpopulations in solutions, making it a promising approach for studying molecules in complex environments. Multiparameter fluorescence detection (MFD) is gradually being adopted in SMF. MFD incorporates additional information into SMF from measurements using fluorescence lifetime, multicolor excitation/detection, and fluorescence anisotropy. Though MFD mitigates the limitation of SMF due to signal weakness by offering enhanced information content, a general framework for data analysis has not yet been established to fully utilize its potential. We recently discovered that independent component analysis (ICA) is ideally suited for analyzing MFD-based SMF data. In this work, we developed an algorithm of ICA optimized for MFD data analysis named IFCA [1]. Applications to static and dynamic mixture systems demonstrate its potential allowing model-free separation of subpopulations with microsecond time resolution in nanomolar concentration regime. To promote its usage, we implemented the developed algorithm with Python and made it compatible with common photon data formats [2].

[1] K. Ishii, M. Sakaguchi, T. Tahara, bioRxiv, https://doi.org/10.1101/2025.03.04.641393 (2025).
[2] K. Ishii, M. Sakaguchi, T. Tahara, Zenodo, https://doi.org/10.5281/zenodo.14869092 (2025).



Imaging Beyond The Visible: advantages of the Shortwave-Infrared spectral range for confocal microscopy and Raman scattering imaging

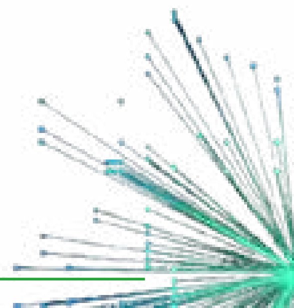
Andriy Chmyrov[1,2,3], Bernardo Arus[1,2,3], Georg Hartl[1,2], Tulio A. Valdez[4], Christopher J. Rowlands[5], Ellen Sletten[6], Oliver T. Bruns[1,2,3]

[1]National Center for Tumor Diseases, Dresden, Germany
[2]German Cancer Research Center DKFZ, Heidelberg, Germany
[3]Helmholtz Pioneer Campus, Helmholtz Zentrum München, Neuherberg, Germany
[4]Department of Otolaryngology, Head and Neck Surgery, Stanford University, Palo Alto, CA, USA
[5]Department of Bioengineering, Imperial College London, London, SW7 2AZ UK
[6]Department of Chemistry and Biochemistry, University of California, Los Angeles, CA, 90095 USA

In the realm of biomedical imaging, the utilization of the short wave infrared (SWIR) range of the electromagnetic spectrum offers substantial advantages, including deeper tissue penetration and reduced autofluorescence. These properties significantly enhance the clarity and accuracy of biological imaging. Historically, access to SWIR detectors was restricted to ordinary users due to military regulations on dual-use technologies. However, this landscape has shifted recently, with SWIR cameras and detectors becoming deregulated. Major market players have started producing InGaAs sensors at more affordable prices. This newfound accessibility paves the way for leveraging SWIR technology in bioimaging applications, including fluorescence microscopy and label-free methods.
We will report about:
[1] a SWIR line-scan one-photon confocal microscope that is capable of deep imaging of biological specimens, as demonstrated by visualization of labelled glomeruli in a fixed uncleared kidney at depths up to 400µm.
[2] benefits of SWIR range for wide field spontaneous Raman imaging on a scale of a whole small animal. With fields of view surpassing 50 cm2, we showcase the versatility of SWIR Raman imaging by monitoring body composition dynamics in living mice, non-invasively detecting liver lipid content in metabolically challenged mice, and identifying calcified areas and lipid-rich deposits in human atherosclerotic plaques.

[1] Jakob GP Lingg, Thomas S Bischof, Bernardo A Arús, Emily D Cosco, Ellen M Sletten, Christopher J Rowlands, Oliver T Bruns, Andriy Chmyrov, Laser & Photonics Reviews, 17, 2300292 (2023)

[2] Bernardo A Arus, Joycelyn Yiu, Jakob GP Lingg, Anja Hofmann, Amy R Fumo, Honglei Ji, Carolin Jethwa, Roy K Park, James Henderson, Kanuj Mishra, Iuliia Mukha, Andre C Stiel, Donato Santovito, Christian Weber, Christian Reeps, Maria Rohm, Alexander Bartelt, Tulio A Valdez, Andriy Chmyrov, Oliver T Bruns, bioRxiv, 2024.06. 10.597863 (2024)



Fluorescence-detected two-dimensional electronic spectroscopy of a single molecule

Sanchayeeta Jana, Simon Kehrer, Markus Lippitz

Experimental Physics III, University of Bayreuth, Germany

Single-molecule fluorescence spectroscopy is a powerful method that avoids ensemble averaging, but its temporal resolution is limited by the fluorescence lifetime to nanoseconds at most. At the ensemble level, two-dimensional spectroscopy provides insight into ultrafast femtosecond processes such as energy transfer and line broadening, even beyond the Fourier limit, by correlating pump and probe spectra. Here, we combine these two techniques and demonstrate coherent 2D spectroscopy of individual dibenzoterrylene (DBT) molecules at room temperature [1]. We excite the molecule in a confocal microscope with a phase-modulated train of femtosecond pulses and detect the emitted fluorescence with single-photon counting detectors. Using a phase-sensitive detection scheme, we were able to measure the nonlinear 2D spectra of most of the DBT molecules we studied. Our method is applicable to a wide range of single emitters and opens new avenues for understanding energy transfer in single quantum objects on ultrafast time scales.

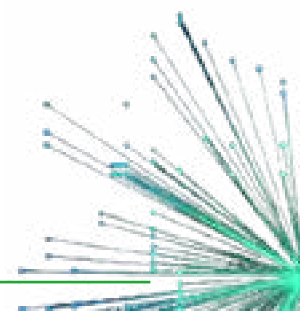
[1[S. Jana, S. Durst, M. Lippitz, Nano Letters, 24, 12576-12581 (2024)

Advancing super-resolution and single-molecule microscopy for studying molecular interactions in membranes

Christian Eggeling

Institute of Applied Optics and Biophysics, Friedrich-Schiller-University & Leibniz Institute of Photonic Technology e.V., Jena, Germany

Molecular interactions in membranes are key in cellular signalling. They are usually ruled by the organization and mobility of the involved molecules such as lipids and proteins. However, the direct and non-invasive observation of the interactions in the living cell membrane is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution and information content. Here, we present an advanced optical microscopy study involving tools such super-resolution STED microscopy in combination with spectral imaging and fluorescence correlation spectroscopy (FCS) and single-molecule tracking on a MINFLUX and iSCAT (interferometric SCATtering) microscope. We highlight issues and how to overcome them, and present how these approaches can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts e.g. during pathogen invasion.



Fluorophore blinking in superresolution microscopy, and as a rich source of moleular-scale information

Jerker Widengren

Department of Applied Physics, Royal Inst of Technology (KTH), Stockholm, Sweden

Reversible dark state transitions of fluorophores are central for all forms of fluorescence-based, single-molecule and super-resolution microscopy and spectroscopy, both as limiting factors and as prerequisites. An additional aspect of such transitions is that they also can be used to sense a manifold of biomolecular environments, dynamics and interactions. To make monitoring of dark state transitions widely applicable for studies on biological samples, we have developed so-called transient state (TRAST) imaging [1]. In TRAST, fluorophore dark state transitions are monitored via the time-averaged fluorescence intensity, and from how it varies with the modulation of the excitation light.Here, recent work within the H2020 project NanoVIB will be presented where TRAST is used to characterize dark state transitions in near-IR cyanine fluorophores, how such transitions can compromise MINFLUX super-resolution imaging, and how this knowledge allowed us to formulate strategies to overcome these problems and make near-IR MINFLUX imaging possible [2]. Also, examples will be given, demonstrating how biologically relevant environmental and molecular interaction parameters can be monitored via fluorophore dark state transitions, in solutions, live cells, and tissues, which are difficult, if possible at all, to follow via regular fluorescence readout parameters. Acknowledgements: European Commission (H2020, NanoVIB, 101017180), Swedish Research Council (VR), Swedish Foundation for Strategic Research (SSF).

[1] Widengren, J. in Fluorescence Spectroscopy and Microscopy in Biology, Springer 2023 (Sachl, R., Amaro, M.M. Eds)

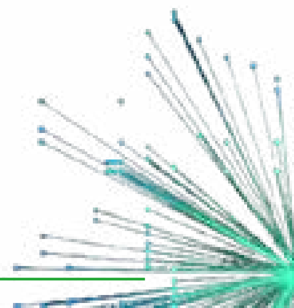
[2] Venugopal Srambickal, C. Esmaeeli H. et al., in revision 2025, BioRXiv: 10.1101/2024.08.27.609859

Adaptive Optics for Aberration Control in STED and (STED)-FCS: Advancing High-Resolution Single-Molecule Studies

Julius Trautmann, Christian Eggeling

Institute for Applied Optics and Biophysics, Friedrich-Schiller University Jena, Philosophenweg 7, 07743 Jena

We investigate the application of adaptive optics (AO) to systematically introduce and control optical aberrations in Stimulated Emission Depletion (STED) microscopy and Fluorescence Correlation Spectroscopy (FCS). FCS enables precise analysis of molecular dynamics and interactions, while STED microscopy provides super-resolved imaging beyond the diffraction limit. Their combination, STED-FCS, merges the spatial resolution of STED with the temporal sensitivity of FCS, offering a powerful approach for nanoscale single-molecule investigations. However, optical aberrations—introduced for example through optically penetrating layers of different refractive indices such as in deep biological samples or through nanomaterials—can significantly degrade imaging resolution and spectroscopic precision. To address this challenge, adaptive optical elements such as deformable mirrors (DMs) have been employed for aberration correction. By deliberately introducing and correcting specific aberrations, we assess their impact on spatial resolution, fluorescence signal, and correlation dynamics, providing critical insights for optimizing single-molecule spectroscopy in both biological and nanomaterial environments.



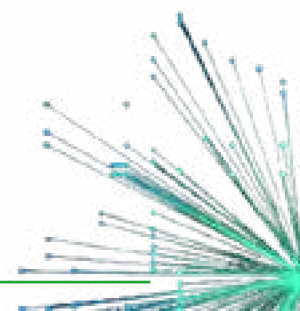
3. Müller R, König A, Groth S, Zarnowski R, Visser C, Handrianz T, Maufrais C, Krüger T, Himmel M, Lee S, Priest EL, Yildirim D, Richardson JP, Blango MG, Bougnoux ME, Kniemeyer O, d'Enfert C, Brakhage AA, Andes DR, Trümper V, Nehls C, Kasper L, Mogavero S, Gutsmann T, Naglik JR, Allert S, Hube B. Secretion of the fungal toxin candidalysin is dependent on conserved precursor peptide sequences. Nat Microbiol. 2024 Mar;9(3):669-683.

Dynamic Burst smFRET in Slow Motion: A Microfluidic Approach for Probing Biocondensates and Liposomes

Stijn Dilissen[1,2], Pedro L. Silva[1], Tom Kache[1], Ronald Thoelen[2,3], Jelle Hendrix[1]

[1]UHasselt, Dynamic Bioimaging Lab, Advanced Optical Microscopy Centre and Biomedical Research Institute, Agoralaan C (BIOMED), B3590 Diepenbeek, Belgium
[2]UHasselt, Biomedical Device Engineering group, Institute for Materials Research (IMO-IMOMEC), Wetenschapspark 1, B3590 Diepenbeek, Belgium
[3]IMOMEC Division, IMEC vzw, Wetenschapspark 1, B3590 Diepenbeek, Belgium

Burst-wise single-molecule FRET (smFRET) is a well-established technique for studying protein dynamics in solution, where molecules diffuse through the confocal volume. However, applying smFRET on slow-diffusing molecules within macromolecular assemblies, such as liposomes or biomolecular condensates formed by liquid-liquid phase separation (LLPS), is challenging. These embedded molecules diffuse slowly, and LLPS condensates often sink in solution, making Brownian diffusion-based smFRET less effective. As a result, only a small fraction of the sample is detected, and the longer burst durations increase the risk of photobleaching.
To address these challenges, we introduce a microfluidic chip that delivers precisely controlled, slow flows just above the diffusion rate of these macromolecular assemblies. By maintaining laminar flow, a more significant fraction of the sample can be efficiently probed. The adjustable flow rate allows fine-tuning burst durations: faster flows enable rapid sampling at higher laser powers. In comparison, slower flows extend bursts, enhancing temporal resolution and capturing slower molecular dynamics. Beyond flow control, the chip allows for on-chip reagent mixing or the introduction of conformational change triggers.
Altogether, this microfluidic platform enables a new way to investigate single-molecule behaviour within macromolecular assemblies like liposomes or LLPS condensates, offering deeper insight into cellular organisation and function.



Dissecting the GPCR conformational landscape using biorthogonal click chemistries and multicolor single molecule FRET

Léo Bonhomme[1], Ecenaz Bilgen[2], Nathalie Lecat-Guillet[3], Hongkang Liu[3], Caroline Clerté[1], Jean-Philippe Pin[3], Philippe Rondard[3], Don C. Lamb[2], Emmanuel Margeat[1], Robert B. Quast[1]

[1]Centre de Biologie Structurale (CBS), Univ. Montpellier, CNRS, INSERM, Montpellier, France.
[2]Department of Chemistry, Ludwig-Maximilians-Universität München (LMU), Munich, Germany.
[3]Institut de Génomique Fonctionnelle, Univ. Montpellier, CNRS, INSERM, 141 rue de la Cardonille, 34094, Montpellier Cedex 05, France.

G protein-coupled receptor (GPCR) activation is mediated by a complex interplay of conformational changes. To decipher the conformational landscape of the human metabotropic glutamate receptors 2 (mGluR2), we developed several conformational 2-color FRET sensors through incorporation of a single[1] and two distinct, reactive ncAAs combined with bioorthogonal conjugation of donor and acceptor fluorophores. We then studied ligand induced conformational rearrangements of single receptors in a carefully optimized detergent mixture[2] by smFRET using confocal, pulsed interleaved excitation and multiparameter fluorescence detection. We find that the natural full agonist glutamate is sufficient to close the Venus flytrap domains. In contrast a synergy with positive allosteric modulators, acting over a long-range functional link, is required to fully activate these multidomain neuroreceptors. Finally, using 3-color smFRET sensors, by combining double ncAA and SNAP-tag labeling, we provide evidence for a previously unknown, pre-active, intermediate state in equilibrium with the active state upon ligand activation that we could not resolve by classical 2-color smFRET[3]. We conclude an activation model where orthosteric and allosteric ligands act on different conformational equilibria between coexisting states to fine-tune mGluR2 activation. Our study highlights the power of minimally invasive, ncAA-based, bioorthogonal labeling to dissect domain-specific conformational rearrangements of GPCRs using smFRET.

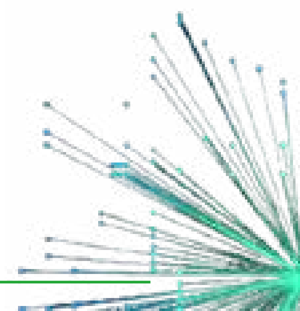
[1] Quast RB*, Lecat-Guillet N*, Liu H, Bourrier E, Møller TC, Rovira X, Soldevila S, Lamarque L, Trinquet E, Liu J, Pin JP, Rondard P, Margeat E. Sci. Adv. 9, eadf1378 (2023), https://doi.org/10.1126/sciadv.adf1378
[2] Quast RB*, Cao AM*, Fatemi F, Rondard P, Pin JP, Margeat E. Nat Commun 12, 5426 (2021), https://doi.org/10.1038/s41467-021-25620-5
[3] Bonhomme L, Bilgen E, Clerté C, Pin JP, Rondard P, Margeat E, Lamb DC, Quast RB. bioRxiv 2024, https://doi.org/10.1101/2024.10.31.621373

Applications of fluorescence lifetime measurements in flow cytometry

Jessica P. Houston

Jessica P. Houston, Ph.D., Chemical & Materials Engineering, New Mexico State University, Las Cruces, NM 88003, USA

Methods for high throughput single-cell analyses have become quite complex over the last decade with emerging technologies that advance the speed of imaging and sorting as well as enhance the number of parameters that can be measured from a single cell. Many instruments, cytometers, or similar devices provide essential features about cells because optical measurements provide not only spatial but also temporal information about the intracellular environ-ment. Time-resolved flow cytometry (TRFC) is one form of cytometry that captures temporal information about fluo-rescent molecules inside the cell. Such information does not rely on brightness and often correlates to signaling events, molecular movement, and dynamics of molecular interaction. Various TRFC technologies will be presented as well as applications that focus on metabolic mapping of tamoxifen resistant breast cancer cells using autofluorescence. Focus will also be placed on a chip-based cytometer that utilizes acoustic focusing for more accurate fluorescence lifetime measurements. The long-term impact of this work is to develop new tools that provide more quantitative fluo-rescence information at the throughput of a flow cytometer.



FRET nanoscopy maps molecules of life

Claus Seidel

Heinrich Heine University Düsseldorf, Germany

Multimodal fluorescence spectroscopy and microscopy with multiparameter detection provide rich insights on biomolecular systems with respect to structural and kinetic properties and spatial localization. Single-molecule FRET experiments offer the required nanosecond time resolution to study the motions biomolecules over a large dynamic time range from nano- to milliseconds [1,2]. If more than two FRET states are present, an appropriate analysis of a kinetic network is challenging. Thus, I introduce parametric relations (FRET-lines) [3,4] between two FRET indicators, the donor fluorescence lifetime and the intensity-based FRET efficiency, that significantly facilitate an interpretation. Model-based FRET-lines serve as pathfinders to decode the dynamic spectroscopic fingerprint by: (i) identifying the number of conformational states, (ii) resolving their dynamic connectivity, (iii) comparing different kinetic models, and (iv) inferring polymer properties of unfolded or intrinsically disordered proteins. We assessed the accuracy of this approach in a comparative single-molecule study [5] using two protein systems with distinct conformational changes and dynamics. We obtained an interdye distance precision of ≤2 Å and accuracy of ≤5 Å. Finally, I introduce a framework for FRET nanoscopy with seamless resolution accessing distances from µm down to 5 nm with a precision < 0.7 nm. To enable dissemination of all results according to the FAIR principle, we introduce the flrCIF data representation, which extends established data standards from the Protein Data Bank and allows for archiving fluorescence-aided integrative structures for multiple states together with associated kinetic data on exchange in the PDB-Dev repository.

[1] Sisamakis, E. et al.; Methods Enzymol. 475, 455-514 (2010). doi.org/10.1016/S0076-6879(10)75018-7.

[2] Lerner, E. et al; eLife 10, e60416 (2021). doi.org/10.7554/eLife.60416

[3] Barth, A. et al.; J. Chem. Phys. 156, e141501 (2022). doi.org/10.1063/5.0089134

[4] Opanasyuk, O. et al.; J. Chem. Phys. 156, e031501 (2022). doi.org/10.1063/5.0095754

[5] Agam, G. et al.; Nat. Methods 20, 523–535 (2023). doi.org/10.1038/s41592-023-01807-0

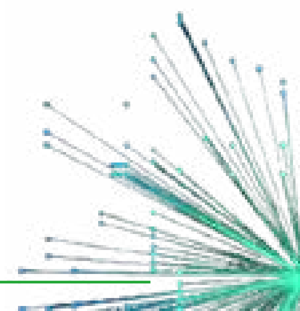
Instant FLIM in SMLM via SPAD Array Imaging

Léa Brito, Sandrine Lévêque-Fort

Institut des Sciences Moléculaires d‘Orsay, 598 Rue André Rivière, 91400 Orsay, France

Single Molecule Localization Microscopy provides a unique access to 3D nanometric cellular organization. Beyond this gain in spatial information, complementary parameters such as fluorescence lifetime imaging (FLIM) also represents a major interest for a detection at the single molecule level. This approach enables not only the identification of various dyes, but also the probing of the cellular environment. This can be achieved through time gating, where photons are collected only during a defined time window, enabling precise lifetime measurements but which should also preserve the photon budget and associated precision. As sequential gating can lead to a loss of fluorophores which turn ON-OFF stochastically, alternative faster implementation is needed.

A new technology, a 512 x 512 SPAD array initially designed for classical wide field FLIM, enables time-gated imaging with a high signal-to-noise ratio compatible with single molecule detection. Our detection module, developed in combination with this large SPAD array, enables the simultaneous acquisition of two temporal gates. This implementation allows for the concurrent reconstruction of SMLM and ratio-FLIM images over a 50 × 25 µm field of view. A complete characterization of the Instant FLIM system, along with demonstrations on biological samples, will be presented.



Investigating Spectral Fluctuations in the Emission of Halide-Perovskite Nanoparticles using Heralded Spectroscopy

Philipp Gebauer[1], Frieder Conradt[1], Chenglian Chu[2], Eva Haage[1], Ihor Cherniukh[2], Claudio Bruschini[3], Gabriele Rainò[2], Edoardo Charbon[3], Maksym Kovalenko[2], Alfred Leitenstorfer[1], Ron Tenne[4]

[1]Department of Physics and Center for Applied Photonics, University of Konstanz, D-78464 Konstanz, Germany
[2]Institute of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland
[3]School of Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Neuchâtel 2002, Switzerland
[4]Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, 3200003 Haifa, Israel

Thanks to their bright and tunable emission, colloidal nanoparticles are strong candidates for use in modern electro-optical applications and biological imaging. Halide-perovskite nanoparticles have recently emerged as a promising candidate for generating quantum states of light [1]. However, instabilities in the spectrum of emitted light remain a major challenge, significantly hindering their practical integration into future quantum systems [2].
To better understand the mechanisms behind these spectral instabilities, we employ Heralded Spectroscopy - a recently developed method to measure spectrally resolved photon correlations [3]. It enables the detection of single-photon events with high temporal (~ 100 ps) and spectral (~ 0.2 meV) resolution using a Single-Photon Avalanche Diode (SPAD) array. We use this setup to measure spectral fluctuations of individual nanoparticles under cryogenic conditions. This enables us to extend the statistical analysis to visually trace spectral fluctuations across more than ten orders of magnitude in the time domain, down to nanoseconds. We find a critical dependence of the magnitude of spectral fluctuations on the laser excitation power. Surprisingly, we observe a power-induced transient increase in the lattice symmetry. To understand this exciting finding, we intend to perform a systematic investigation of particles with varying sizes and shapes.

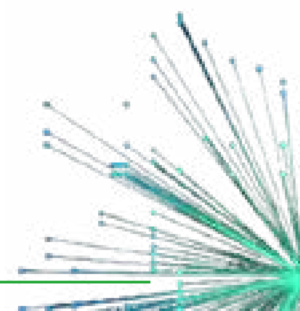
[1] Kaplan, A.E.K., Krajewska, C.J., Proppe, A.H. et al., Nat. Photon. 17, 775-780 (2023).
[2] F. Conradt, V. Bezold, V. Wiechert, S. Huber, S. Mecking, A. Leitenstorfer and R. Tenne, Nano Lett., 23, 21 (2023)
[3] G. Lubin, R. Tenne, A. C. Ulku, I. M. Antolovic, S. Burri, S. Karg, V. J. Yallapragada, C. Bruschini, E. Charbon and D. Oron, Nano. Lett., 21, 16 (2021)

Characterizing Barrier Crossing Dynamics of Protein Folding Through Transition Paths Using Single-molecule FRET in Zero-mode Waveguides

Chi-Jui Feng, Ulrich Baxa, John M Louis, Hoi Sung Chung

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, United States

Transition paths (TPs) describe short-lived, single-molecule barrier crossing events that encode conformational dynamics of the molecular transitions, including folding and binding of proteins and nucleic acids. Single-molecule spectroscopy has been hailed to study properties associated with the TPs including TP times around 10–100 μs, free energy landscape along one-dimensional distance coordinate, and diversity of the TPs. However, in single-molecule FRET (smFRET), limited brightness impedes characterization of the TPs around microsecond or shorter. To enhance the brightness to study protein folding TPs, we combined smFRET spectroscopy with zero-mode waveguides. Waveguides successfully enhanced the brightness to several MHz to study microsecond TPs. We surveyed several proteins with varied sizes, secondary structures, and folding rates to discuss relations between various physicochemical properties, kinetics, and barrier crossing dynamics. We found a strong determinant of the TP times is curvature modulated by the barrier height, rather than number of native contacts or size of proteins. We also found that diffusivity of native contact formation empirically scales with the absolute contact order, which is a manifestation of kinetic cooperativity in contact formation. We expect this approach serves as an effective modality to study fast kinetics and dynamics in general folding and binding processes.



Leveraging DNA Nanotechnology for Single-Molecule Optical Sensing

Viktorija Glembockyte

Max Planck Institute for Medical Research, Heidelberg, Germany

Optical labels and sensors are invaluable tools for understanding biological processes and dissecting disease mechanisms. The advent of single-molecule and super-resolution imaging techniques has placed increasingly stringent demands on these probes, necessitating high photostability for labels and single-molecule sensitivity with high optical contrast for sensors. This presentation will detail our recent efforts to address these challenges by combining single-molecule imaging with DNA nanotechnology. Specifically, we utilize DNA origami to decouple sensing from signal output, enabling the creation of modular and tunable sensor platforms that exhibit both large Förster Resonance Energy Transfer (FRET) contrast and single-molecule sensitivity (Nat. Nanotech. 2025, 20, 303). The inherent modularity of this DNA origami approach allows for the development of single-molecule sensors targeting diverse analytes, including nucleic acids, antibodies, and enzymes, simply by exchanging sensing elements. Furthermore, the incorporation of multiple sensor elements facilitates cooperativity, tunable dynamic range, and advanced logic sensing operations.While DNA nanotechnology holds immense promise for biomedical applications, its widespread utility is currently limited by the inherent instability of DNA nanostructures within complex biochemical environments. We will also discuss our ongoing research aimed at understanding and enhancing the addressability and functionality of DNA nanodevices, leveraging the power of single-molecule and super-resolution imaging (Adv. Mater. 2023, 35, 2212024; Small 2025, in press).

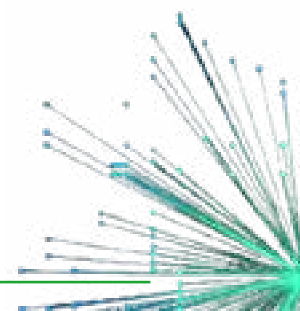
Probing rapid biomolecular dynamics with single-molecule spectroscopy

Ben Schuler

Universität Zürich, Switzerland

Single-molecule spectroscopy enables biomolecular dynamics to be investigated across more than twelve orders of magnitude in time1, which allows us to probe the molecular mechanisms of a wide range of biological processes. By combining single-molecule FRET with nanosecond FCS, dynamics in the submicrosecond range can be resolved, even in complex environments, such as biomolecular condensates2 and live cells3. I will illustrate recent advances in probing the nanosecond dynamics of disordered proteins4, 5 and nucleic acids6, the increasing synergy with molecular simulations, and the accuracy with which distance distributions and dynamics in such systems can be obtained7.

1. Nettels D., Galvanetto N., Ivanovic M. T., Nüesch M., Yang T. J., Schuler B. Single-molecule FRET for probing nanoscale biomolecular dynamics. Nat. Rev. Phys. 6, 587-605 (2024).
2. Galvanetto N., Ivanovic M. T., Chowdhury A., Sottini A., Nuesch M. F., Nettels D., . . . Schuler B. Extreme dynamics in a biomolecular condensate. Nature 619, 876-883 (2023).
3. König I., Soranno A., Nettels D., Schuler B. Impact of In-Cell and In-Vitro Crowding on the Conformations and Dynamics of an Intrinsically Disordered Protein. Angew. Chem. Int. Ed. 60, 10724-10729 (2021).
4. Nüesch M. F., Ivanovic M. T., Claude J. B., Nettels D., Best R. B., Wenger J., Schuler B. Single-molecule Detection of Ultrafast Biomolecular Dynamics with Nanophotonics. J. Am. Chem. Soc. 144, 52-56 (2022).
5. Chowdhury A., Nettels D., Schuler B. Interaction Dynamics of Intrinsically Disordered Proteins from Single-Molecule Spectroscopy. Annu. Rev. Biophys. 52, 433-462 (2023).
6. Nüesch M. F., Pietrek L., Holmstrom E. D., Nettels D., von Roten V., Kronenberg-Tenga R., . . . Schuler B. Nanosecond chain dynamics of single-stranded nucleic acids. Nat. Commun. 15, 6010 (2024).
7. Nüesch M., Ivanovic M. T., Nettels D., Best R. B., Schuler B. Accuracy of distance distributions and dynamics from single-molecule FRET. Biophys. J., in press (2025).



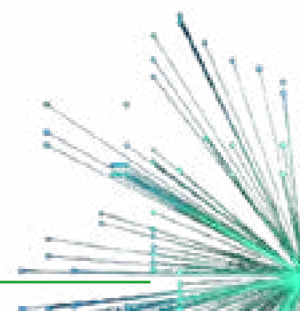
Investigation of DNA and DNA-Protein-Interaction on the Nanometer Scale using Graphene Energy Transfer

Jakob Hartmann[1], Lars Richter1, Giovanni Ferrari[1], Alan Szalai[1], Merve-Zeynep Kesici[1], Bosong Ji1, Izabela Kamińska[1,2] and Philip Tinnefeld[1]

[1] Department of Chemistry and Center for Nanoscience (CeNS), Ludwig Maximilian University of Munich, Germany
[2] Institute of Physical Chemistry of the Polish Academy of Sciences, 01-224 Warsaw, Poland

Graphene energy transfer (GET) has been successfully used to achieve nanometer-scale spatial resolution by accurately measuring fluorophore distances of up to 40 nm from the graphene surface.[1, 2, 3, 4]
In the novel method for Graphene Energy Transfer with vertical Nucleic Acids (GETvNA), a single-strand DNA/double-strand DNA hybrid is immobilized on the graphene surface with the double-stranded DNA part standing vertically.[5] At the upper end of the DNA a fluorescent dye is positioned, allowing for precise distance determination to the graphene with sub-nanometer accuracy.
With this high axial resolution, GETvNA enabled the detection of a single base pair difference corresponding to 0.35 nm. In addition to resolving individual molecular heights, it allowed the characterization of DNA conformational changes induced by A-tracts, bulges, or mismatches, some of which exhibited multiple bending states. Incorporation of an abasic site within the DNA construct further facilitated the observation of conformational changes upon interaction with E. coli endonuclease IV and AP endonuclease 1. The fluorescence time traces revealed dynamic bending transitions occurring on the millisecond timescale.
In addition, through the direct contact of the DNA to the graphene, novel photophysical phenomena can be recognized and precisely characterized by shrinking gate fluorescence correlation spectroscopy.[6]

[1] A. Ghosh, A. Sharma, A.I. Chizhik, et al., Nat. Photonics, 2019, 13, 860–865.
[2] I. Kaminska, J. Bohlen, S. Rocchetti, F. Selbach, G. P. Acuna, P. Tinnefeld, Nano letters 2019, 19, 4257–4262.
[3] I. Kamińska, J. Bohlen, R. Yaadav, P. Schüler, M. Raab, T. Schröder, J. Zähringer, K. Zielonka, S. Krause, P. Tinnefeld, Advanced materials 2021, 33, e2101099.
[4] J. Zähringer, F. Cole, J. Bohlen, F. Steiner, I. Kamińska, P. Tinnefeld, Light science & applications 2023, 12, 70.
[5] A. M. Szalai, G. Ferrari, L. Richter, J. Hartmann, M.-Z. Kesici, B. Ji, K. Coshic, A. Jaeger, A. Aksimentiev, I. Tessmer, I. Kamińska, A. M. Vera, P. Tinnefeld, Nat. Methods 2024, 22, 135-144.
[6] T. Schröder, J. Bohlen, S.E. Ochmann, P. Schüler, S. Krause, D.C. Lamb, P. Tinnefeld, Proc. Natl. Acad. Sci. U.S.A. 2023, 120 (4), e2211896120.



Pinpointing Polymer–Active-Catalyst Speciation in Solution

Vy Pham

Department of Chemistry, University of California, Irvine; Irvine, California, 92697, United States

A small fraction of catalysts can dictate the bulk chemical reactivity observed on scale. However, the assignment of activity to specific catalytic species is often obscured by the limits of detection sensitivity and/or dynamic range of traditional analytical techniques. Here, a fluorescence correlation spectroscopy (FCS) method is developed to assign catalyst activity to polymers of a specific apparent size in solution. This method was enabled by doping a fluorescent monomer into growing polydicyclopentadiene (polyDCPD) or polynorbornene during ring-opening metathesis polymerization (ROMP). By design, only polymers with active catalyst chain ends were detected, without convolution by species bearing inactive complexes. Data showed that polymers continued to aggregate, and catalytic activity continued to decrease, despite no detectable changes in the polymers or catalysts by 1H NMR spectroscopy and gel-permeation chromatography. Catalysts in polyDCPD aggregates were more persistent than those in polynorbornene aggregates. Assigning such behaviors underpins long-term goals in the development of latent catalysis and of nanostructures that possess size-dependent catalytic activity.

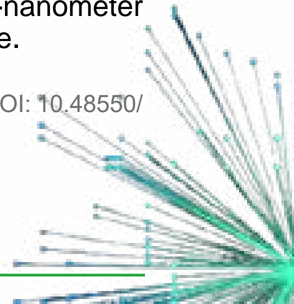
Performing and Analyzing FRET Nanoscopy Measurements on DNA-Origami Platforms with sub-Nanometer Precision

Noah Salama[1], Jan-Hendrik Budde[1], Nicolaas T. M. van der Voort1, Suren Felekyan[1], Julian Folz1, Ralf Kühnemuth[1], Christian Hanke[1], Paul Lauterjung[1,2], Michelle Rademacher[1,3], Markus Köhler[4], Andreas Schönle[4], Julian Sindram[5], Marius Otten[5], Matthias Karg[5], Christian Herrmann[2], Anders Barth[1,6], Claus A. M. Seidel[1]

[1]Chair for Molecular Physical Chemistry, Heinrich-Heine-University Düsseldorf, Germany
[2]Physical Chemistry I, Ruhr-Universität Bochum, Germany
[3]Institute for Physical Chemistry II, Heinrich-Heine-Universität Düsseldorf, Germany
[4]Abberior Instruments GmbH, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany
[5]Lehrstuhl für Kolloide und Nanooptik, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany
[6]PicoQuant, Rudower Chaussee 29, 12489 Berlin, Germany

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, DOI: 10.48550/arXiv.2108.00024



Single-Molecule FRET in Living Cells

Thorsten Hugel

Institute of Physical Chemistry, University of Freiburg, Germany, Signalling research centers BIOSS and CIBSS, University of Freiburg, Germany

Time-resolved single-molecule Förster Resonance Energy Transfer (smFRET) measurements provide quantitative insights into protein dynamics. While extensively applied in vitro, their use in living systems remains limited.
Here, we integrate smFRET with single-protein tracking in the cytosol of living HeLa cells [1], allowing us to obtain the first time-resolved smFRET traces of the heat shock protein Hsp90 in its native cellular environment. This approach enables direct comparisons between the in vitro and in vivo behavior of Hsp90.
Previous in vitro studies have revealed large conformational changes in Hsp90, closely associated with cochaperone and substrate interactions [2]. Our findings show that these dynamic structural transitions also occur in the cytosol of living cells. This advancement paves the way for investigating how drugs and protein modifications influence these conformational changes and, consequently, Hsp90's chaperone functionality in vivo.
In summary, our results highlight the necessity of combining smFRET and tracking measurements to explore protein conformational dynamics in living cells, providing a powerful tool to observe Hsp90 in action within its physiological context.

[1] A. Anandamurugan et al., bioRxiv (2023)
[2] L. Vollmar et al., Nat. Commun. 15, 569 (2024)

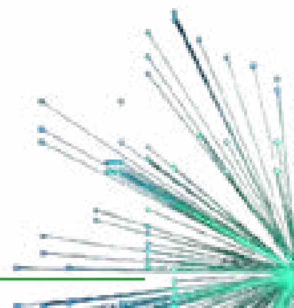
Material properties of biomolecular condensates emerge from nanoscale dynamics

Nicola Galvanetto[1], Milos Ivanovic[1], Aritra Chowdhury[1], Daniel Nettels[1], Robert Best[2], Ben Schuler[1]

[1]University of Zurich, Zurich, Switzerland
[2]National Institutes of Health, Bethesda, MD, USA

Biomolecular condensates are droplets-like structures originating from phase-separation of biomolecules. The functions of condensates within living cells span many length scales: From the modulation of chemical reactions at molecular scale to the compartmentalization of the cell. We employed single-molecule experiments (1) to study the conformations and dynamics of proteins within single droplets (2), combined with microrheology to assess cell-scale properties. We found that material properties relevant for subcellular organization — such as condensate fusion times and viscosity —quantitatively emerge from the nanosecond dynamics of individual proteins (3). Atomistic simulations reveal that the rapid exchange of inter-residue contacts we observe may be a general mechanism for preventing dynamic arrest in compartments densely packed with polyelectrolytes, such as the cell nucleus. Overall, these results indicate that phase-separated systems, despite their high macroscopic viscosity, allow for rapid local biomolecular rearrangements essential for efficient molecular-scale reactions.

1. D. Nettels, N. Galvanetto, et al., Nat Rev Phys 6, 587–605 (2024).
2. N. Galvanetto, et al., Nature 619, 876–883 (2023).
3. N. Galvanetto, et al., arXiv:2407.19202 (2024).



Single-cell time-resolved multiparameter fluorescence spectroscopy of phytoplankton

Eitan Lerner[1,2], Nir Keren[3,4], Nadav Ben-Eliezer[3], Paul David Harris[1]

[1]Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel
[2]The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Israel
[3]Department of Plant Sciences, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel
[4]The Interuniversity Institute for Marine Sciences, Eilat, Israel

Aquatic phytoplankton are in charge of the majority of photosynthesis on earth, and serve as the main contributors to primary productivity. Cells of these species carry molecular systems that specialize in funneling excitation energy for use in photosynthesis-related charge separation, and subsequently respiration. These systems include photosystems embedded in membranes, and phycobilisomes on top of photosystems to optimize funneling of energy of the visible range of the electromagnetic spectrum. Such well-controlled highly-efficient use of light is driven by the spatial organization of specific pigments and by excitation energy transfer between them at different coupling regimes. As such, the autofluorescence of unicellular phytoplankton species is meaningful and therefore can be used for identifying different species and their metabolic responses to certain environmental changes.
In this talk I will showcase a single-cell time-resolved multiparameter fluorescence spectroscopy approach to track photo-physiological changes that phytoplankton species undergo when acclimating to different external stresses with direct relevance to ecology.

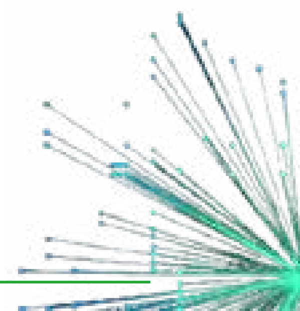
Paul David Harris, Nadav Ben Eliezer, Nir Keren, Eitan Lerner. (2024). Phytoplankton cell-states: multiparameter fluorescence lifetime flow-based monitoring reveals cellular heterogeneity. FEBS Journal 291(18): 4125

Pulsed Interleaved Excitation for enhanced FRET sensing in an Anti-Brownian ELectrokinetic (ABEL) Trap: ABEL-PIE

Allison Squires

University of Chicago, USA

Single-molecule measurements capture rare and asynchronous events, revealing rich mechanistic detail that complements and deepens our understanding from bulk experiments. The Anti-Brownian ELectrokinetic (ABEL) Trap is a versatile platform for single-molecule fluorescence spectroscopy that can record and control complex, dynamic energy transfer pathways in both natural and engineered macromolecular systems. In the ABEL trap, single nanoscale particles are confined within a confocal spot using closed-loop electrokinetic feedback forces to counteract the particle's Brownian motion, allowing photon-by-photon acquisition of rich, multi-parameter spectroscopic data. Away from the perturbative influence of surfaces and tethers, the ABEL trap provides an isotropic, single-molecule level view of biomolecules dynamically interacting in free solution, either by co-localization or FRET. Recently, we have introduced modulation of excitation in the ABEL trap by pulsed interleaved excitation (ABEL-PIE), which opens up new possibilities for precision spectroscopy at the single-molecule level. In addition to providing an excellent built-in reference for FRET measurements, ABEL-PIE enables direct readout of stoichiometry and protein-protein interactions in macromolecular complexes. Here, I will present how ABEL-PIE has enhanced our measurements on systems including light-harvesting supercomplexes, phase-separated protein condensates, and engineered spectroscopic labels constructed from a minimal set of chemical building blocks.



Ligand-Binding Kinetics to G-Quadruplex DNA: Insights from FCS and Molecular Simulations

Sobhan Sen

School of Physical Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Guanine-rich DNA sequences can fold into G-quadruplex (GqDNA) structures in the presence of monovalent cations. These non-canonical DNA conformations are implicated in key biological processes, offering selective binding sites for small molecules (ligands) with potential applications in anticancer therapy and gene regulation. Understanding the kinetics of ligand binding and unbinding to GqDNA is critical for both biological insight and pharmacological development. This talk will demonstrate how fluorescence correlation spectroscopy (FCS) and molecular dynamics (MD) simulations can be synergistically employed to dissect the thermodynamics and kinetics of ligand–GqDNA interactions under both dilute and molecularly crowded conditions, simulating the intracellular environment [1–5]. Our experiments reveal that saccharide and polyethylene glycol (PEG) crowders modulate ligand binding kinetics to GqDNA differentially. Atomistic MD simulations elucidate the molecular underpinnings of ligand stabilization and destabilization in the absence and presence of various crowders. Additionally, metadynamics simulations uncover the free energy landscapes and rate-limiting steps governing these interactions, in strong agreement with our experimental data. Together, these results provide a comprehensive, multiscale view of how ligand–GqDNA interaction dynamics occur in dilute and crowded conditions.

[1] Alam, P. et al. J. Phys. Chem. B 2025, 129, 2958.

[2] Alam, P. et al. Methods Appl. Fluoresc. 2024, 12, 045002.

[3] Clovis, N.S. et al. J. Photochem. Photobiol. A Chem. 2023, 437, 114432.

[4] Clovis, N. S. and Sen, S. J. Phys. Chem. B 2022, 126, 6007.

[5] Verma, S. D. et al. Anal. Chem, 2012, 84, 7218.

Simultaneous high-resolution fluorescence and voltage clamp measurements on free-standing membranes on a chip

Jan C. Behrends

Department of Physiology, University of Freiburg., Hermann-Herder-Str. 7, Freiburg, Germany

Voltage clamp analysis in the form of single ion channel recording was the first biophysical technique to attain single molecule resolution 50 years ago. The first speculation as to the feasibility of combined spectroscopic and electrical recordings techniques in single channel studies dates back 30 years.1 Such experiments are notoriously difficult to set up, especially if high resolution is to be preserved for both modalities. In addition the compounded propensities for the rupturing of membranes, bleaching of fluorophores and other mishaps makes successful experiments a rarity when single membranes are used.2

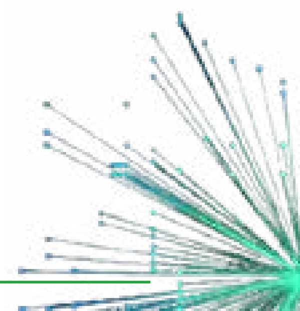
I will report on recent progress based on a variant of the microelectrode cavity array (MECA) chip3 with microelectrodes shaped to leave an optical window through which a free-standing membrane can be addressed with high-NA optics.4 This MECAopto system is currently used both for widefield and confocal/FCS analysis of membranes with pore-forming peptides and proteins and promises to enable combined electrical-optical recording from reconstituted protein ion channels.

[1] MacDonald, A. G.; Wraight, P. C. Combined Spectroscopic and Electrical Recording Techniques in Membrane Research: Prospects for Single Channel Studies. Prog Biophys Mol Bio 1995, 63, 1-29.

[2] Borisenko, V., T. Lougheed, J. Hesse, E. Fureder-Kitzmuller, N. Fertig, J.C. Behrends, G.A. Woolley, and G.J. Schutz. 2003. Simultaneous optical and electrical recording of single gramicidin channels. Biophys. J. 84:612-622.

[3] Baaken, G., M. Sondermann, C. Schlemmer, J. Rühe, and J.C. Behrends. 2008. Planar microelectrode-cavity array for high-resolution and parallel electrical recording of membrane ionic currents. Lab Chip. 8:938-944. doi:10.1039/b800431e.

[4] Ensslen, T., and J.C. Behrends. 2022. A chip-based array for high-resolution fluorescence characterization of free-standing horizontal lipid membranes under voltage clamp. Lab Chip. 22:2902-2910.



SPAD array detector enables a large localization range in MINFLUX

Eli Slenders[1], Sanket Patil[1,2], Marcus Oliver Held[1], Alessandro Zunino[1], Giuseppe Vicidomini[1]

[1]Molecular Microscopy and Spectroscopy, Istituto Italiano di Tecnologia, Genoa, Italy
[2]Department of Informatics, Bioengineering, Robotics and Systems Engineering (DIBRIS), University of Genoa, Italy

The MINFLUX concept overcomes a fundamental limitation of conventional single-molecule localization microscopy (SMLM): i.e., the localization uncertainty is limited by the number of photons emitted by the molecule [1]. MINFLUX-based microscopes [2,3] achieve a low uncertainty by scanning a zero-intensity focus around the molecule, typically in a circular trajectory. For a given number of photons, a smaller trajectory diameter results in a better localization uncertainty. However, MINFLUX requires the molecule to be inside the scanned trajectory. This requirement is typically fulfilled with an iterative scheme with a decreasing trajectory diameter in every iteration, thus demanding extra photons and increasing the microscope complexity.
Here, we demonstrate how single-photon avalanche diode (SPAD) array detectors [4] can solve these limitations. We propose a simple MINFLUX system based on a conventional confocal laser-scanning microscope equipped with a SPAD array detector, providing true MINFLUX localization uncertainty within the scanned trajectory and conventional photon-limited uncertainty outside it. We call our technique ISM-FLUX [5] and demonstrate its large localization range and single-digit localization uncertainty on proof-of-concept measurements of fixed fluorophores and DNA-origami nanorulers with 20 nm and 40 nm spacings. We expect the robustness and simplicity of this MINFLUX implementation to facilitate the widespread adoption of MINFLUX.

[1] E. Betzig et al., Science, 313, 1642-1645 (2006).
[2] F. Balzarotti et al., Science, 355, 606-612 (2016).
[3] L. Masullo et al., Light: Sci. Appl., 11, 1-9 (2022).
[4] M. Castello et al., Nat. Methods, 16, 175-178 (2019).
[5] E.Slenders and G. Vicidomini, Phys. Rev. Res. 5, 023033 (2023).

Uncovering Shared Routes of Nuclear Import and Export Using Dual-Color MINFLUX

Abhishek Sau[1], Sebastian Schnorrenberg2, Ziqiang Huang[2], Debolina Bandyopadhyay[1], Ankith Sharma[1], Clara-Marie Gürth[3], Sandeep Dave[1], Siegfried M. Musser*[1]

[1]Department of Cell Biology and Genetics, Texas A&M University; College Station, TX, USA
[2]EMBL Imaging Centre, European Molecular Biology Laboratory; Heidelberg, Germany
[3]Abberior Instruments GMBH, Göttingen, Germany

The nuclear pore complex (NPC) facilitates nucleocytoplasmic transport, enabling the bidirectional exchange of proteins and nucleic acids with remarkably high throughput, a process disrupted in diseases such as ALS, Alzheimer's, Huntington's, and viral infection. While distinct import and export pathways could, in principle, prevent collisions and streamline opposing traffic, direct visualization of the three-dimensional (3D) nanoscale dynamics within the NPC remains a major challenge—particularly at the millisecond timescales relevant to transport. Recently, we employed 3D MINFLUX microscopy to first identify the NPC scaffold, and subsequently to simultaneously track nuclear import and export. Our results show that both processes occur within overlapping regions of the central channel. Translocation-arrested import complexes localized to the periphery, whereas translocating complexes favored a ~40–50 nm diameter annular region, exhibiting minimal circumferential motion. Strikingly, both import and export events were largely confined to a single octant, likely reflecting the rotational symmetry and structural constraints of the NPC. The apparent absence of transport near the central axis suggests the presence of a plug or structural occlusion. Furthermore, transport within the pore was approximately 1000-fold slower than in free solution and characterized by intermittent pauses, implying a constrained environment shaped by structural hindrances or transient interactions. These findings underscore the benefits of MINFLUX in achieving high spatiotemporal resolution with minimal photobleaching and reveal that the NPC permeability barrier comprises at least three concentric zones: a non-binding central core, a dynamic transport annulus, and a peripheral zone with high-affinity interactions (Ref.1)

[1] Sau, Abhishek; Schnorrenberg, Sebastian; Huang, Ziqiang; Bandyopadhyay, Debolina; Sharma, Ankith; Gürth, Clara-Marie; Dave, Sandeep; Musser, Siegfried M, Nature, (2025) doi.org/10.1038/s41586-025-08738-0

From Bio Sensing to Soft Robotics with DNA Na notec

Philip Tinnefeld

Ludwig-Maximilians-University Munich, Germany

Merging DNA nanotech with single molecule detection allows visualizing molecular processeswith ultimate resolution and sensitivity. Here, we show how DNA origami can be the key toadvance biosensing schemes with respect to sensitivity, specificity and programmability. Fluorescence signals are physically enhanced by DNA origami nanoantennas for attomolardetection of pathogenic nucleic acids towards points of care molecular diagnostics. 1,2Biosensors are adapted in the working range and cooperativity without changing thebiorecognition elements using avidity and lever effects. 3 Combining different d y namic DNAelements allows DNA computing that benefits from Brownian moti on and does not protect thestability of s tates against it. The vision is to develop molecular robotic systems that processcomplex inputs, compute autonomously and provide light signals or cargo release as output. 4,5

[1] Yaadav, R., Trofymchuk, K., Dass, M., Behrendt, V., Hauer, B., Schütz, J., . . . Tinnefeld, P. Bringing Attomolar Detection to the Point of Care with Nanopatterned DNA Origami Nanoantennas. bioRxiv , 2024.10.14.618183 (2024).

[2] Trofymchuk, K., Glembockyte, V., Grabenhorst, L., Steiner, F., Vietz, C., Close, C., . . . Tinnefeld, P. Addressable nanoantennas with cleared hotspots for single molecule detection on a portable smartphone microscope. Nat Commun 12 , 950 (2021)

[3] Grabenhorst, L., Pfeiffer, M., Schinkel, T., Kummerlin, M., Bruggenthies, G.A., Maglic, J.B., . . . Glembockyte, V. Engineering modular and tunable single molecule sensors by decoupling sensing from signal output. Nat Nanotechnol 20 , 303 310 (2025).

[4] Pfeiffer, M., Cole, F., Wang, D., Ke, Y. & Tinnefeld, P. Spring loaded DNA origami arrays as energy supplied hardware for modular nanorobots. bioRxiv , 2024.09.30.615428 (2024)

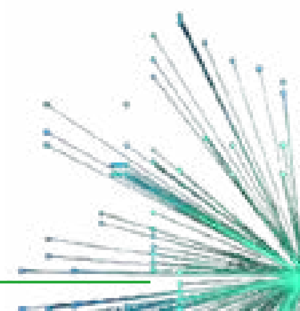
[5] Cole, F., Pfeiffer, M., Wang, D., Schroder, T., Ke, Y. & Tinnefeld, P. Controlled mechanochemical coupling of anti junctions in DNA origami arrays. Nat Commun 15 , 7894 (2024)

Resolving individual multi-molecular interactions in living cells

María García-Parajo

ICFO, Barcelona, Spain

In the last two decades, plasma membrane compartmentalization has emerged as a dominant feature present at different spatiotemporal scales and regulating key cell functions. Super-resolution microscopy and single molecule imaging have shown that receptor functioning in the plasma membrane is influenced by their dynamic interaction with other molecules and the surrounding environment. However, having access to the dynamic re-modeling of the environment, impact in receptor function, and real-time interactions between different molecules remains challenging. I will discuss an approach based on high-density, multicolor single particle tracking to map how individual molecules explore their dynamic environment, and to uncover dynamic multi-molecular interactions in real time. We have applied this methodology to capture real-time interactions between individual virus-like-particles (VLPs) and three different viral (co-) receptors on the plasma membrane of immune cells. Together with quantitative tools, our approach revealed the existence of a coordinated spatiotemporal diffusion of the three different (co-)receptors prior to viral engagement. Such a concerted diffusion impacted on the residence time of HIV-1 and SARS-CoV-2 VLPs on the host membrane and potential viral infectivity. Overall, our methodology can be easily implemented for the investigation of other multi-molecular systems at the single-molecule level.

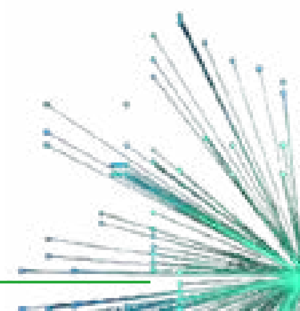


Molecular resolution fluorescence imaging in cells

***Markus Sauer*[1,2]**

1Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
[2]Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, Germany

Over the past decade, super-resolution fluorescence imaging by single-molecule localization has evolved as a powerful method for subdiffraction-resolution fluorescenceimaging of cells and structural investigations of subcellular structures. However, although refined single-molecule localization microscopy (SMLM) methods can now provide a spatial resolution in the one-digit nanometer range on isolated molecules, that is, well below the diffraction limit of light microscopy, translation of such high spatial resolutions to sub-10 nm imaging in cells or tissues remains challenging. This is mainly caused by the insufficient labeling density and linkage error achieved using standard labeling methods. Furthermore, even if high density labeling can be realized fluorophore communication via different energy pathways can prevent reliable molecular resolution fluorescence imaging in cells. In my contribution I will introduce and discuss different methods to bypass these limitations. One is based on physical expansion of the cellular structure by linking a protein of interest into a dense, cross-linked network of a swellable polyelectrolyte hydrogel. By combining ~8-fold Expansion Microscopy (ExM) with direct stochastic optical reconstruction microscopy (dSTORM) on post-expansion immunolabeled samples we resolve the 8-nm periodicity of a,ß-heterodimers in microtubules and the polyhedral lattice in clathrin-coated pits with nanometer resolution in intact cells. Furthermore, I will demonstrate that 2-color Ex-dSTORM reveals the molecular organization of endogeneous RIM scaffolding proteins and Munc13-1, an essential synaptic vesicle priming protein, in ring-like structures with diameters of 20-30 nm at the presynapse in hippocampal neurons. Furthermore, I will discuss an alternative approach that uses genetic code expansion (GCE) and click labeling of unnatural amino acids to introduce fluorophores site-specifically into multimeric proteins with minimal linkage error. Using resonance energy transfer between fluorophores separated by less than 10 nm, information about the distance of the fluorophores in cells separated by only a few nanometers can be unraveled using fluorescence photoswitching characteristics. Using time-resolved fluorescence detection in combination with this so-called photoswitching fingerprint analysis interfluorophore distances of only a few nanometers can be reliably resolved, even in living cells. Finally, I will demonstrate that the use of these tools in combination with fixed and live-cell lattice-light-sheet microscopy can be used advantageously to decode the molecular interplay of endogenous CD20 on tumor cells with therapeutic antibodies.



Fast volumetric fluorescence lifetime imaging of multicellular systems using single-objective light-sheet microscopy

Valentin Dunsing-Eichenauer[1,4], Johan Hummert[2], Claire Chardès[1], Thomas Schönau[2], Léo Guignard[1], Max Tillmann[2], Felix Koberling[2], Corinna Nock[2], Ivan Michel Antolovic3, Rainer Erdmann[2], Pierre-François Lenne[1]

[1]Aix-Marseille Université & CNRS, IBDM—UMR7288 & Turing Centre for Living Systems, Marseille, France
[2]PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
[3]Pi Imaging Technology SA, EPFL Innovation Park, 1015 Lausanne, Switzerland
[4]Department of Infectious Diseases and Respiratory Medicine, Charite-Universitätsmedizin Berlin

Fluorescence lifetime imaging (FLIM) is widely used for functional and multiplexed bioimaging. The lifetime of autofluorescence or fluorescent sensors encodes physiologically relevant parameters. Thus, FLIM is especially relevant for the investigation of living systems. However, application of FLIM to live specimen is hampered by its slow speed and high phototoxicity. To enable faster and gentler FLIM, we integrated single-objective light-sheet microscopy with pulsed excitation and time-resolved detection on a novel SPAD array detector [1]. We achieved 10-100-fold acceleration compared to confocal FLIM, down to 100 ms acquisition time per image, with excellent quantitative agreement. The massively enhanced speed enables volumetric FLIM acquisitions on live multicellular specimens, which we demonstrate with lifetime-based multiplexing in 3D and time-lapse FLIM of tension probes on living embryonic organoids. We benchmark both scanned and static light-sheet modalities to facilitate adding FLIM capability to a large variety of light-sheet microscopes.

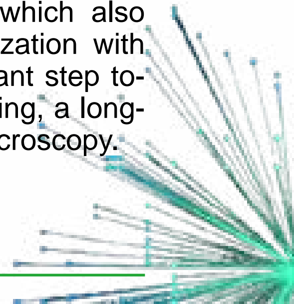
[1] bioRxiv 2024.03.24.586451; doi: https://doi.org/10.1101/2024.03.24.586451

Fast and multiplexed super-resolution imaging of cells

Roman Tsukanov[1], Samrat Basak[1,8], Nazar Oleksiievets1, Daniel C. Jans[2,3], Stefan Jakobs[2,3,4,5], Felipe Opazo[6,7,9], Jörg Enderlein[1,5]

[1]III. Institute of Physics – Biophysics, Georg August University, 37077 Göttingen, Germany.
[2]Department of NanoBiophotonics, Max Planck Institute for Multidisciplinary Sciences, 37077 Göttingen, Germany
[3]Department of Neurology, University Medical Center Göttingen, 37073 Göttingen, Germany.
[4]Translational Neuroinflammation and Automated Microscopy, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, 37073 Göttingen, Germany.
[5]Cluster of Excellence “Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells” (MBExC), Georg August University, 37077 Göttingen, Germany.
[6]Institute of Neuro-and Sensory Physiology, University Medical Center Göttingen, 37073 Göttingen, Germany.
[7]NanoTag Biotechnologies GmbH, 37079 Göttingen, Germany.
[8]Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 München, Germany
[9]Center for Biostructural Imaging of Neurodegeneration (BIN), University of Göttingen Medical Center, 37075 Göttingen, Germany.

DNA-PAINT is a powerful Single-Molecule Localization Microscopy (SMLM) technique that enables imaging with nanometer localization precision. It is a perfect fit for multi-target super-resolution imaging - such as through Exchange-PAINT. A key advantage of Exchange-PAINT is that the same fluorophore can be used to image all targets, completely avoiding chromatic aberration. However, its main drawback is long acquisition times, which scale linearly with the number of targets. Additionally, extensive sample washing may compromise the integrity of delicate structures.
I present an elegant solution for parallel multi-target super-resolution imaging: Fluorescence Lifetime DNA-PAINT (FL-PAINT) [1], a fast multiplexed imaging technique that uses fluorescence lifetime for target identification. FL-PAINT has been implemented with both wide-field and confocal FL-SMLM [2].
Fluorescence lifetime can also be used to determine axial position with nanometer precision via Metal-induced Energy Transfer (MIET). Combining MIET's exceptional axial resolution with the lateral resolution of DNA-PAINT, MIET-PAINT emerges as a powerful tool for multiplexed 3D super-resolution imaging [3].
Precise environmental control within the experimental chamber was achieved using a custom-designed microfluidic system [4], which also enables automation and synchronization with the acquisition process - an important step toward automated, high-content imaging, a long-standing goal in super-resolution microscopy.



step toward automated, high-content imaging, a long-standing goal in super-resolution microscopy.

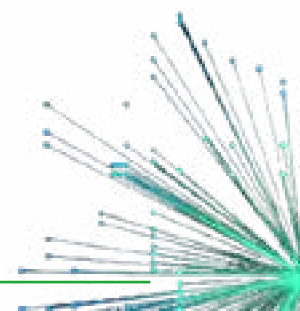
[1] Oleksiievets, N.; Sargsyan, Y.; Thiele, J.C.; Mougios, N.; Sograte-Idrissi, S.; Nevskyi, O.; Gregor, I.; Opazo, F.; Thoms S.; Enderlein, J.*; <u>Tsukanov, R.
[2] Oleksiievets, N., Mathew, C., Thiele, J.C., Gallea, J.I., Nevskyi, O., Gregor, I., Weber, A., <u>Tsukanov, R.
[3] Oleksiievets, N.; Mougios, N.; Jans, D.C.; Hauke L.; Thiele J.C.; Basak, S.; Jakobs, S.; Opazo, F.; Enderlein, J.*; <u>Tsukanov, R.
[4] Sograte-Idrissi, S.; Oleksiievets, N.; Isbaner, S.; Eggert-Martinez, M.; Enderlein, J.; <u>Tsukanov, R.* ; Opazo, F.*, “Nanobody Detection of Standard Fluorescent Proteins Enables Multi-Target DNA-PAINT with High Resolution and Minimal Displacement Errors”. Cells, 8 (1), 48 (2019).

Single molecule localization imaging of Env clustering in native HIV-1 viruses

Cecilia Zaza[1], David J. Willliamson[2,3], Irene Carlon-Andres[2,3,] Alessia Gentili1, Harry Holmes[1], James Daly2, Joseph Thrush[2,3], Tobias Starling[2,] Stuart Neil[2,] Ray Owens[2,4], Michael Malim[2], Sabrina Simoncelli[1,5], Sergi Padilla-Parra[2,3,6]

[1]London Centre for Nanotechnology, Faculty of Maths & Physical Sciences, University College London
[2]Department of Infectious Diseases, School of Immunology & Microbial Sciences, King’s College London
[3]Structural Biology Department, Rosalind Franklin Institute
[4]Division of Structural Biology, University of Oxford, Oxford
[5]UCL Department of Chemistry, London
[6]Randall Centre for Cell & Molecular Biophysics, King’s College London

Viral envelope proteins are essential for mediating virus entry into host cells. Typically existing as glycosylated trimers, these proteins undergo significant conformational changes during receptor binding and membrane fusion. In HIV-1, the envelope glycoprotein (Env) trimers are critical for infection of CD4+ T cells and macrophages. Super-resolution fluorescence microscopy, such as STED, has revealed that Env is not randomly distributed but instead forms distinct clusters on the surface of mature HIV-1 virions—structures thought to be key for viral entry and immune targeting.
Here, I will present high-resolution visualizations and quantitative analyses of HIV-1 Env clusters using DNA-PAINT, a single-molecule super-resolution fluorescence microscopy technique capable of achieving 10 nm resolution. To preserve Env’s structural and functional integrity, we used AlphaFold2 to guide the insertion of a synthetic epitope at an optimal site, allowing precise labeling with high-affinity single-domain antibodies (sdAbs) without disrupting native conformation.
Our findings provide novel insights into the spatial organization of Env on both mature and immature HIV-1 virions. Notably, DNA-PAINT imaging enables the visualization of Env trimers in both open and closed conformations—key states for viral entry. These results enhance our understanding of Env microclusters and their role in HIV-1 biology, highlighting their importance as potential targets for immune responses.



Single molecule tracking of mismatch repair in vivo and in vitro

Taekjip Ha

Program in Cellular and Molecular Medicine, Boston Children‘s Hospital and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, United States

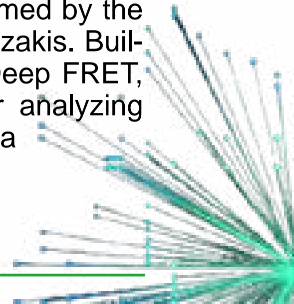
Unrepaired DNA mismatches become sources of genetic variation that alters cellular phenotypes causing dysfunction and diseases. To understand how mismatches in diverse sequence contexts are repaired, we developed a high-throughput approach to track single mismatch repair (MMR) events in vivo. We discovered hypervariable MMR efficiencies of TT, AG, and CT mismatches that were primarily determined by local sequence context. Single-molecule FRET analysis showed that well-repaired mismatches achieve a higher flux of MutS sliding clamp formation through faster mismatch binding, slower dissociation, faster conformational switching into sliding clamp, and faster departure from the mismatch. The hypervariable mismatch repair imparts enhanced mutability if a repair failure causes only synonymous or conservative codon change, suggesting MMR may have influenced codon usage and the genetic code. Moreover, sequence-dependent repair can explain the patterns of substitution mutations in MMR -deficient tumors, human cells, and C. elegans. Comparison to biophysical and biochemical analyses indicate that DNA physics is a central determinant of MMR efficiency by impacting MutS progressions to an activated sliding clamp.

PIE and CAKE: How Sweet! ;)

Don C. Lamb

LMU München, Germany

At the Biophysical Society Metting in 2002, I saw a poster by Achilles Kapanidis from the group of Shimon Weiss regarding millisecond Alternating Laser Excitation (ALEX) for separating out dual-labeled molecules for single-molecule Förster Resonance Energy Transfer (smFRET) experiments. I thought, if this could be performed significantly faster, it could be an interesting approach for getting rid of spectral crosstalk in fluorescence cross-correlation spectroscopy (FCCS) experiments. Hence, I pushed the alternation cycle into the MHz regime and developed what is now known as Pulsed Interleaved Excitation or (PIE), which is synonymous with nanosecond ALEX. PIE uses two or more pulsed excitation sources that are interleaved with each other. The ease of which one can control the pulses of the picosecond pulsed lasers from PicoQuant together with their photon counting detection hardware has developed a tremendous synergy between my research and PicoQuant. When combining PIE with other fluorescence methods, new capabilities become possible. For example, it is possible to perform FCCS experiments without spectral crosstalk and also obtain the correct amplitudes for the autocorrelation functions when using PIE. When combining PIE with smFRET experiments, it is possible to extract the correction factors necessary for the determination of accurate FRET efficiencies directly from the same measurement. In the meanwhile, PIE has been combined with a number of methods including Raster Image Correlation Spectroscopy and MinFLUX. When expanding FRET to three-colors, it becomes possible to determine three distances within the same molecule at the same time. This makes it possible to investigate coordinated motions within biomolecules. For three-color smFRET experiments, PIE is not only advantageous, it is essential. When performing smFRET experiments on immobilized molecules, it is possible to follow the dynamics of individual molecules with time. Thus, it is possible to extract kinetic information directly from an experiment. The difficulty with these experiments is the intensive time necessary to select the high-quality traces for performing the analysis. With the development of machine learning methods, it is now possible to analyze traces automatically. Pioneering work on using machine learning to analyze smFRET traces was performed by the groups of Nil Walters and Nikos Hatzakis. Building on their results, in particular Deep FRET, we developed a software suite for analyzing one-, two- and three-color FRET data



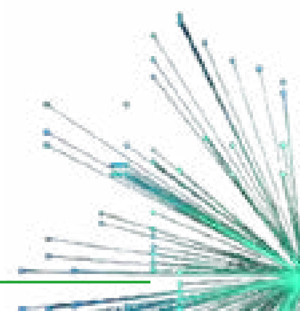
and extracting kinetics from them with the aid of machine learning. The software is known as Deep-Learning Assisted Single molecule Image analysis (Deep-LASI), but I affectionately call it Computer Assisted Kinetic Extraction, or CAKE.

From Single Molecule Insights to Real-World Impact: Optical Mapping for Life Sciences

Johan Hofkens

Katholieke Universiteit Leuven, Belgium

Over the past three decades, single molecule spectroscopy has transformed our ability to interrogate molecular processes, enabling discoveries across catalysis, plasmonics, polymer science, biophysics, and genomics. In my laboratory, we have coupled these capabilities with the design of innovative fluorescent labels, precision linker chemistries, and novel imaging modalities to push the boundaries of resolution and applicability. This presentation will highlight our latest developments of FLUOROCODE, a super-resolution optical DNA mapping technology that delivers nanoscale structural insights into genomic sequences. I will discuss how fundamental advances in photophysics, molecular design, and data analysis have converged to enable applications—most notably in microbiome analysis—driving translation into biotechnology and diagnostics. By uniting cutting-edge microscopy with tailored chemistry, we aim to open new frontiers in both fundamental science and applied life sciences.



Molecular behavior of disordered translation factor eIF4B: from monomers to oligomers and condensates

Mikayel Aznauryan

University of Bordeaux, Inserm, CNRS, ARNA Laboratory, U1212, UMR 5320, Institut Européen de Chimie et Biologie, 33600, Pessac, France

Eukaryotic translation initiation factor eIF4B is essential for efficient cap-dependent translation, particularly for mRNAs with extended and structured 5' untranslated regions. It is tightly regulated to ensure proper physiological functions and responses, but is frequently dysregulated in various pathologies. Despite its significant functional importance, eIF4B is rarely observed in cryo-EM structures of translation complexes due to its high intrinsic disorder. As a result, the molecular details of eIF4B and especially its long intrinsically disordered region (IDR) remain largely unknown.

By integrating single-molecule and ensemble experiments with molecular simulations we demonstrate that eIF4B IDR orchestrates and fine-tunes an intricate transition from monomers to a condensed phase [1, 2]. Across this transition variable-size dynamic oligomeric clusters form as nucleation hot-spots to favor mesoscopic phase separation. Our single-molecule FRET assays allow following the conformation and dynamics (from ns to ms) of the protein throughout all these molecular states. The observed complex self-association landscape displays strong sensitivity to even marginal changes of ionic strength and molecular crowding. This translates into sensitive regulation of eIF4B nanoscopic and mesoscopic behaviors driven by protein post-translational modifications, binding partners or changes to the cellular environment. Unsurprisingly, the molecular driving forces that govern the in vitro self-association of eIF4B play a pivotal role in determining the protein condensation behavior during cellular stress and its assembly into stress granules.

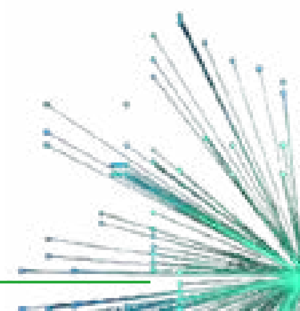
[1] Swain B.C., et al. Nat Commun 15, 8766 (2024)
[2] Mondal S. et al. Biomol NMR Assign. 17, 199–203 (2023)

Single-Molecule Sensors for Mapping Crowding and Ionic Strength in Live Cells

Yuhan Wang[1], Valentin von Roten[1], Daniel Nettels[1], Benjamin Schuler[1,2]

[1]Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[2]Department of Physics, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Intrinsically disordered proteins (IDPs) are abundant in cells and are highly sensitive to intracellular crowding and ionic strength—two key factors that regulate the cellular environment. Observing conformational changes in IDPs within cells raises a critical but underexplored question: are these changes driven by variations in crowding or ionic strength? To address this challenge, we developed an approach utilizing single-molecule FRET spectroscopy in live cells, and identified a set of IDPs that serve as effective orthogonal sensors for intracellular crowding and ionic strength. This method enables the quantification of both factors with subcellular resolution, allowing us to disentangle their individual effects on local IDP behavior and map these influences across distinct cellular regions.



Brightness demixing for simultaneous multi-target imaging in 3D single-molecule localization microscopy

Laurent LE[1], Surabhi. K. SREENIVAS[1,2], Emmanuel FORT[2], Sandrine LEVEQUE-FORT[1]

[1]Institut des Sciences Moléculaires d'Orsay, CNRS, Université Paris Saclay, 91400 Orsay, France
[2]Institut Langevin, ESPCI, 75005 Paris

Revealing the complex nanoscale organization of different proteins with Single-Molecule Localization Microscopy is usually based on a combination of fluorophores with spectral differences. While a single excitation laser associated to a simultaneous ratiometric detection is now widely developed [1-6], the spectral-based separation is inherently constrained by spectral overlap. We propose here Brightness Demixing [7], a novel method for fluorophore discrimination that exploits brightness, which directly depends on the fluorophores extinction coefficient and quantum yield. By oversampling blinking events, we can precisely quantify photon flux as a proxy for brightness, enabling robust differentiation of fluorophores independent of their spectral properties, without requiring additional spectral separation. Brightness Demixing operates within a single detection channel, eliminating the need for additional spectral filters or cameras. Simultaneous two- and three-target imaging in both 2D and 3D configurations can be retrieved. By maintaining single-wavelength excitation and minimizing chromatic aberrations, this method significantly enhances multiplexing in SMLM while remaining fully compatible with existing setups. Brightness Demixing thus offers a simple yet powerful approach to expanding multi-target imaging capabilities in super-resolution microscopy. Beyond its role in multiplexing, precise photon flux measurement opens new avenues for probing quantum yield variations, which are inherently linked to fluorescence lifetime.

[1] Bossi, M., Flling, J., Belov, V.N., Boyarskiy, V.P., Medda, R., Egner, A., Eggeling, C., Schnle, A., Hell, S.W.: Multicolor Far-Field Fluorescence Nanoscopy through Isolated Detection of Distinct Molecular Species. Nano Letters 8(8), 2463–2468 (2008) https://doi.org/10.1021/nl801471d

[2] Testa, I., Wurm, C.A., Medda, R., Rothermel, E., Middendorf, C., Flling, J., Jakobs, S., Schonle, A., Hell, S.W., Eggeling, C.: Multicolor Fluorescence Nanoscopy in Fixed and Living Cells by Exciting Conventional Fluorophores with a Single Wavelength. Biophysical Journal 99(8), 2686–2694 (2010) https://doi.org/10.1016/j.bpj.2010.08.012 . Accessed 2023-06-28

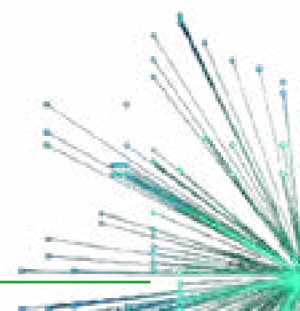
3] Lampe, A., Haucke, V., Sigrist, S.J., Heilemann, M., Schmoranzer, J.: Multi-colour direct STORM with red emitting carbocyanines. Biology of the Cell 104(4), 229–237 (2012) https: //doi.org/10.1111/boc.201100011

4] Zhang, Y., Schroeder, L.K., Lessard, M.D., Kidd, P., Chung, J., Song, Y., Benedetti, L., Li, Y., Ries, J., Grimm, J.B., Lavis, L.D., De Camilli, P., Rothman, J.E., Baddeley, D., Bewersdorf, J.:Nanoscale subcellular architecture revealed by multicolor three-dimensional salvaged fluorescence imaging. Nature Methods 17(2), 225–231 (2020) https://doi.org/10.1038/s41592-019-0676-4

[5] Li, Y., Shi, W., Liu, S., Cavka, I., Wu, Y.-L., Matti, U., Wu, D., Koehler, S., Ries, J.: Global fit ting for high-accuracy multi-channel single-molecule localization. Nature Communications 13(1), 3133 (2022) https://doi.org/10.1038/s41467-022-30719-4 .

[6] Friedl, K., Mau, A., Boroni-Rueda, F., Caorsi, V., Bourg, N., Leveque-Fort, S., Leterrier, C.: Assessing crosstalk in simultaneous multicolor single-molecule localization microscopy. Cell Reports Methods 3(9) (2023) https://doi.org/10.1016/j.crmeth.2023.100571 .

[7] Brightness demixing for simultaneous multi-target imaging in 3D single-molecule localization microscopy, Laurent Le, Surabhi K. Sreenivas, Emmanuel Fort, Sandrine Lévêque-Fort, bioRxiv 2025.03.02.639924; doi: https://doi.org/10.1101/2025.03.02.639924



Ångström Super-resolution in Structural Biology: Cryogenic Light Microscopy of Proteins in Their Native Environment

Hisham Mazal[1,2], Franz-Ferdinand Wieser[1,2,3], Vahid Sandoghdar[1,2,3]

[1]Max Planck Institute for the Science of Light, 91058 Erlangen, Germany
[2]Max-Planck-Zentrum für Physik und Medizin, 91054 Erlangen, Germany
[3]Department of Physics, Friedrich-Alexander University of Erlangen-Nürnberg, 91058 Erlangen, Germany

Optical microscopy at the nanoscale holds great promise for studying membrane proteins in their native cellular membrane environment. A few recent reports have demonstrated subnanometer resolution in light microscopy, but these works considered chemically fixed samples (1,2). To achieve near-native preservation of transmembrane proteins with Ångstrom precision, we have developed a high-vacuum cryogenic shuttle system that allows us to transfer shock-frozen vitrified samples in and out of a cryostat (3) for single-particle cryogenic light microscopy (spCryo-LM) at liquid helium temperature (4,5). We benchmark our method by resolving the complete configuration of alphahemolysin (αHL) as a heptameric membrane protein model system in a supported lipid bilayer. Moreover, we apply this technique to decipher the conformational states of the mouse PIEZO1 (mPIEZO1) mechanosensitive ion channel within its native cell membrane. Using our approach, we localize fluorescent labels placed at the extremities of the three blades of the mPIEZO1, allowing us to ascertain three distinct configurations with side lengths of 9, 19, and 34 nm (3). Aside from preserving the near-native state of biological sample, our approach promises a seamless integration into the pipeline of correlative imaging with Cryo-EM and ushers in a new regime of structural biology at the Ångstrom level.

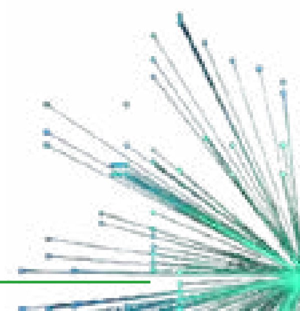
1. S. J. Sahl et al., Direct optical measurement of intramolecular distances with angstrom precision. Science 386, 180-187 (2024)

2. S. C. M. Reinhardt et al., Ångström-resolution fluorescence microscopy. Nature 617, 711-716 (2023).

3. Mazal, H.; Schambony, A.; Sandoghdar, V., bioRxiv 2024,12.22.629944 (2024).

4. Mazal, H.; Wieser, F.-F.; Sandoghdar, V., eLife, 11:e76308 (2022).

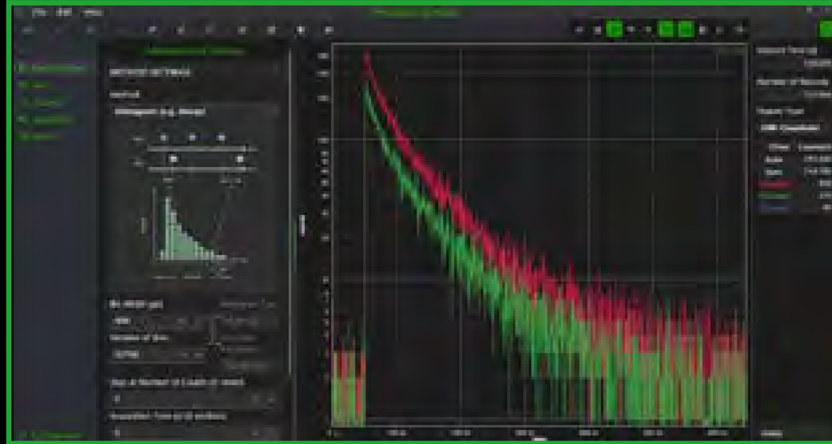
5. Weisenburger, S.; Boening, D.; Schomburg, B.; Giller, K.; Becker, S.; Griesinger, C.; Sandoghdar, V., Nat Methods, 14, 141-144 (2017).



UniHarp Software

Fast, Poweful,Intergrated

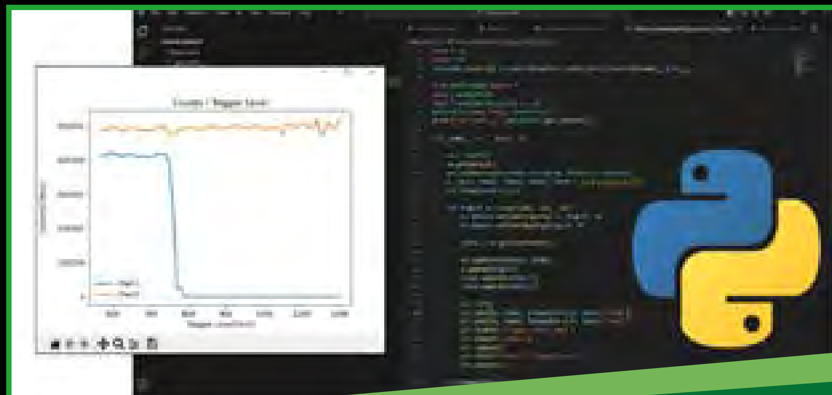
- Quick Setup & Live View: Intuitive GUI with real-time visualization
- Advanced Analysis: Histograms, g^2, FCS, and more with PTU/PHU support
- Full Automation: Scripted workflows and seamless PicoQuant integration



snAPI Software

Fast,Flexible,Python-Powered

- Intuitive Interface: Real-time access and direct PTU handling built on a robust C++ core
- On-the-Fly Processing: Built-in tools for coincidence, heralding, count rates, delays, and stream merging
- Custom Workflows: Full flexibility to create your own analysis pipelines in Python

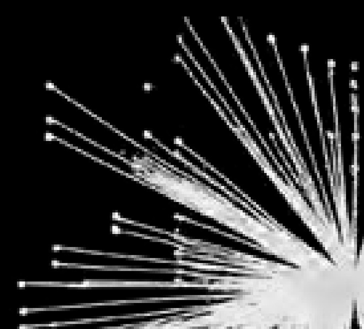


Website

PicoQuant

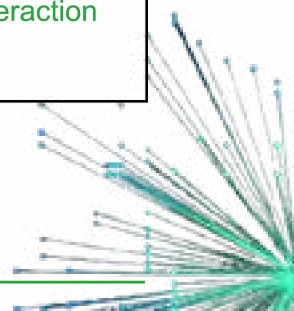
List of Poster Presentations

as of September, 18



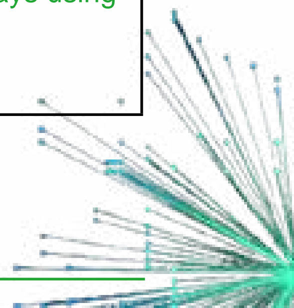
Poster presentations Session I
(in alphabetical order)

Presenter	Session	Poster Number	Title
Acconcia, Giulia	TUE	P41F	Revolutionizing TCSPC Speed: Achieving 400% of the Excitation Rate with Near-Zero Distortion Flash talk
Ashwin Balakrishnan	TUE	P84F	Fast and long-term super-resolution STED microscopy of nanostructural organellar dynamics using a neural network Flash talk
Belhadji, Kémil	TUE	P67F	Temperature-dependent conformational signatures of membrane protein, BmrA using single-molecule FRET Flash talk
Berger, Julia	TUE	P1	Single-Molecule Spectroscopy of the Excited-State Proton Transfer
Börner, Richard	TUE	P53	smFRET-guided integrative modelling of RNA - from single structures to a structural ensemble
Del Bufalo, Francesco	TUE	P65	A unified computational strategy for multi-target super-resolution imaging with SPAD array detector
Cabrejos, Anthony Monteza	TUE	P57	Super-Resolution Mapping of T Cell Re-ceptor Forces via tension-PAINT
Choi, Bok-Eum	TUE	P71F	Tether-free single-molecule FRET uncovers hidden hairpin dynamics of CRISPR RNA Flash talk
Conradie, Francois	TUE	P75	Exciton annihilation evident in TCSPC-FCS study of aggregating photosynthetic antenna complexes from plants
Elena-Real, Carlos A	TUE	P91	FCHO homologous disordered proteins ex-plore different conformational landscape to initiate Endocytosi5
Fillipek, Klaudia	TUE	P95	New analysis options push the limits of FLIM imaging modalities
Franchini, Lucia	TUE	P3	Elucidating the Free-Energy Landscape of Histone H1 and Prothymosin α Interaction by Single-Molecule Techniques



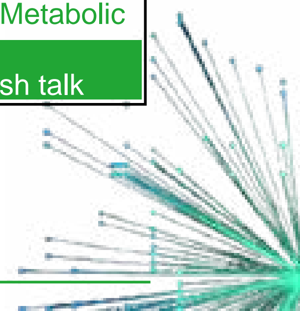
Poster presentations Session I
(in alphabetical order)

Presenter	Session	Poster Number	Title
Gensch, Thomas	TUE	P27	Imaging Cell Parameters with Organic Dyes and Genetically Encoded Biosensors based on Fluorescence Lifetime Imaging
Gligonov, Ivan	TUE	P29	Spectral decomposition of two molecule intensity images in polarized excitation fluorescence microscopy: reconstruction algorithms and simulations
Górecki, Andrzej	TUE	P31	Single-Molecule FRET Analysis of the Structural Dynamics of the human transcription factor YY1
GUZMAN-ROCHA, DULCE	TUE	P33	Plasmonic material whit magneto-optical properties for biomedical applications
Hartsch, Muriel	TUE	P5	Structural dynamics and long-range interactions controlling timing of the Neurospora circadian clock
Hemmen, Katherina	TUE	P35	Towards understanding the role of transcription factor oligomerization in regulating gene expression in live cells
Jorissen, Lambert-Paul	TUE	P37	Programmable heating for fluorescence microscopy using Printed Circuit Board (PCB) technology
Kalra, Priyanshi	TUE	P39	Probing Biomolecular Condensates: Insights from Fluorescence Studies
Kölbl, Niklas	TUE	P7	Development of new photostabilization strategies
Konthalapalli, Hradini	TUE	P43	Capturing protein-protein interactions in live cells: APC/C and CDC20 in mitosis
Lisibach, Matteo	TUE	P47	Toward multiple coordinates: Resolving the intrinsic heterogeneity of a group II intron folding process
Majer, Katharina	TUE	P49	Following ribozyme splicing pathways using escape-time stereometry (ETs)



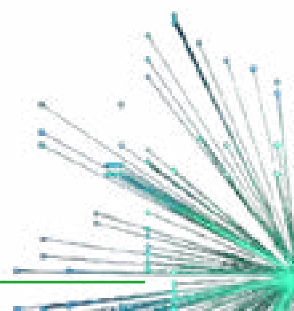
Poster presentations Session I
(in alphabetical order)

Presenter	Session	Poster Number	Title
Marin, Zach	TUE	P51F	Evaluating MINFLUX experimental performance in silico Flash talk
Marx, Daniel	TUE	P9	Mapping complex optical light field distribution with single fluorescence molecules
Meghnagi, Maxime	TUE	P60F	Modulated illumination with an optical nanochip for in-depth SMLM Flash talk
Mhanna, Rana	TUE	P55	SPECTRAL CHARACTERIZATION OF TERRYLENE PHOTOPRODUCTS
Moya, Gabriel	TUE	P45F	Single-molecule FRET on a minimalistic 3D-printed setup using optimized dyes in the blue-green spectral region Flash talk
Nandy, Atanu	TUE	P61	Exploring the Conformational Dynamics with Metal Induced Energy Transfer
Orekhova, Daria	TUE	P63	Hexagonal Boron Nitride for Single-Molecule Biophysics
Papagiannoula, Andromachi	TUE	P11	Conformational dynamics of the endocytic protein Eps15
Pincet, Lancelot	TUE	P99F	Revisiting multichannel processing with in-depth multitarget 3D ModLoc imaging Flash talk
Platz, Adrian	TUE	P13	Towards kilohertz structured illumination microscopy with random pattern
Punter, Eva	TUE	P69	Integrating Coarse-Grained Molecular Dynamics and Fluorescence Spectroscopy to Unravel Tau Protein
Rastogi, Harshita	TUE	P105F	Temperature-dependence and Crowding-Induced Modulation in PFK1-Driven Metabolic Regulation Flash talk



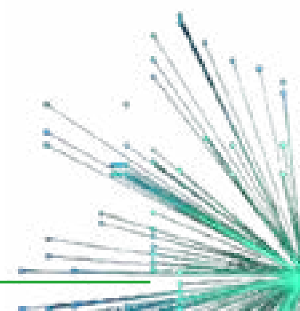
Poster presentations Session I
(in alphabetical order)

Presenter	Session	Poster Number	Title
Rech, Valentin	TUE	P15	DNA Origami Nanoantennas for Real-Time Monitoring of Polymerase Activity and Prospective DNA Sequencing
Rohmann, Mostofa	TUE	P103	Tunable linker systems for broad use of functional dyes in single-molecule imaging
Salam, Abdul	TUE	P17	A zinc complex as an NIR emissive probe for real-time dynamics and in vivo embryogenic evolution of lysosomes using super-resolution microscopy
Schmidt, Christoph	TUE	P19	Manipulation of the Energy Landscape of Tethered Fluorophores for Enhanced L-PAINT
Schröder, Tim	TUE	P81	A DNA-based exciton collider to monitor one-dimensional exciton diffusion
Seth, Sudipta	TUE	P83F	Imaging Functional Microstructures to Understand the Working Mechanism of Perovskite Solar Cells in Operation
Shkarin, Alexey	TUE	P79F	Fourier-limited electronic transitions in surface-adsorbed quantum emitters Flash talk
Soulias, Dimitrios	TUE	P85	Escape-time stereometry (ETs) for measuring mRNA poly(A) tail length
Tevosian, Margarita	TUE	P89	ClearFinder: a Python GUI for annotating cells in cleared mouse brain
Venugopal Srambickal, Chinmaya	TUE	P93	Combined MINFLUX – SRS – TPE FLIM imaging of bacteria and their targeted host cells



Poster presentations Session I
(in alphabetical order)

Presenter	Session	Poster Number	Title
Vogler, Bela T. L.	TUE	P23	Rethinking the Mindset: Unlocking the Potential of MINFLUX enabled Single-Particle Tracking
Whaley-Mayda, Lukas	TUE	P87F	Exploring the resolution limits of single-molecule FRET Flash talk
Wu, Hongbin	TUE	P25	A fluorescence-based platform for monitoring osmolyte transport
Zhu, Xin	TUE	P101	Examining protein multimerization using Escape-time Stereometry
Zhurgenbayeva, Gaukhar	TUE	P21	Characterization of Candidalysin peptide self-aggregation on lipid membranes using Fluorescence Correlation Spectroscopy.



Poster presentations Session II
(in alphabetical order)

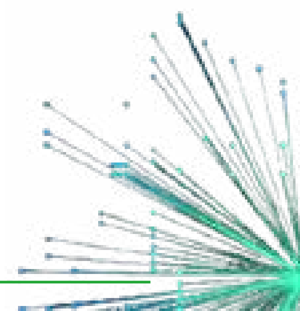
Presenter	Session	Poster Number	Title
Azad, Shaf	THU	P2	Using single-molecule techniques to study the telomeric shelterin complex
Balakrishnan, Ashwin	THU	P84F	Fast and long-term super-resolution STED microscopy of nanostructural organellar dynamics using a neural network Flash talk
Barentine, Andrew E. S.	THU	P4	Detection Limits of Stimulated Emission Imaging
Basak, Samrat	THU	P34	Smart Multiplexing and 3D Super-Resolution with Local PAINT and Lifetime-Encoded Imaging
Becker, Mailin	THU	P16	A Link between Neural Development and Neurological Disorders-Molecular Switch of the Dendrite-to-Spine Transport of TDP-43/ FMRP-Bound Neuronal mRNA and Its Impairment in ASD
Belhadji, Kémil	THU	P70F	Temperature-dependent conformational signatures of membrane protein, BmrA using single-molecule FRET Flash talk
Börsch, Michael	THU	P10F	Monitoring subunit rotation in a single membrane enzyme FoF1-ATP synthase by quantum sensing ABEL-FLIM and ABEL-FRET Flash talk
Brasselet, Sophie	THU	P106	Using Light Field Microscopy to measure the orientation of Single Molecules in 3D
Brüggenthies, Gereon	THU	P24F	Monitoring the Coating of Single DNA Origami Nanostructures with a Molecular Fluorescence Lifetime Sensor Flash talk

Poster presentations Session II
(in alphabetical order)

Presenter	Session	Poster Number	Title
Budiarta, Made	THU	P12	Protein-Based Nanorulers for Validating Sub-10 nm Resolution in Super-Resolution Microscopy
Bukvin, Ivana	THU	P54	Towards a single-molecule approach to capture the delicate interplay between translation and misfolding on the ribosome
Buzón, Pedro	THU	P14F	Real-time single-particle kinetics to reveal the mechanisms of virus assembly Flash talk
Chu, Jen-Fei	THU	P16	A Link between Neural Development and Neurological Disorders-Molecular Switch of the Dendrite-to-Spine Transport of TDP-43/FMRP-Bound Neuronal mRNA and Its Impairment in ASD
Dunsing-Eichenauer, Valentin	THU	P20	FCS as a tool to quantify morphogen dynamics and interactions in living embryos
Esmaeeli, Hanie	THU	P22	Effects of Photoswitching Dynamics on MINFLUX Performance with Far-Red and Near-Infrared Fluorophores
Gallea, Jose I.	THU	P66	T4 Bacteriophage as a Nature-Crafted 3D Nanoruler for Super-Resolution Microscopy
Garg, Richa	THU	P72	Carbon Nanodots as a Red Emissive Fluorescent Probe for the Super-Resolution Microscopy of DNA Dynamics during Paclitaxel Treatment
Gennerich, Arne	THU	P26F	The Power of Three: Dynactin associates with three dyneins under load for greater force production Flash talk
Gentsch, Gregor J.	THU	P28	Nanotexture – a universal approach of AI-based computational multiplexing and phenotyping of super-resolution data.NanTex - a start to finish framework for multiplexing super-resolution data
Goppelt, Stefan	THU	P78	Technical Challenges in Single-Complex Fluorescence-Excitation CD Spectroscopy at Cryogenic Temperatures
Günther, Lisa M.	THU	P32	Empowering Research with Time-Resolved Fluorescence Methods: A KeyLab Approach

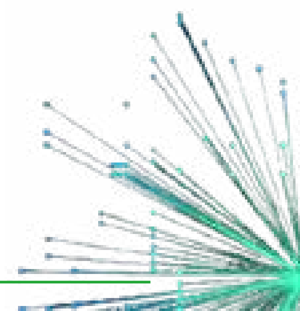
Poster presentations Session II
(in alphabetical order)

Presenter	Session	Poster Number	Title
Jaworek, Michel W.	THU	P36	Kinetic ligand binding mechanisms of bacterial substrate binding proteins
Jungblut, Marvin	THU	P38	Revealing oligomerization states of membrane receptors at sub-5 nm resolution using multicolor ExM-SMLM
Kenesei, Adam	THU	P40	Identification of cancer-induced protein rearrangement in platelets evidenced by STED super-resolution microscopy
Kirchmair, Bernhard	THU	P42	RNA-Quantification in Lipid Nanoparticles with Fluorescence Correlation Spectroscopy
Koenig, Marcelle	THU	P102	Expanding the Horizon of FCS with SPAD Arrays: A Promising Outlook for New Applications
Kumar, Charitra Sree Senthil	THU	P82F	Single Molecule 3D Orientation and Localization Microscopy (SMOLM) via ratiometric 4-polarization projection microscopy on dense actin structures Flash talk
Lengauer, Maximilian	THU	P44	PSF Engineering for Single-Molecule Circularly Polarized Luminescence (CPL) Detection: Sensitivity Analysis and Parameter Estimation
Leslie, Sabrina	THU	P73F	New eyes on medicines and vaccines: seeing how they work one molecule at a time Flash talk
Lindner, Lennart	THU	P46	Binding or Unwinding? The role of an RNA binding protein in ribozyme activity
Liu, Haichun	THU	P48	Photophysical structured illumination flowmetry based on the long-lasting emission response of lanthanide luminescent nanoparticles
Loidolt-Krüger, Maria	THU	P104	Single-molecule and time-resolved fluorescence microscopy studies of the interaction between synapsin-1/α-synuclein condensates and membranes



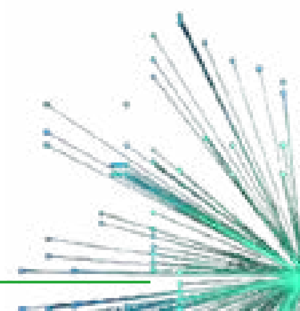
Poster presentations Session II
(in alphabetical order)

Presenter	Session	Poster Number	Title
Malsbenden, David	THU	P50	Rotational Dynamics of Single Molecules at the Interfaces of Thin Polymer Films
Martinez, Luciana	THU	P52	Unraveling CAR-T Cell Activation and Synapse Dynamics Using DNA-PAINT Single-Particle Tracking
Mitra, Shrutarshi	THU	P56	Mapping heterodimerization and domain interplay controlling pioneer activity of Ascl1 with single-molecule FRET
Morella, Fabio	THU	P58	Dissecting the TATA box sequence using smFRET
Nevskyi, Oleksii	THU	P62F	Fluorescence-Lifetime SMLM as a Versatile Tool for Multiplexed, Environment-Sensitive and 3D Super-Resolution Imaging Flash talk
Peulen, Thomas-Otavio	THU	P64	Integrative Design of Fluorescence Experiments: From Labeling Strategy to Coarse-Grained Prediction of Fluorescence Observables
Prokazov, Yury	THU	P68	Pushing the Limits of FLIM: Ultrafast Photon Detection with LINCam, PhotonPix, and LINTag
Rodríguez, Leandro Cruz	THU	P18F	Revealing α-Synuclein Phase Transitions with ACDAN-Based Phasor Analysis Flash talk
Sadiq, Abdul Rahman	THU	P74	RNA-Inhibitor Interactions using Multisite smFRET
Sarkar, Subhartha	THU	P76	Nanofluidic-Assisted Single-Molecule Fluorescence Burst Size Distribution Analysis
Schmauder, Ralf	THU	P80	Using changes in photophysical properties to detect ligand binding at moderate concentrations



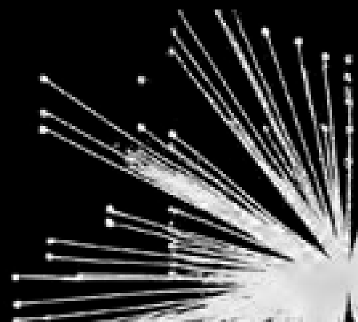
Poster presentations Session II
(in alphabetical order)

Presenter	Session	Poster Number	Title
Spantzel, Lukas	THU	P86	Single-molecule spectroscopy of GPCR oligomers in lipid nanodiscs using an Anti-Brownian Electrokinetic Trap (ABELtrap)
Szalai, Alan	THU	P94	Monitoring Dynamic Conformations of a Single Fluorescent Molecule Inside a Protein Cavity
Terterov, Ivan	THU	P88	Model-free photon analysis of diffusion-based single-molecule FRET experiments
Trottenberg, Leon	THU	P90	Combined spectral and lifetime measurements of Nile Red in pNIPAM microgels
Vankerkhoven, György	THU	P110	INTRACRINE SIGNALING OF THE EGF RECEPTOR
Vámosi, Yarne	THU	P92	Raster Image Correlation Spectroscopy in the Presence of Frame-to-frame Diffusing Artifacts
Zaza, Cecilia	THU	P96	Super-Resolution Imaging in Whole Cells and Tissues via DNA-PAINT on a Spinning Disk Confocal with Optical Photon Reassignment
ZHANG, Cheng	THU	P98	Plasmonic Nanotaper Meta-surfaces for High-Contrast Live-Cell Imaging
Zhao, Ziqing Winston	THU	P108	Imaging, Quantifying and Mapping Human Chromatin Remodeler Dynamics: From Phase-separation-mediated Intranuclear Organization to Cancer-mutant-specific Regulatory Landscape
Zhou, Jimeng	THU	P100	Extending volumetric imaging in single molecule localization microscopy



Abstracts:
Poster Presentations

according to poster number



P1

Single-Molecule Spectroscopy of the Excited-State Proton Transfer

Julia Berger, Gregor Jung

Biophysical Chemistry, Saarland University, Campus B2.2, 66123 Saarbrücken, Germany

Single-molecule spectroscopy applied to chemical reactions can reveal competing reaction pathways or microheterogeneities in the sample that remain hidden in ensemble studies.[1,2] Excited-state proton transfer (ESPT), as one of the few photochemical reactions compatible with fluorescence, is therefore particularly suitable for single-molecule investigations. The cyclic nature of the reaction involving the electronic excitation, proton migration, spontaneous emission and reprotonation[3,4] moreover allows the ESPT to be repeatedly studied on one, individual single molecule through its fluorescence spectrum.

Here I present our approach to catch and characterise intermediates of the ESPT at the single-molecule level. By embedding highly fluorescent and photostable photoacid molecules[5] in a solid phosphine oxide matrix, intermediates of the ESPT can be monitored using total internal reflection fluorescence microscopy (TIRFM). Emission spectra of individual photoacid/phosphine oxide complexes can be recorded by adding a transmission grating in front of the CMOS-camera.[6] Deconvolution of the obtained single-molecule fluorescence spectra revealed an extremely heterogeneous environment in the matrix, which is directly affecting the ESPT. These heterogeneities are not covered by ensemble studies[7] and serve as an excerpt of the microenvironments that are ubiquitous in chemical reaction dynamics.

[1] A. Rybina, C. Lang, M. Wirtz, K. Grußmayer, A. Kurz, F. Maier, A. Schmitt, O. Trapp, G. Jung, D.-P. Herten, Angew. Chem. Int. Ed. 2013, 52, 6322-6325.

[2] R. Mhanna, J. Berger, M. Jourdain, S. Muth, R. J. Kutta, G. Jung, ChemPhysChem 2025, e202400996.

[3] M. Vester, T. Staut, J. Enderlein, G. Jung, J. Phys. Chem. Lett. 2015, 6, 1149-1154.

[4] M. Vester, A. Grueter, B. Finkler, R. Becker, G. Jung, Phys. Chem. Chem. Phys. 2016, 18, 10281.

[5] B. Finkler, C. Spies, M. Vester, F. Walte, K. Omlor, I. Riemann, M. Zimmer, F. Stracke, M. Gerhards, G. Jung, Photochem. Photobiol. Sci. 2014, 13, 548-562.

[6] M. N. Bongiovanni, J. Godet, M. H. Horrocks, L. Tosatto, A. R. Carr, D. C. Wirthensohn, R. T. Ranasinghe, J.-E. Lee, A. Ponjavic, J. V. Fritz, C. M. Dobson, D. Klenerman, S. F. Lee, Nat. Commun. 2016, 7, 1-9.

[7] A. Grandjean, J. L. Pérez Lustres, S. Muth, D. Maus, G. Jung, J. Phys. Chem. Lett. 2021, 12, 1683-1689.

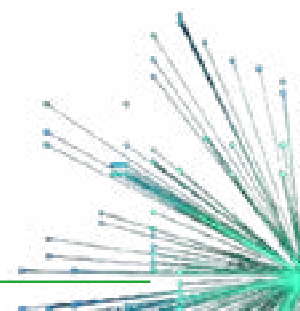
P2

Using single-molecule techniques to study the telomeric shelterin complex

Shafayat Azad[1,2], Paul Girvan[3,4], Oviya Inian[1,2], Korak Kumar Ray3,4, David S. Rueda[3,4], Sebastian Guettler[1,2]

[1]Division of Structural Biology, The Institute of Cancer Research, London, UK.
[2]Division of Cell and Molecular Biology, The Institute of Cancer Research, London, UK.
[3]Section of Virology, Department of Infectious Disease, Faculty of Medicine, Imperial College London, UK.
[4]Single-Molecule Biophysics Group, MRC Laboratory of Medical Sciences, London, UK.

The shelterin complex is essential for telomere maintenance: it protects telomeres from an unwarranted DNA damage response and contributes to regulating their length. TRF1, a component of shelterin, along with tankyrase, a shelterin binding partner, forms a critical partnership responsible for precise telomere elongation in human cells. Cell-based and bulk biochemical studies suggest that TRF1 is a negative regulator of telomere length and that its function is counteracted by tankyrase-dependent poly-ADP-ribosylation (PARylation). The current model lacks a precise mechanistic and real-time dynamic understanding of this process. This project aims to biochemically reconstitute a minimal telomere system for interrogation by single-molecule TIRF microscopy and optical tweezers to study the molecular details of telomere length control by PARylation. This system will further be used to investigate alterations in disease-relevant contexts in various telomeropathies.



P3

Elucidating the Free-Energy Landscape of Histone H1 and Prothymosin α Interaction by Single-Molecule Techniques

Lucia Franchini, Marie Synakewicz, Daniel Nettels, Ben Schuler

Department of Biochemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland

The paradigm that the specificity and affinity of biomolecular interactions rely on well-defined structures is challenged by the complex formed between the highly and oppositely charged intrinsically disordered proteins linker histone H1.0 (H1, net charge +53) and prothymosin α (ProTα, net charge -44). H1 and ProTα form a highly dynamic, disordered complex that lacks a structured binding interface [1]. Diffusion-limited binding kinetics and molecular dynamics simulations suggest that the electrostatically driven formation of the complex follows a barrierless downhill potential, rather than exhibiting a barrier as typical for canonical, structure-based complexes [1,2]. We aim to experimentally reconstruct the free energy landscape of H1 and ProTα binding. We will use single-molecule force spectroscopy and single-molecule FRET experiments to investigate the binding energetics and dynamics of the H1-ProTα complex. For this investigation, we designed fusion constructs in which H1 and ProTα are linked by an uncharged peptide linker. By varying the linker length, we are able to tune the effective local concentration of H1 and ProTα, providing additional insight into how tethering proteins affects their interaction compared to freely diffusing binding partners. Tethering the binding partners might also be a promising way of probing such interactions with single-molecule spectroscopy in live cells.

[1] Borgia, A., Borgia, M., Bugge, K. et al., Nature, 555, 61–66 (2018).
[2] Sottini, A., Borgia, A., Borgia, M.B. et al., Nat. Commun., 11, 5736 (2020).

P4

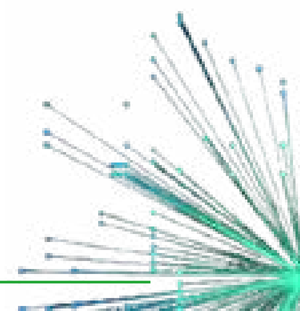
Detection Limits of Stimulated Emission Imaging

Andrew E. S. Barentine, W.E. Moerner

Department of Chemistry, 364 Lomita Drive, Stanford University, Stanford, California, USA

Stimulated emission (StE) has served as a valuable tool in biological imaging as a quenching mechanism for fluorescence, yet has itself remained relatively unused as an image-forming signal. Often thought of as a photon-copying mechanism, StE has potential speed and resolution advantages over fluorescence as an imaging contrast due to it being driven-by and coherent-with an experimentally controlled field (the probe). The ultimate problem in imaging StE is how to detect the StE light generated in the sample without also detecting the probe, which is typically a powerful laser. Unsolved, this problem contaminates StE images with the shot noise (and technical noise) of the probe laser, which is orders of magnitude higher than the StE signal that can typically be generated with a single organic dye molecule, blocking the possibility of single-molecule imaging.
Here, we use simultaneous detection of fluorescence depletion as a rigorous control and calibration [1] for the previously-developed approach to transmission StE imaging [2], whose sensitivity limit is bounded by the shot noise of the probe. With the same controls, we then attempt to detect StE without the background of the probe, the success of which could ultimately open the possibility of single-molecule StE imaging.

[1] A. E. S. Barentine and W. E. Moerner, Optica, 11, 464 (2024)
[2] W. Min, S. Lu, S. Chong, R. Roy, G. R. Holtom, and X. S. Xie, Nature, 461, 1105–1109 (2009)



P5

Structural dynamics and long-range interactions controlling timing of the Neurospora circadian clock

Muriel Hartsch[1], Kathrin Motzny[1], Ida Marie Vedel[1], Michael Brunner[2], Sigrid Milles[1]

1Leibniz-FMP Berlin, Robert-Roessle-Str. 10, 13125 Berlin
2Heidelberg University, Biochemistry Center (BZH), Im Neuenheimer Feld 328, 69120 Heidelberg

The function and maintenance of the circadian clock in Neurospora crassa are governed by a feedback loop involving both negative and positive regulatory elements, which together drive the oscillating circadian rhythm with a period of approximately 24 hours. The dimeric, intrinsically disordered protein FREQUENCY (FRQ) is a key component of the negative feedback complex and subject to post-transcriptional hyperphosphorylation by casein kinase 1a (CK1a). Phosphorylation of clock proteins is highly conserved across species, from fungi to mammals, with the human PERIOD (PER) protein being a notable example. However, the precise functions associated with hyperphosphorylation remain poorly understood. We hypothesize that time-dependent hyperphosphorylation of FRQ at multiple sites facilitates a transition from closed to open conformation, regulating interactions with its partners. Using nuclear magnetic resonance (NMR) and single-molecule fluorescence resonance energy transfer (smFRET), we investigate the conformational dynamics going along with FRQ phosphorylation by recombinant CK1a. This will allow, combined with molecular modeling of the intrinsically disordered protein, to understand how phosphorylation alters the conformation and triggers a switch in FRQ.

P6

Conformational Selection in Liquid-Liquid Phase Separation: Decoding SOD1 Folding and Aggregation Pathways

Mailin Becker[1,2], Nirnay Samanta[3], Sara Ribeiro[1,2], Linda Kartaschew[1,2], Simon Ebbinghaus[1,2]

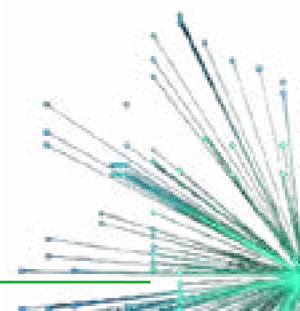
[1]Chair of Biophysical Chemistry, Ruhr-Universität Bochum, Bochum, Germany.
[2]Research Center Chemical Sciences and Sustainability, Research Alliance Ruhr, Bochum, Germany.
[3]Department of Biochemistry and Molecular Biophysics, Washington University in St Louis, Saint Louis, MO, USA.

Liquid-liquid phase separation (LLPS) drives the formation of stress granules (SGs), which are dynamic condensates critical for cellular stress responses. SG formation has been observed under heat stress which also leads to the unfolding of proteins [1]. Using superoxide dismutase 1 (SOD1), a protein linked to amyotrophic lateral sclerosis (ALS), we investigate how conformational selection modulates partitioning between the cytoplasm and SGs, and how these environments reshape unfolding and aggregation pathways.

Single-molecule FRET (smFRET, Picoquant Microtime2000) will be employed to resolve real-time conformational dynamics of SOD1 variants (e.g., destabilized mutant A4V) in reconstituted in vitro condensates vs the diluted phase, probing folded, partially unfolded, and misfolded states. Complementary, Fast Relaxation Imaging (FReI) quantifies folding equilibria within the SGs and the surrounding cytoplasm, allowing direct comparison of different cellular environments. We have shown that destabilized SOD1 mutants with higher hydrophobicity and flexibility exhibit enhanced partitioning into SGs [2].

This work establishes a mechanistic link between conformational selection and LLPS, offering insights into how SGs act as protective hubs or pathogenic reservoirs in proteostasis. The integration of smFRET with cellular biophysics provides a transformative approach to dissect LLPS-mediated regulation of protein misfolding in neurodegenerative diseases.

[1] D. Mateju et al., EMBO, 36: 1669 – 1687(2017).
[2] N. Samanta and S. Ribeiro et al., JACS, 143, 47, 19909–19918(2021).



P7

Development of new photostabilization strategies

Niklas Kölbl[1], Michael Scheckenbach[1], Benedikt Baumgartner[1], Carmen Zecha[1], Alexander Wiegand[1], Martin Reynders[3], Oliver Thorn-Seshold[3], Philip Tinnefeld[1], Viktorija Glembockyte[2]

[1]Ludwig-Maximilians-University Munich, Department of Chemistry , Butenandtstr. 5 – 13 (Gerhard-Ertl-Building), D–81377 Munich
[2]Max Planck Institute for Medical Research, Jahnstraße 29, D-69120 Heidelberg
[3]TU Dresden, Faculty of Chemistry and Food Chemistry, Bergstr. 66, 01069 Dresden

Due to the fast progress in technological advancements regarding detector sensitivity and resolution in the field of single molecule spectroscopy, the properties of organic dyes have become the limiting factor. Therefore, it is now of great interest to improve the dyes' properties like brightness and survival time. The problems occur, when the dye undergoes intersystem crossing ending up in a triplet state and opening photobleaching pathways. By now, this problem was mostly tackled by the use of triplet state quenchers in solution. Those have the tendency to be cytotoxic and need to be applied in high concentrations, limiting their applications. This work aims to conquer that by introducing azobenzenes to the dye using different approaches to quench occurring triplet states. The first approach aims to compare the already established quenchers to the used azobenzenes. The second and the third approach use different methods (DNA mediation and self-healing constructs) to bring photostabilizer and azobenzene in close proximity, increasing the local concentration of stabilizer and reducing the phototoxicity inherited by the solution-based stabilization. All approaches were tested using dyes commonly used for single-molecule and superresolution imaging together with several azobenzenes yielding promising results for photostabilization in single molecule microscopy.

J. Vogelsang, R. Kasper, C. Steinhauer, B. Person, M. Heilemann, M. Sauer, P. Tinnefeld, Angewandte Chemie (International ed. in English), 47, 5465 (2008).

J. L. Alejo, S. C. Blanchard, O. S. Andersen, Biophysical journal, 104, 2410 (2013).

P. Tinnefeld, T. Cordes, Nature methods, 9, 426-7; author reply 427-8 (2012).

M. Isselstein, L. Zhang, V. Glembockyte, O. Brix, G. Cosa, P. Tinnefeld, T. Cordes, The journal of physical chemistry letters , 11, 4462 (2020).

P9

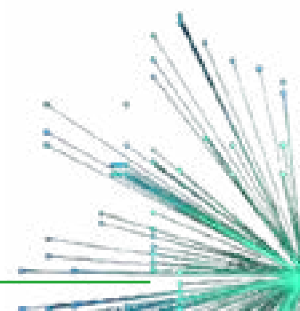
Mapping complex optical light field distribution with single fluorescence molecules

Daniel Marx[1], Oleksii Nevskyi[1], David Malsbenden[2], Dominik Wöll[2], Jörg Enderlein[1]

[1]III. Institute of Physics – Biophysics, Georg-August-University Göttingen
[2]Institute for Physical Chemistry – RWTH Aachen University

Confocal laser-scanning microscopy is a widely used technique in biological and medical research. In densely labeled samples or when imaging fast-rotating molecules, the polarization of the excitation laser can often be neglected. However, in the case of fixed single molecules, the interaction between the molecule's dipole orientation and the laser's polarization can result in distinct and orientation-dependent image patterns. A theoretical understanding of these patterns enables accurate determination of the molecule's orientation and localization without introducing bias.
To simulate the expected 3D point-spread functions (PSFs), we employ a theoretical framework that first computes the electromagnetic field distribution within the focused laser beam for the specific polarization state, and then incorporates the dipole orientation of the emitter [1].
We utilize, as an ideal model sample for systematically measuring confocal PSFs of fixed dipoles, the highly photostable dye Perylene Diimide (PDI) embedded in a thin polystyrene (PS) film.
Using this system, we experimentally recorded 3D point-spread functions under left- and right-handed circular, as well as linearly polarized excitation, each for a range of dipole orientations. The resulting PSFs were then compared to simulations generated with our theoretical model, showing strong agreement and thereby validating the approach.

[1] M. Fazel, K. S. Grussmayer, B. Ferdman, A. Radenovic, Y. Shechtman, J. Enderlein, Reviews of Modern Physics, 96 (2024).



P10F

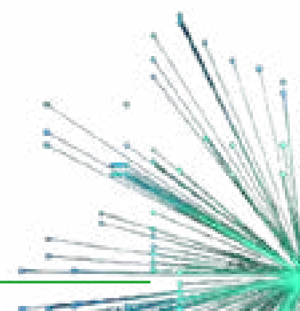
Monitoring subunit rotation in a single membrane enzyme FoF1-ATP synthase by quantum sensing ABEL-FLIM and ABEL-FRET

Michael Börsch, Iván Pérez, Thomas Heitkamp, Lukas Spantzel, Fahimeh Rashidi

Single-Molecule Microscopy Group, Jena University Hospital, Nonnenplan 2 - 4, 07743 Jena, Germany

For 28 years we have focused on subunit rotation of the enzyme FoF1-ATP synthase in solution [1,2]. We introduced single-molecule FRET (smFRET) measurements to study subunit rotation and regulatory conformational changes in individual FoF1-ATP synthases in liposomes. The rotary motors of these large membrane-embedded enzymes are either driven by ATP hydrolysis, or by a proton motive force for ATP synthesis. However, observation times of single, freely diffusing proteoliposomes are limited to tens of milliseconds by Brownian motion using a confocal microscope. To counteract diffusive motion actively in real time, we have built a fast anti-Brownian electrokinetic trap (ABEL trap, invented by A. E. Cohen and W. E. Moerner [3]) with a laser focus pattern and electrode feedback controlled by a FPGA. Increased observation times for about several seconds in the ABEL trap was achieved for smFRET measurements. Fast subunit rotation in FoF1-ATP synthases was recorded at different ATP concentrations revealing broad distributions of ATP hydrolysis rates from enzyme to enzyme, and changing speeds in time traces of a single enzyme [4]. ABEL-smFRET was used to unravel the mechanism of ADP inhibition of single FoF1-ATP synthase [5].
However, the ABEL trap observation times are still limited by photobleaching of the FRET fluorophores. How can we overcome this limit? Nitrogen-vacancy (NV) centers in nanodiamonds (10 to 100 nm diameter) can be applied as single fluorescent quantum sensors. The extraordinary photo-physical properties such as very high photo-stability and non-blinking behavior allow for optical detection of magnetic resonance due to the NV- triplet spin states as well as nanoscale distance measurements. To develop a nanodiamond-based alternative for smFRET, we determined the different molecular brightness, spectral ratio, diffusion coefficient, surface charge and multiexponential fluorescence lifetimes for nanodiamonds one by one in solution [6]. Now, we evaluate monitoring the fluorescence lifetime changes of the NV- center due to the Zeeman effect of local magnetic fields as a novel approach to record conformational changes like subunit rotation of a single diffusing FoF1-ATP synthase for tens to hundreds of seconds.

M. Börsch et al., FEBS lett. 527, 147-152 (2002)M. Diez et al., Nat. Struct. Mol. Biol. 11, 135-141 (2004)A. E. Cohen, W. E. Moerner, Proc. Natl. Acad. Sci. U. S. A. 103, 4362–4365 (2006) T. Heitkamp et al., J. Phys. Chem. B 125, 7638−7650 (2021) I. Pérez et al., Intl. J. Mol. Sci. 24, 8442 (2023) I. Pérez et al., Proc. of SPIE 12849, 1284906 (2024)



P11

Conformational dynamics of the endocytic protein Eps15

Andromachi Papagiannoula, Ida M Vedel, Mihai Bodescu, Kathrin Motzny, Sigrid Milles

Leibniz-Forschungsinstitut für Molekulare Pharmakologie im Forschungsverbund, Berlin

Eps15 is one of the earliest initiators in clathrin mediated endocytocis and is of complex architecture: Its N-terminal domain comprises three small Eps15 homology (EH) domains, followed by a coiled coil domain and a C-terminal intrinsically disordered region (IDR) of more than 400 residues in length. The EH domains contribute towards the establishment of a complex interaction network within clathrin mediated endocytosis by interacting with Asn-Pro-Phe (NPF) motifs within IDRs of other endocytic proteins. We investigated the binding from the side of the EH domains as well as from the side of the IDR of the endocytic partner Dab2 using nuclear magnetic resonance (NMR) spectroscopy. In addition to NPF binding, we detect a high level of binding promiscuity leading to significant interaction with non-NPF binding sites. This behavior also leads to interactions between Eps15's EH domains and Eps15IDR. When EH domains are expressed in row, as they occur in the wild type full length protein (EH123), EH2 and EH3 tumble together as one entity, while EH1 moves independently. Using single molecule Förster resonance energy transfer (smFRET), we assess the three dimensional organization of the three EH domains with respect to each other and assess binding with both Dab2 and Eps15IDR.

P12

Protein-Based Nanorulers for Validating Sub-10 nm Resolution in Super-Resolution Microscopy

Made Budiarta[1], Dominic Helmerich[2], Marvin Jungblut[1], Danush Taban[2], Sören Doose[2], Markus Sauer[1,2], Gerti Beliu[1]

[1]Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, 97080 Germany
[2]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, Würzburg, 97074 Germany

Super-resolution microscopy (SRM) has transformed biological imaging, enabling unprecedented insights into molecular arrangements and cellular architecture at resolutions below 10 nm. However, the lack of robust, biologically relevant reference structures has hindered accurate validation of these high-resolution techniques. Existing approaches, such as DNA origami-based rulers, while precise, often face limitations in mimicking native biological environments. P
Previously, we introduced PicoRulers based on the homotrimeric proliferating cell nuclear antigen (PCNA), featuring fluorophores arranged at fixed 6 nm intervals.[1] While effective, these rulers were constrained by their symmetrical architecture and limited labeling sites. To address these challenges, we have developed a new class of PicoRulers using Circular Tandem Repeat Proteins (cTRPs), which naturally assemble into rings with a diameter of 10 nm and up to 24 repeated units. By incorporating clickable unnatural amino acids into the flexible loop regions of cTRPs, we achieved site-specific labeling with high precision. This modular design allows for diverse fluorophore geometries, multi-site labeling, and multiplexed imaging using orthogonal bioorthogonal chemistries.
The performance of cTRP-based PicoRulers was validated using cutting-edge SRM techniques, including photoswitching fingerprint analysis with dSTORM, DNA-PAINT, and fluorescence lifetime imaging microscopy (FLIM). These experiments highlight their ability to serve as versatile and stable calibration tools, offering precise control over fluorophore placement with minimal linkage errors. Furthermore, the Picorulers exhibit excellent structural integrity, even under biologically relevant conditions, positioning them as reliable benchmarks for sub-10 nm resolution across diverse imaging platforms.
By bridging the gap between molecular precision and biological relevance, our PicoRulers represent a significant advancement for SRM.

They provide a powerful framework for studying molecular interactions, protein architectures, and dynamic processes at near-atomic resolution, paving the way for future innovations in bioimaging and nanotechnology.

[1] D. A. Helmerich, M. Budiarta, D. Taban, S. Doose, G. Beliu, M. Sauer, PCNA as Protein-Based Nanoruler for Sub-10 nm Fluorescence Imaging. Adv. Mater., 36, 2310104 (2024).

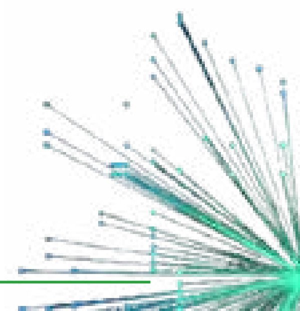
P13

Towards kilohertz structured illumination microscopy with random pattern

Adrian Platz, Gregor Gentsch, Andreas Stark, Christian Franke

Faculty of Physics and Astronomy, Institute of Applied Optics and Biophysics, Friedrich Schiller University Jena, Jena, Germany

Classical super-resolution microscopy techniques, such as Structured Illumination Microscopy (SIM), are often limited by the speed of pattern switching processes in Spatial Light Modulators (SLMs) or diffraction gratings. These speed limitations can often hinder the ability to capture fast biological processes. To overcome this challenge, we present a novel SIM approach that utilizes random pattern structured illumination. Our straight-forward method not only achieves sub-diffraction resolution but also achieves ultra-fast pattern switching, significantly improving imaging speeds compared to state of the art SIM implementation. We demonstrate imaging speeds approaching kilohertz framerates, while maintaining spatial resolutions in the order of 100 nm. This combination of high speed and spatial resolution opens new possibilities for imaging of biological processes, e.g. endosomal trafficking processes and ER fusion dynamics.



P14F

Real-time single-particle kinetics to reveal the mechanisms of virus assembly

Pedro Buzón[1], Daniel Nettels[1], Ben Schuler[1,2]

[1]Department of Biochemistry, University of Zurich, Zurich 8057, Switzerland.
[2]Department of Physics, University of Zurich, Zurich 8057, Switzerland.

Viral self-assembly is governed by balanced interactions between capsid proteins and the viral genome, where proteins and nucleic acids spontaneously assemble to form new viral particles. This is the most common assembly strategy for many small RNA viruses. However, the detailed mechanisms involved in this process remain poorly understood. Certainly, one of the major hurdles in the field has been dealing with the stochasticity associated with viral particle formation—inherent in the assembly process. Here, we present a method based on state-of-the-art single-molecule fluorescence spectroscopy and microscopy, including single-molecule Förster resonance energy transfer (smFRET), which allows the assembly of individual viral particles to be followed in real time. We show that the stochastic formation of viral particles can be modulated by ionic strength and initial protein concentration. In addition, we propose a kinetic model that quantitatively reproduces the main features of assembly and reveals the fine-tuned energetics of the process. Overall, we present a strategy that allows the study of viral particle formation with unprecedented mechanistic detail.

P15

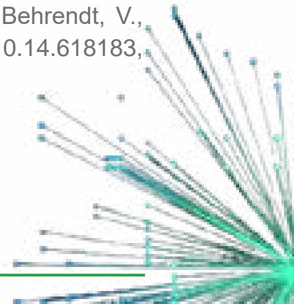
DNA Origami Nanoantennas for Real-Time Monitoring of Polymerase Activity and Prospective DNA Sequencing

Valentin Rech

Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-University, Butenandtstraße 5−13, 81377 Munich, Germany

The DNA origami technique is a powerful nanotechnology that enables the precise construction of complex 2D and 3D nanostructures using the programmability of DNA base pairing. These scaffolds allow for the spatially controlled attachment of diverse macromolecules, such as proteins, nucleic acids, and nanoparticles, through sequence-specific hybridization.
In our previous work, we developed surface-anchored DNA nanoantennas capable of capturing fluorescently labelled DNA probes. By integrating two nanoparticles into the origami scaffold, we created plasmonic hotspots—regions of enhanced electromagnetic fields—leading to significant fluorescence signal enhancement from probes captured within the hotspot.
Building upon this concept, we have now engineered a DNA origami system that immobilizes a DNA polymerase directly within such a plasmonic hotspot. This configuration enables real-time observation of strand synthesis by the elongating DNA polymerase via fluorescently labelled probes. Beyond monitoring polymerase activity, we envision this platform for single-molecule DNA sequencing based on the detection of fluorescently labelled deoxynucleoside triphosphate incorporation events. Our approach eliminates the need for prior template amplification and benefits from uniform enzyme positioning and high signal-to-noise ratios enabled by plasmonic enhancement, offering a promising route toward long-readlength and scalable sequencing technologies.

[1] Acuna, G. P., Möller, F. M., Holzmeister, P., Beater, S., Lalkens, B., Tinnefeld, P., Science, 338, 506–510 (2012).
[2] Puchkova, A., Vietz, C., Pibiri, E., Wünsch, B., Sanz Paz, M., Acuna, G. P., Tinnefeld, P., Nano Letters, 15, 8354–8359 (2015).
[3] Glembockyte, V., Grabenhorst, L., Trofymchuk, K., Tinnefeld, P., Accounts of Chemical Research, 54, 2918–2929 (2021).
[4] Close, C., Trofymchuk, K., Grabenhorst, L., Lalkens, B., Glembockyte, V., Tinnefeld, P., Advanced Materials Interfaces, 9, 2200255 (2022).
[5] Yaadav, R., Trofymchuk, K., Dass, M., Behrendt, V., Hauer, B., Schütz, J., et al., bioRxiv, 2024.10.14.618183, 1–21 (2024).



P16

A Link between Neural Development and Neurological Disorders-Molecular Switch of the Dendrite-to-Spine Transport of TDP-43/FMRP-Bound Neuronal mRNA and Its Impairment in ASD

Pritha Majumder[1,2], Biswanath Chatterjee[1], Khadiza Akter[1], Asmar Ahsan[1], Su Jie Tan[2], Chi-Chen Huang[1], Jen-Fei Chu[1], Che-Kun James Shen[1,3]

[1]PhD Program in Medical Neuroscience, Taipei Medical University, Taipei, Taiwan.
[2]Institute of Molecular Medicine, College of Medicine, National Chen Kung University, Tainan, Taipei.
[3]Institute of Molecular Biology, Academia Sinica, Nangang, Taipei, Taiwan

Regulation of messenger RNA (mRNA) transport and translation in neurons is essential for dendritic plasticity and learning/memory development. The trafficking of mRNAs along the hippocampal neuron dendrites remains translationally silent until they are selectively transported into the spines upon glutamate-induced receptor activation. However, the molecular mechanism(s) behind the spine entry of dendritic mRNAs under metabotropic glutamate receptor (mGluR)-mediated neuroactivation and long-term depression (LTD) as well as the fate of these mRNAs inside the spines are still elusive. Different molecular and imaging techniques, e.g., biochemical analyses, and optical imaging including live-cell imaging, live-cell tracking of RNA using molecular beacon, high-resolution imaging and mouse model study are used to elucidate a novel mechanism regulating dendritic spine transport of mRNAs in mammalian neurons. We demonstrate here that brief mGluR1 activation-mediated dephosphorylation of pFMRP (S499) results in the dissociation of FMRP from TDP-43 and handover of TDP-43/Rac1 mRNA complex from the dendritic transport track on microtubules to myosin V track on the spine actin filaments. In contrast, during mGluR1-mediated neuronal LTD, FMRP (S499) remains phosphorylated and the TDP-43/ Rac1 mRNA complex, being associated with kinesin 1-FMRP/cortactin/drebrin, enters the spines owing to Ca^2-dependent microtubule invasion into spines, but without translational reactivation. In a VPA-ASD mouse model, this regulation becomes anomalous. The misregulation of this switch could contribute to the pathogenesis of FMRP-related neurodisorders including the autism spectrum disorder (ASD). It also could indicate a molecular connection between ASD and neurodegenerative disease-related protein TDP-43 and opens up a new perspective of research to elucidate TDP-43 proteinopathy among patients with ASD.

P17

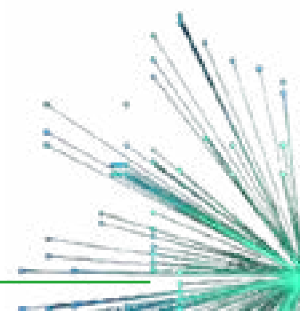
A zinc complex as an NIR emissive probe for real-time dynamics and in vivo embryogenic evolution of lysosomes using super-resolution microscopy

Abdul Salam[1], Kush Kaushik[1], Bodhidipra Mukherjee[2], Farhan Anjum[2], Chayan Kanti Nandi[1]

[1]School of Chemical Sciences, Indian Institute of Technology Mandi, Himachal Pradesh-175005, India
[2]School of Biosciences and Bioengineering, Indian Institute of Technology Mandi, Himachal Pradesh-175005, India

Zn-based fluorescent metal complexes have gained increasing attention due to their non-toxicity and high brightness with marked fluorescence quantum yield (QY). However, they have rarely been employed in super-resolution microscopy (SRM) to study live cells and in vivo dynamics of lysosomes. Here, we present an NIR emissive highly photostable Zn-complex as a multifaceted fluorescent probe for the long-term dynamical distribution of lysosomes in various cancerous and non-cancerous cells in live conditions and in-vivo embryogenic evolution in Caenorhabditis elegans (C. elegans). Apart from the normal fission, fusion, and kiss & run, the motility and the exact location of lysosomes at each point were mapped precisely. A notable difference in the lysosomal motility in the peripheral region between cancerous and non-cancerous cells was distinctly observed. This is attributed to the difference in viscosity of the cytoplasmic environment. On the other hand, along with the super-resolved structure of the smallest size lysosome (-77 nm) in live C. elegans, the complete in-vivo embryogenic evolution of lysosomes and lysosome-related organelles (LROs) was captured. We were able to capture the images of lysosomes and LROs at different stages of C. elegans, starting from a single cell and extending to a fully matured adult animal.

1. A. Salam et al., Chem. Sci., 15, 15659-15669 (2024)
2. N. Gustafsson et al., Nat. Commun., 7, 12471 (2016)
3. D. E. Johnson et al., J. Cell Biol., 212, 677 (2016)
4. Jankele et al., Elife, 10, e61714 (2021)



P18F

Revealing α-Synuclein Phase Transitions with ACDAN-Based Phasor Analysis

Leandro Cruz Rodríguez[1,] Nahuel N. Foressi[2], Pedro Silva[1], Leonel Malacrida[3,4], Maria Soledad Celej[5], Jelle Hendrix[1]

[1]UHasselt, Dynamic Bioimaging Lab, Advanced Optical Microscopy Centre, Biomedical Research Institute, Agoralaan C (BIOMED), B3590 Diepenbeek, Belgium
[2]Neurobiology & Biophysics, University of Washington School of Medicine, 1705 NE Pacific Street, 98195, Seattle, Washington, USA
[3]Advanced Bioimaging Unit, Institut Pasteur de Montevideo and Universidad de la República, Montevideo, Uruguay
[4]Unidad Académica de Fisiopatología, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[5]Departamento de Química Biológica Ranwel Caputto, Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC, CONICET), Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

Protein coacervation and phase transitions have emerged as a new paradigm in subcellular organization and disease biogenesis, consequently, novel approaches are increasingly needed to decipher the complexity of these phenomenon. In recent years, phasor analysis of spectral and lifetime measurements has proven valuable for interpreting biological phenomena in cells or biomimetic systems. In our work, we investigated the phase transitions of α-synuclein in the presence of spermine using 6-acetyl-2-dimethylaminonaphthalene (ACDAN) fluorescence, combined with hyperspectral imaging and two-photon fluorescence lifetime imaging microscopy. Cuvette measurements were able to capture subtle spectral shifts of ACDAN, indicating early changes in polarity and solvent relaxation that mark the onset of aggregation—details often overlooked by conventional probes such as Thioflavin-T. Complementary, HSI provided spatial resolution of dipolar relaxation within condensates, uncovering emerging heterogeneities during maturation. FLIM data revealed that while liquid condensates display a uniform fluorescence lifetime, the transition to mature amyloid fibrils gives rise to two distinct lifetime components, highlighting the coexistence of different microenvironments throughout fibril formation. These results demonstrate that monitoring ACDAN's spectral shifts and lifetime changes effectively tracks the evolution of the protein microenvironment, setting the stage for further research in molecular biology and protein biophysics, particularly in neurodegenerative diseases.

P19

Manipulation of the Energy Landscape of Tethered Fluorophores for Enhanced L-PAINT

Christoph Schmidt,[1] Julian Bauer,[1] Patrick Schüler[1], Samrat Basak[1], Tim Schröder[1], Jonas Zähringer[2] and Philip Tinnefeld[1]

[1]Department of Chemistry and Center for Nanoscience (CeNS), Ludwig Maximilian University of Munich, Germany
[2]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, USA and Department of Pediatrics, Harvard Medical School, Boston, USA

In Local-PAINT, a DNA-tethered fluorophore probes its nanoscale environment by transiently binding to specific docking sites, enabling site-selective fluorescence imaging at the single-molecule level.Compared to regular DNA-PAINT with imager molecules in solution, L-PAINT offers the advantages of having an increased local imager concentration leading to higher imaging speed while simultaneously reducing background.[1,2] Especially for serial applications such as MINFLUX that are not photon limited, substantial speed increase and reduced drift sensitivity can become a game changer. The technique relies on the diffusive motion of the DNA tether, whose inherent spatial bias limits access to distant binding sites.[3] In this work, we investigate how the energy landscape explored by the tethered probe can be actively shaped by altering the physical properties of the DNA pointer and its binding environment. Using models from polymer physics, we systematically vary parameters such as contour length, Kuhn length, the spatial arrangement of docking sites on model DNA origamis to manipulate the conformational freedom of the tether. By tuning the system's design, we aim to overcome the positional bias of tethered probes and achieve more uniform binding probabilities across a defined area. This approach lays the foundation for programmable, tunable control over molecular interactions at the nanoscale and opens new possibilities for engineered Local-PAINT systems.

[1] Zähringer, J. et al. Combining pMINFLUX, graphene energy transfer and DNA-PAINT for nanometer precise 3D super-resolution microscopy. _Light Sci Appl_ 12, 70 (2023).
[2] Ralf Jungmann, Christian Steinhauer, Max Scheible, Anton Kuzyk, Philip Tinnefeld, and Friedrich C. Simmel, Nano Letters 2010 10 (11), 4756-4761, DOI: 10.1021/nl103427w
[3] M. Schickinger, M. Zacharias, & H. Dietz, Tethered multifluorophore motion reveals equilibrium transition kinetics of single DNA double helices, Proc. Natl. Acad. Sci. U.S.A. 115 (32) E7512-E7521, https://doi.org/10.1073/pnas.1800585115 (2018).

P20

FCS as a tool to quantify morphogen dynamics and interactions in living embryos

Valentin Dunsing-Eichenauer[1,2], Pierre Recouvreux[1], Gayatri Mundhe[1], Pritha Pai[1], Vincent Bertrand1, Claudio Collinet1, Thomas Lecuit[1,3], Pierre-François Lenne[1]

[1]Aix Marseille Université, CNRS, IBDM UMR7288, Turing Center for Living Systems, Marseille, France
[2]Department of Infectious Diseases and Respiratory Medicine, Charité-Universitätsmedizin Berlin
[3]Collège de France, Paris, France

Its dynamic nature and sensitivity make FCS a powerful tool for studying molecular dynamics in living systems, despite challenges such as autofluorescence and low endogenous expression levels. Here, we applied FCS to quantify morphogen dynamics during highly dynamic morphogenetic processes in C. elegans and Drosophila embryos.
We show with point and scanning FCS that Wnt ligands produced in the posterior half of the C. elegans embryo spread extracellularly into the anterior half by diffusion over a timescale shorter than the cell cycle [1]. Through time integration of ligand arrival, the polarity established at the tissue level by the posterior Wnt source is transferred to the cellular level and induces asymmetric divisions of target cells.
In Drosophila embryos, we show that the GPCR ligand Fog, expressed in the posterior endoderm, diffuses and acts in a concentration-dependent manner to activate actomyosin contractility at a distance during a wave of tissue invagination [2]. While Fog is uniformly distributed in the extracellular space, it forms a surface-bound gradient that activates Myosin-II via receptor oligomerization, which we detect by FCS based diffusion and brightness analysis. This activity gradient self-renews as the wave propagates and is shaped by receptor endocytosis and a feedback mechanism involving integrin adhesion.

[1] Recouvreux, P. et al. (2024), Current Biology, Volume 34, Issue 9, 1853 - 1865.e6
[2] Mundhe, G., Dunsing-Eichenauer, V., et al. (2025), bioRxiv 2025.04.11.648359; doi: https://doi.org/10.1101/2025.04.11.648359

P21

Characterization of Candidalysin peptide self-aggregation on lipid membranes using Fluorescence Correlation Spectroscopy.

Gaukhar Zhurgenbayeva, Francisco Larios Paez, Christian Eggeling

Institute for Applied Optics and Biophysics, Friedrich-Schiller University Jena, Philosophenweg 7, 07743 Jena

Candidalysin is a cytolytic peptide toxin, secreted by the Candida albicans fungus during the invasion of the intestinal epithelia. This toxin plays a major role in direct cell membrane damage. To damage the cell, the secreted toxin must localize and accumulate at the plasma membrane. Before the secretion, Candidalysin is incorporated into a polyprotein precursor Ece1, composed of a signal peptide, the precursor peptide for CaL, and seven additional non-CaL Ece1 peptides (NCEPs). The accurate processing of Ece1 and the delivery of CaL to the extracellular space are crucial for causing damage to host epithelial cells. Here, we use fluorescence correlation spectroscopy to measure the diffusion rates of fluorescently labeled peptide toxins. The aim of our project was to study and compare the aggregation of Candidalysin its ability to specifically target the plasma membrane of host epithelial cells. We employed the synthetic peptide toxin fluorescently labeled with AlexaFluor647 and measured its diffusion in the aqueous solution and on the supported lipid bilayers composed of phosphatidylcholine and cholesterol. Additionally, we investigated how the NCEPs contribute to preventing auto-aggregation of Candidalysin, by co-incubating the peptide with NCEPs for 30 minutes before the measurement.

1. Moyes DL, Wilson D, Richardson JP, Mogavero S, Tang SX, Wernecke J, Höfs S, Gratacap RL, Robbins J, Runglall M, Murciano C, Blagojevic M, Thavaraj S, Förster TM, Hebecker B, Kasper L, Vizcay G, Iancu SI, Kichik N, Häder A, Kurzai O, Luo T, Krüger T, Kniemeyer O, Cota E, Bader O, Wheeler RT, Gutsmann T, Hube B, Naglik JR. Candidalysin is a fungal peptide toxin critical for mucosal infection. Nature. 2016 Apr 7;532(7597):64-8.
2. Richardson JP, Mogavero S, Moyes DL, Blagojevic M, Krüger T, Verma AH, Coleman BM, De La Cruz Diaz J, Schulz D, Ponde NO, Carrano G, Kniemeyer O, Wilson D, Bader O, Enoiu SI, Ho J, Kichik N, Gaffen SL, Hube B, Naglik JR. Processing of Candida albicans Ece1p Is Critical for Candidalysin Maturation and Fungal Virulence. mBio. 2018 Jan 23;9(1):e02178-17.
3. Müller R, König A, Groth S, Zarnowski R, Visser C, Handrianz T, Maufrais C, Krüger T, Himmel M, Lee S, Priest EL, Yildirim D, Richardson JP, Blango MG, Bougnoux ME, Kniemeyer O, d'Enfert C, Brakhage AA, Andes DR, Trümper V, Nehls C, Kasper L, Mogavero S, Gutsmann T, Naglik JR, Allert S, Hube B. Secretion of the fungal toxin candidalysin is dependent on conserved precursor peptide sequences. Nat Microbiol. 2024 Mar;9(3):669-683.

P22

Effects of Photoswitching Dynamics on MINFLUX Performance with Far-Red and Near-Infrared Fluorophores

Hanie Esmaeeli a, Chinmaya Venugopal Srambickal a, Mark Bates b, Joachim Piguet a, Lenny Reinkensmeier b, René Siegmund b, Alexander Egner b, Jerker Widengren a*

a Experimental Biomolecular Physics, Department of Applied Physics, Royal Institute of Technology (KTH), Stockholm, Sweden.b Institut für Nanophotonik Göttingen, Germany.

MINimal fluorescence photon FLUXes (MINFLUX)1 is an advanced super-resolution microscopy technique delivering nanometer precision through patterned illumination and reversible photoswitchable fluorophores. Like other single molecule localization methods, MINFLUX performance de-pends critically on fluorophore photophysical and photoswitching behav-iors, necessitating thorough characterization.We systematically studied the blinking/switching properties of far-red and near-infrared (NIR) cyanine fluorophores across microsecond-to-second timescales. Using Transient State (TRAST)2 spectroscopy and stochastic optical reconstruction microscopy (STORM)3, we examined fast (µs-ms) and slow (ms-s) switching dynamics under MINFLUX-relevant conditions. We established photodynamic models with transition rate parameters and simulated fluorophore behaviors under representative MINFLUX excitation beam scans.Results revealed that dark state transitions in the µs to ms range, particu-larly redox state transitions, significantly affect MINFLUX localization, especially with NIR fluorophores. This finding led us to develop a redox-balanced buffer which enabled the extension of MINFLUX to the NIR spec-tral range4. Furthermore, we discovered that nearby fluorophores pho-toswitching to off-states and bleaching in the ms to s timescales impacts MINFLUX image quality, providing crucial insights for optimizing super-resolution imaging protocols.

1. Balzarotti et al, Science 2017
2. Sandberg et al, J Phys Chem B 2023
3. Rust et al, Nature Methods 2006
4. Srambickal & Esmaeeli et al, Bioarxive 2024

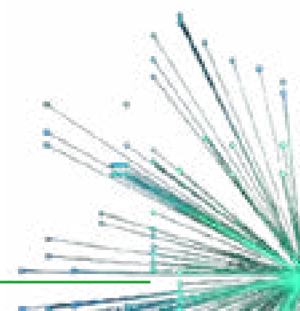
P23

Rethinking the Mindset: Unlocking the Potential of MINFLUX enabled Single-Particle Tracking

Bela T. L. Vogler[1,2], Francesco Reina[2,3], Christian Eggeling[1,2,4,5]

[1]Faculty of Physics and Astronomy, Institute of Applied Optics and Biophysics, Friedrich Schiller University Jena, Jena, Germany
[2]Leibniz Institute of Photonic Technology e.V., Jena, Germany, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
[3]Max Perutz Labs, Department of Structural and Computational Biology, University of Vienna, Dr.-Bohr-Gasse 9, 1030 Vienna, Austria
[4]Jena Center for Soft Matter, Friedrich Schiller University Jena, Jena, Germany
[5]Abbe Center of Photonics, Friedrich Schiller University Jena, Jena, Germany

MINFLUX fluorescence microscopy advances the field of super resolution imaging and Single-Particle Tracking (SPT) by achieving unparalleled spatio-temporal resolution with single-digit nanometer precision and kilohertz sampling rates. These capabilities enable the exploration of molecular interactions and dynamic structures in live cells with unprecedented detail. However, the transition from conventional SPT to MINFLUX demands a fundamental shift in methodology and mindset. Traditional analysis techniques, developed for camera-based systems, often fail to account for the iterative position estimation, inhomogeneous time signal, and statistical nuances inherent to MINFLUX. We will discuss the challenges and opportunities of adapting SPT approaches for MINFLUX-enabled studies, highlighting the methodological innovations required to fully leverage its potential. By addressing the need for optimized feedback systems, artifact minimization, and context-aware data interpretation, we aim to showcase how MINFLUX uniquely advances our understanding of cellular structures and their dynamic behavior.



P24F

Monitoring the Coating of Single DNA Origami Nanostructures with a Molecular Fluorescence Lifetime Sensor

Gereon Andreas Brüggenthies, Michael Scheckenbach, Tim Schröder, Karina Betuker, Lea Wassermann, Philip Tinnefeld, Amelie Heuer-Jungemann, Viktorija Glembockyte

Viktorija Glembockyte, Jahnstr. 29 , 69120 Heidelberg, Germany

Current advances in DNA nanotechnology enabled the design and synthesis of complex and functional nanostructures including artificial DNA motors, DNA crystals and DNA nanopores. [1] More recently, the focus shifted to more biological applications like DNA origami-based drug delivery and therapeutics. Those DNA nanostructures have to meet certain biostability thresholds to remain stable under physiologic conditions. [2] Recent approaches take advantage of the use of silica or polymers like oligolysine PEG to shield the structures from harsh conditions including low salt buffers, high temperatures or enzymatic degradation [3,4]. Up to this point a direct readout for the encapsulation of DNA origami on the single nanodevice level is missing. Here we present a method where we use a fluorescence lifetime of a cyanine dye to report on encapsulation of DNA origami nanostructures. Using this strategy, we are now able to study the efficiency and robustness of different DNA origami protection strategies in harsh chemical and biochemical environments.

[1] Pengfei Zhan et al., Chemical Reviews, 123:3976-4050 (2023)

[2] Arun R. Chandrasekaran et al., Nature Reviews Chemistry, 5:225–239 (2021)

[3] Lea M. Wassermann et al., Advanced Materials, 35:2212024 (2023)

[4] Nandhini Ponnuswamy et al., Nature communications. 8:15654 (2017)

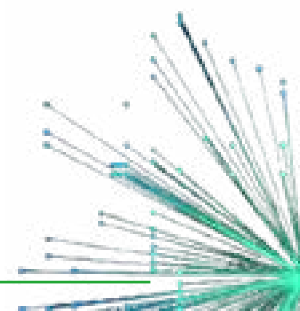
P25

A fluorescence-based platform for monitoring osmolyte transport

Hongbin Wu[1,2], Despoina Kapiki[1,2], Heinrich Jung[3], Christine Ziegler[4], Thorben Cordes[1,2]

[1]Biophysical Chemistry, Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany
[2]Physical and Synthetic Biology, Faculty of Biology, Ludwig-Maximilians-Universität München, Großhadernerstr. 2-4, 82152 Planegg-Martinsried, Germany
[3]Microbiology, Faculty of Biology, Ludwig-Maximilians-Universität München, Großhadernerstr. 2-4, 82152 Planegg-Martinsried, Germany
[4]Department for Structural Biology / Biophysics II, University of Regensburg, Regensburg, Germany

Membrane transporters are involved in a variety of cellular processes. Despite their importance many underlying transport mechanisms or even the transported substrates are unclear, which makes it difficult to monitor transport kinetics or to identify inhibitors. We developed fluorescence-based chemosensor systems for real-time monitoring of active osmolyte transport across biological membranes. Our assay utilizes an indicator displacement principle[1], where the fluorescent dyes are initially quenched upon complexation with a host molecule. The subsequent displacement of the dye by transported osmolytes leads to fluorescence recovery, providing a direct measure of osmolyte concentration in the liposome and thus transport activity. The chemosensor system was successfully integrated into different proteoliposomes, enabling the investigation of osmolyte transport by secondary-active membrane transporters (BetP, BetT, and PutP). Bulk fluorescence assays directly show the active transport of osmolytes, with varying sensitivities depending on the dye-host combination and the target analyte. Furthermore, we show the implementation of total internal reflection fluorescence (TIRF) microscopy for the visualization of single-molecule transporter recordings with the goal to obtain new insights into kinetics the transport. The newly developed chemosensor systems offer a powerful tool for studying molecular mechanisms of transport down to the single liposome level, with potential applications in drug discovery, diagnostics, and the understanding fundamental biological processes.



P26F

The Power of Three: Dynactin associates with three dyneins under load for greater force production

Arne Gennerich

1300 Morris Park Ave, Forchheimer Bldg. Room 628,
Bronx, NY 10461

Cytoplasmic dynein, a highly complex microtubule-associated motor protein, is essential for a wide range of cellular functions. Recent research reveals that dynein's largest cofactor, dynactin, in complex with the cargo-adaptor Bicaudal-D2 (BicD), binds to two dyneins. Through structure-function and single-molecule analyses, we unveil a tension-induced binding of a third dynein. The regulatory protein Lis1 promotes dynein triad formation under tension and fully activates dynein-dynactin-BicD (DDB) when bound to a single dynein (DDB-1). Without Lis1, DDB-1 generates forces of either ~2.5 or ~4.5 pN, depending on its partial or full activation. Fully activated DDB complexes generate forces of ~4.5, ~7, or ~9 pN, depending on the number of bound dyneins, suggesting a staggered arrangement of the motors. Contrasting prior studies, we show that DDB complexes take predominantly 8 nm steps under load. These findings suggest that DDB motor complexes self-assemble when under load, demonstrating adaptation under mechanical tension in cellular functions.

P27

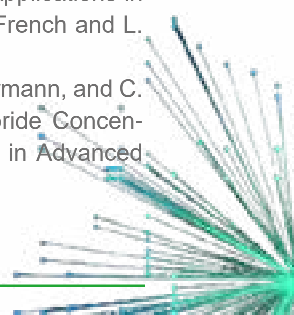
Imaging Cell Parameters with Organic Dyes and Genetically Encoded Biosensors based on Fluorescence Lifetime Imaging

Thomas Gensch

Institute of Biological Information Processing 1 (IBI-1; Molecular and Cellular Physiology); Forschungszentrum Juelich, Leo-Brandt-Str., 45428 Juelich, FRGermany

Fluorescence Microscopy has grown in 1980 - 2000 to one of the most important tools in cell biology and physiology and keeps this role undisputed. Starting from „simple“ fluorescence intensity readout many different, sophisticated and specialized fluorescence microscopy modalities have been and are still developed to this day. This relies on both improved intrumentation hardware, biosensor development, and new data evaluation schemes, to name the most important. Fluorescence Lifetime Imaging (FLIM) is an advanced fluorescence microscopy modality that – though established already in the 1970ies - has been implemented and used rarely.
FLIM can be used to determine vital cell parameters like concentrations of cell constituents, temperature, forces or enzyme activity. In the following an overview is given on how FLIM based on Time-Correlated Single Photon Counting has been used in my lab in the past 25 years to determine pH, ion concentrations and other parameters.

[1] H. Kaneko, I. Putzier, S. Frings, U. B. Kaupp, and T. Gensch J. Neurosci. 24:7931-7938 (2004)
[2] D. Gilbert, C. Franjic-Wuertz, K. Funk, T. Gensch, S. Frings, and F. Moehrlen Int. J. Dev. Neurosci. 25:479-489 (2007)
[3] K. Funk, A. Woitecki, C. Franjic-Wuertz, T. Gensch, F. Moehrlen, and S. Frings Mol. Pain 4:32-43 (2008)
[4] J. Potzkei, M. Kunze, T. Drepper, T. Gensch, K.-E. Jaeger, and J. Buechs BMC Biology 10:28 (2012)
[5] A. Geiger, L. Russo, T. Gensch, T. Thestrup, S. Becker, K.-P. Hopfner, C. Griesinger, G. Witte, and O. Griesbeck Biophys. J. 102:2401 – 2410 (2012)
[6] G. Stölting, R. Campos de Oliveira, R.E. Guzman, E. Miranda-Laferte, R. Conrad, N. Jordan, S. Schmidt, J. Hendriks, T. Gensch, and P. Hidalgo J. Biol. Chem. 290:4561-4572 (2015)
[7] C. Biskup, T. Gensch Fluorescence Lifetime Imaging of ions in biological tissues in “Fluorescence Lifetime Spectroscopy and Imaging. Principles and Applications in Biomedical Diagnostics” D. Elson, P.W.M. French and L. Marcu, eds. Taylor & Francis (2014)
[8] T. Gensch, V. Untiet, A. Franzen, P. Kovermann, and C. Fahlke. „Determination of Intracellular Chloride Concentrations by Fluorescence Lifetime Imaging“ in Advanced Time-Correlated Single Photon Counting



Applications W. Becker (ed.) Springer Series in Chemical Physics 111 Springer International Publishing Switzerland (2015).
[9] V. Untiet, L.M. Moeller, X. Ibarra-Soria, G. Sánchez-Andrade, M. Stricker, E.M. Neuhaus, D. W. Logan, T. Gensch, and M. Spehr Chem. Senses 41:669-676 (2016)
[10] V. Untiet, P. Kovermann, N.J. Gerkau, T. Gensch, C.R. Rose, and Fahlke C. Glia 65:388-400 (2017)
[11] S. Burgstaller, H. Bischof, T. Gensch, S. Stryeck, B. Gottschalk, J. Ramadani-Muja, E. Eroglu, R. Rost, S. Balfanz, A. Baumann, M. Waldeck-Weiermair, J.C. Hay, T. Madl, W.F. Graier, and R. Malli ACS Sensors 4:883-891 (2019).
[12] J. Meyer, V. Untiet, C. Fahlke, T. Gensch, and C.R. Rose J. Gen. Physiol., 151, 1319–1331 (2019)
[13] M. Engels, M. Kalia, S. Rahmati, L. Petersilie, P. Kovermann, M.J.A.M. van Putten, C.R. Rose, H.G.E. Meijer, T. Gensch and C. Fahlke Front. Cell. Neurosci. 15, 735300 (2021)
[14] C. Montali, S. Abbruzzetti, A. Franzen, G. Casini, S. Bruno, P. Delcanale, S. Burgstaller, J. Ramadani-Muja, R. Malli, T. Gensch, C. Viappiani Antioxidants, 11, 2229 (2022)
[15] E. Byvaltcev, M. Behbood, J.-H. Schleimer, T. Gensch, A. Semyanov, S. Schreiber, and U. Strauss Cell Reports, 42, 112934 (2023)
[16] A. Tharmasothirajan, J. Melcr, J. Linney, T. Gensch, K. Krumbach, K.M. Ernst, C. Brasnett, P. Poggi, A.R. Pitt, A.D. Goddard, A. Chatgilialoglu, S.J. Marrink, J. Marienhagen Nat. Comm., 14, 5619 (2023)
[17] M.M. Gonzalez, M.G. Vizoso-Pinto, R. Erra-Balsells, T. Gensch, F.M. Cabrerizo Int. J. Mol. Sci. 25:4966 (2024)

P28

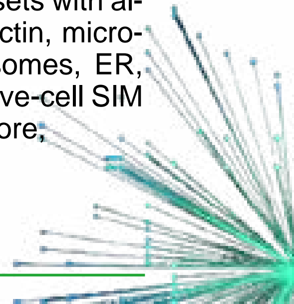
Nanotexture – a universal approach of AI-based computational multiplexing and phenotyping of super-resolution data.

NanTex - a start to finish framework for multiplexing super-resolution data

Gregor J. Gentsch[1], Bela T. L. Vogler[1,2], Pablo Carravilla[1,2,3], Dominic A. Helmerich[4], Teresa Klein[4], Katharina Reglinski[1,2], Markus Sauer[4,5], Christian Eggeling[1,2,6,7], Christian Franke[1,6,7]

[1]Faculty of Physics and Astronomy, Institute of Applied Optics and Biophysics, Friedrich Schiller University Jena, Jena, Germany
[2]Leibniz Institute of Photonic Technology e.V., Jena, Germany, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
[3]Present address: Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, Solna, Sweden
[4]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, Würzburg, Germany
[5]Rudolf Virchow Center, Research Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, Germany
[6]Jena Center for Soft Matter, Friedrich Schiller University Jena, Jena, Germany
[7]Abbe Center of Photonics, Friedrich Schiller University Jena, Jena, Germany

Fluorescence-based super-resolution microscopy (SRM) enables nanometer-scale visualization of cellular organelles. Traditional multicolor SRM relies on spectral multiplexing, but leading methods—STED, SMLM, MINFLUX—require specialized dyes with delicate photophysical properties, limiting multi-color imaging fidelity and live-cell compatibility. Fast acquisition techniques like SIM and Airy-Scan also face speed-resolution trade-offs and lack synchronicity in multi-color applications.
We introduce NanTex, a ML-based context-agnostic multiplexing approach leveraging organelle-specific nanotextures, applicable to SMLM, MINFLUX, STED, SIM, and Airy scan microscopy. NanTex demixes overlapping organelles from single-channel images without spectral separation, using AI-enabled textural demixing via U-Net learning [1].
NanTex trained on SMLM is directly applicable to MINFLUX without retraining, facilitating multiplexing at MINFLUX resolution without the extreme task to gather sensible amounts of training data. We demonstrate multiplexing in artificial overlays and real experimental datasets with almost all major cellular organelles (actin, microtubules, clathrin, endosomes, lysosomes, ER, mitochondria, golgi, etc.), including live-cell SIM and airyscan multiplexing. Furthermore,



we present NanTex on our novel high-speed SIM, based on random pattern structured illumination (speckle SIM) with 100 nm resolution at framerates of >100 Hz [2].
NanTex also enables computational phenotyping, exemplified by quantifying microtubule depolymerization upon nocodazole treatment.
NanTex advances super-resolution multi-organellar imaging in diverse SRM techniques.

[1] B. Vogler, G.J. Gentsch et al. in preparation (will be on biorxiv in April 2025)
[2] A. Platz, G.J. Gentsch et al. in preparation (will be on biorxiv in April 2025)

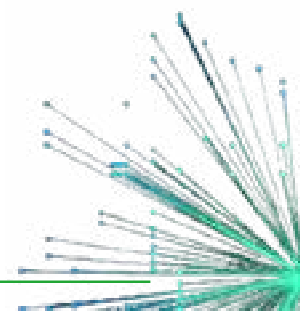
P29

Spectral decomposition of two molecule intensity images in polarized excitation fluorescence microscopy: reconstruction algorithms and simulations

Ivan Gligonov, Arjun Sharma, Oleksii Nevskyi, Jörg Enderlein

Third Institute of Physics – Biophysics, Georg-August-Universität Göttingen, Friedrich-Hund-Platz 1, 37073 Göttingen, Germany

Super-resolution microscopy techniques, in particular Single-Molecule Localization Microscopy (SMLM), rely on molecular blinking to distinguish individual fluorophores within an image. As these methods have advanced, the scientific focus has shifted toward resolving increasingly complex structures, where fluorophores are often separated by less than 10 nanometers. However, recent studies have shown that under such conditions, fluorophores tend to blink synchronously, making it difficult—or even impossible—to resolve them individually using conventional blinking-based approaches.
This work introduces a theoretical framework for a novel strategy that distinguishes two closely spaced molecules based on their orientation, offering a promising alternative to traditional methods. The proposed experimental setup utilizes polarized excitation at varying azimuthal angles and combines this with polarization-resolved image acquisition. This generates images with overlapping point spread functions (PSFs), but with orientation-dependent intensity contributions from each fluorophore.
Simulations demonstrate that it is possible to accurately estimate the orientations of individual fluorophores and use this information to decompose the composite image into two separate images—each corresponding to a distinct fluorophore. These separated images can then be used for the precise colocalization of two emitters. Using simulated data, we show that this method enables the resolution of fluorophores spaced 10 nm or less apart, opening new possibilities for studying densely packed molecular structures beyond the limits of current super-resolution techniques.



P30

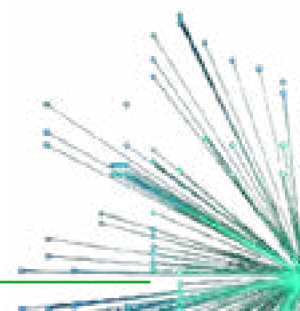
Multiphysics Simulation of Thermal Dispersion in Hydroxylapatite Scaffolds with Fe_3O_4–Au Hybrid Nanoparticles

Rebeca Jacqueline González Macías[1], Ángel David Ramírez Galindo[1], Solange Ivette Rivera Manrique[1], Dulce Araceli Guzmán Rocha[2], René García Contreras2

[1]Universidad de Guanajuato, División de Ciencias e Ingenierías Campus León, León 37150, México
[2]Escuela Nacional de Estudios Superiores Unidad León, Universidad , Nacional Autónoma de México (UNAM), León 37689, México

This work presents a multiphysics simulation aimed at studying heat propagation in a porous hydroxyapatite scaffold containing hybrid nanoparticles composed of a magnetic iron oxide (Fe_3O_4) core and a gold shell synthesized in situ using gum arabic (GA). These nanoparticles combine superparamagnetic properties, making them promising for applications in magnetic hyperthermia.
Using COMSOL Multiphysics®, the three-dimensional geometry of the scaffold was constructed, and the electromagnetic waves (in frequency domain) and heat transfer in solids modules were coupled. The complex optical properties of gold and the superparamagnetic properties of the Fe_3O_4 core were considered, integrating the photonic absorption effect as a heat source in the thermal simulation.
The results reveal a preferential localization of temperature dispersion in areas with higher density of hybrid nanoparticles, highlighting the design potential of therapeutic systems with spatial heating control. This simulation provides a useful prediction for optimizing bioactive structures with hybrid nanomaterials, aligning with current trends in biophotonics and nanomedicine.

[1] D. A. Guzmán-Rocha, B. Aranda-Herrera, L. S. Acosta Torres, M. C. Arenas-Arrocena, R. García-Contreras, Nano Select, 6, e202400077 (2025).
[2] D. Guzmán-Rocha, T. Córdova-Fraga, J. Bernal-Alvarado, E. Cano-González, MRS Advances, 7, 1–11 (2022).
[3] E. Gallegos Nieto, H. I. Medellín Castillo, D. F. de Lange, Ingeniería Mecánica, Tecnología y Desarrollo, 4, 5 (2013).
[4] Y. Zhao, X. Wang, L. Zhang, Y. Chen, Gels, 8, 575 (2022).
[5] T. Fernández Cabada, Caracterización de nanopartículas magnéticas y de oro para posibles aplicaciones biomédicas en diagnóstico y terapia, Tesis Doctoral, Universidad Politécnica de Madrid, Madrid (2014).
[6] Y. Guo, D. Gu, Z. Jin, P.-P. Du, R. Si, J. Tao, W.-Q. Xu, Y.-Y. Huang, S. Senanayake, Q.-S. Canción, C.-J. Jia, F. Schüth, Nanoescala, 7, 15990–15998 (2015).
[7] S. Schwaminger, P. Fraga-García, Nanoscale Adv., 3, 6012–6020 (2021)
[8] M. A. M. Tarkistani, V. Komalla, V. Kayser, Nanomateriales, 11, 1227 (2021).
[9] Z. Ban, Y. A. Barnakov, F. Li, V. O. Golub, C. J. O'Connor, J. Mater. Chem., 15, 4660–4662 (2005).
[10] J. W. Shen, K. Y. Li, L. Cheng, Z. Liu, S. T. Lee, J. Liu, ACS Appl. Mater. Interfaces, 6, 6443–6452 (2014).
[11] S. Hoskins, Y. Min, M. Gueorguieva, C. McDougall, A. Volovick, P. Prentice, Z. Wang, A. Melzer, P. Cuschieri, L. Wang, J. Nanobiotechnol., 10, 27 (2012).



P31

Single-Molecule FRET Analysis of the Structural Dynamics of the human transcription factor YY1

Andrzej Górecki, Jakub Bartuś, Katarzyna Gołębiowska-Mendroch

Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Kraków, Gronostajowa 7, 30-387 Kraków, Poland

Ying Yang 1 (YY1) is a multifunctional transcription factor involved in numerous developmental and regulatory processes. Its structure comprises a C-terminal zinc finger DNA-binding domain and a largely disordered N-terminal regulatory region [1]. The disordered character of the N-terminal domain poses challenges in understanding YY1's molecular mechanism of action.
To investigate YY1's conformational flexibility, we designed protein constructs suitable for fluorescence-based structural studies, with particular emphasis on single-molecule Förster Resonance Energy Transfer (smFRET). As part of this work, we developed and successfully applied a site-specific dual fluorescent labeling strategy, allowing precise insertion of donor and acceptor dyes at defined positions within the protein [2].
Initial spectroscopic analyses indicate that YY1 adopts distinct conformations depending on environmental factors such as salt concentration and zinc ion availability. These observations suggest that structural transitions within the N-terminal region are sensitive to external conditions and may underlie YY1's regulatory functions.
While smFRET data collection is still in progress, the dual-labeling strategy and preliminary findings provide a strong foundation for deeper insight into the dynamic behavior of YY1 and its functional modulation via conformational flexibility.
Keywords: YY1, intrinsically disordered protein, single-molecule FRET, site-specific labeling, structural dynamics

[1] Górecki, A., Bonarek, P., Górka, A. K., Figiel, M., Wilamowski, M., & Dziedzicka-Wasylewska, M., Proteins, 83(7), 1284–1296. (2015)
[2] Górka, A. K., Górecki, A., & Dziedzicka-Wasylewska, M., Protein science, 27(2), 390–401. (2018)

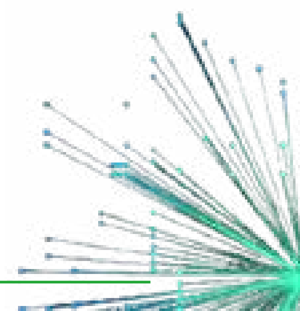
P32

Empowering Research with Time-Resolved Fluorescence Methods: A KeyLab Approach

Lisa M. Günther

Spectroscopy of Soft Matter, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany.
Bavarian Polymer Institute, University of Bayreuth, Universitätsstraße 30, 95447 Bayreuth, Germany.

Addressing today's complex scientific questions often requires interdisciplinary approaches that combine advanced analytical methods with diverse sample systems. However, researchers frequently encounter challenges due to limited access to specialized setups or expert technical and scientific support. KeyLabs provide a solution by offering shared environments where expertise, state-of-the-art equipment, and collaboration converge.
The KeyLab Optical Spectroscopy at the Bavarian Polymer Institute exemplifies this approach by providing access to a broad range of time-resolved (single-molecule) fluorescence techniques. At its core is a flexible MicroTime 200 system, complemented by a fully customizable setup built with PicoQuant components. This infrastructure supports researchers across disciplines such as macromolecular chemistry, biophysics, inorganic chemistry, and materials science.
From investigating polymer aggregates and perovskite nanostructures to studying energy transfer in biological complexes, the KeyLab offers customized experimental strategies to address specific scientific questions. By lowering technical barriers and promoting collaboration, it empowers users to explore complex photophysical phenomena without requiring extensive expertise in optics.



P33

Plasmonic material whit magneto-optical properties for biomedical applications

Dulce Guzmana-Rocha

Nanostructures and Biomaterials Area, Interdisciplinary, Research Laboratory (LII), National School of Higher, Studies (ENES), Leon Unit, National Autonomous, University of Mexico (UNAM), Leon, Mexico
Quantum applications laboratory, División de Ciencias e Ingenierías Campus Leon, Loma del Bosque 103, Lomas del campestre, Leon, Gto, México
Centro de Investigaciones en Óptica, Loma del Bosque 115, Colonia Lomas del Campestre, león, Gto, Mexico

Were synthesized hybrids nanoparticles consisting of an iron oxide nucleus and coatings of Gum Arabic (GA) and gold nanoparticles. The aim is to take advantage of the photothermal (PTT) properties of gold and the magnetic properties of iron oxide to obtain a material that works for a dual therapy: photothermal and magnetic hyperthermia (MHT) for cancer treatment. The structural, morphological, and magnetic characterization of the hybrid nanoparticles was obtained. The presence of gold nanoparticles was confirmed by X-ray diffraction: the hybrid diffraction patterns show the peaks corresponding to the NP–Au; also, the TEM images show a crystal size of 12 nm. The colloidal stability increment with the presence of gold nanoparticles obtained a zeta potential value of 21 mV. The magnetization saturation for hybrids was 47 emu/g and a blocking temperature was 336 K. These results manifest that MNP–GA–Au could be a promising alternative for dual treatment, PTT and MHT for cancer treatment. The synergistic effect of the magnetic and optical properties of the hybrid material are an option for the treatment of cancer and its use as a contrast medium for diagnostic imaging.

1. Y. Deng, D. Qi, C. Deng, X. Zhang, D. Zhao, J. Am. Chem. Soc.
130, 28–29 (2008)
2. J. Park, K.J. An, Y.S. Hwang, J.G. Park, H.J. Noh, J.Y. Kim, J.H.
Park, N.M. Hwang, T. Hyeon, Nat. Mater. 3, 891–895 (2004)
3. A.P. Zhu, L.H. Yuan, S. Dai, J. Phys. Chem. C 112, 5432–5438
(2008)

P34

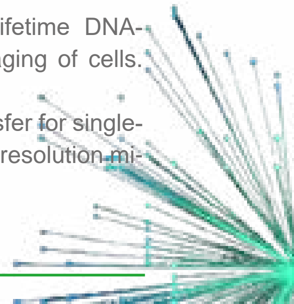
Smart Multiplexing and 3D Super-Resolution with Local PAINT and Lifetime-Encoded Imaging

Samrat Basak[1], Christoph Schmidt[1], Fiona Cole[1], Jonas Zähringer[1], Susanne C.M. Reinhardt[2,3], Julian Bauer[1], Giovanni Ferrari[1], Jakob Hartmann[1], Roman Tsukanov[4], Ralf Jungmann[2,3], Philip Tinnefeld[1]

[1]Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 München, Germany
[2]Faculty of Physics and Center for Nanoscience, Ludwig Maximilian University, Munich, Germany
[3]Max Planck Institute of Biochemistry, Martinsried, Germany
[4]III. Institute of Physics - Biophysics, Georg August University, 37077, Göttingen, Germany

Local PAINT1 is a variant of DNA PAINT 2,3 super-resolution where imager strands are tethered near their docking sites via long flexible linkers, enabling transient binding without relying on diffusion. This spatial confinement reduces background and improves imaging speed for local clusters avoiding complex drift correction.
We here explore how local PAINT can be used for intracellular targets and dense environments. Its spatial confinement and programmable kinetics not only improve localization accuracy but enables tracking of faster biomolecular processes that standard DNA-PAINT2 cannot resolve.
We establish local PAINT on both widefield and pMINFLUX4 platforms for precise, high-speed imaging in biological and synthetic systems. Together, spatially confined blinking, lifetime multiplexing, and GET-based z-localization form a powerful, versatile imaging strategy for next-generation single-molecule microscopy.

[1] Zähringer, J. et al. Combining pMINFLUX, graphene energy transfer and DNA-PAINT for nanometer precise 3D super-resolution microscopy. Light Sci Appl 12, 70 (2023).
[2] Jungmann, R. et al. Single-Molecule Kinetics and Super-Resolution Microscopy by Fluorescence Imaging of Transient Binding on DNA Origami. Nano Lett. 10, 4756-4761 (2010).
[3] Jungmann, R. et al. Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. Nat. Methods 11, 313–318 (2014).
[4] Masullo, L. A. et al. Pulsed interleaved MINFLUX. Nano Lett. 21, 840–846 (2021).
[5] Oleksiievets, N. et al. Fluorescence lifetime DNA-PAINT for multiplexed super-resolution imaging of cells. Commun. Biol. 5, 38 (2022).
[6] Kamińska, I. et al. Graphene energy transfer for single-molecule biophysics, biosensing, and super-resolution microscopy. Adv. Mater. 33, 2101099 (2021).



P35

Towards understanding the role of transcription factor oligomerization in regulating gene expression in live cells

Katherina Hemmen[1], Anay Fernanda Lazaro Alfaro[2], Thomas-Otavio Peulen[4], Exequiel A. Medina[3], Katrin G. Heinze[1], Hugo Sanabria[2]

[1]Julius-Maximilians University Wuerzburg, Rudolf Virchow Center for Translational and Integrative Bioimaging, Wuerzburg, Germany
[2]Clemson University, Department of Physics and Astronomy, Clemson, South Carolina, U.S. A.
[3]University of Chile, Department Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Santiago, Chile
[4]Faculty for Chemistry and Chemical Biology, Physical Chemistry, TU Dortmund University, Dortmund, Germany

Transcription Factors (TFs) are often multidomain proteins that regulate gene expression by binding specific DNA sequences. The FoxP family includes a conserved Forkhead-box DNA-binding domain (FKH), a leucine zipper (ZIP) domain, an unstructured linker, and a low-complexity, poly-Q-rich N-terminal region. Both FKH and ZIP domains dimerize, but their organization and dynamics in physiological conditions remain unclear.
We aim to investigate FoxP1 dimer formation in live cells, focusing on the contributions of the FKH, ZIP, and poly-Q-rich domains to dimerization and gene regulation. Using multiparameter fluorescence imaging spectroscopy, we analyze full-length and truncated FoxP1 variants—ZIP-FKH, FKH, and point mutants (R514H, A500P)—tagged with eGFP or mCherry.
Data from polarization-resolved confocal PIE-FRET-FLIM are processed using an automated pipeline (e.g., tttrlib[1]), which computes fluorescence lifetimes and anisotropies. This is integrated with machine learning-based segmentation and nuclear classification to map spatially resolved molecular complexes.
Preliminary results suggest the poly-Q-rich domain plays a key role in FoxP1 dimerization and oligomerization. Additionally, the R514H mutation, which disrupts DNA binding, causes nuclear condensation which may affect nuclear organization.

[1] Thomas-Otavio Peulen, Katherina Hemmen, Annemarie Greife, Benjamin M Webb, Suren Felekyan, Andrej Sali, Claus A M Seidel, Hugo Sanabria, Katrin G Heinze, tttrlib: modular software for integrating fluorescence spectroscopy, imaging, and molecular modeling, Bioinformatics, Volume 41, Issue 2, February 2025, btaf025, https://doi.org/10.1093/bioinformatics/btaf025

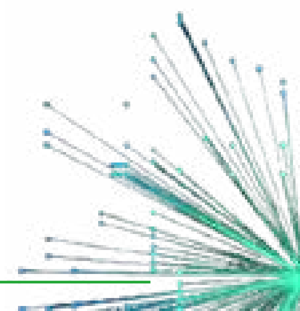
P36

Kinetic ligand binding mechanisms of bacterial substrate binding proteins

Pazit Con[2], Michel W. Jaworek[1], Thomas R. Weikl[3], Thorben Cordes[1,2]

[1]Biophysical Chemistry, Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany
[2]Physical and Synthetic Biology, Faculty of Biology, Ludwig-Maximilians-Universität München, Großhadernerstr. 2-4, 82152 Planegg-Martinsried, Germany
[3]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany

Periplasmic substrate-binding proteins (SBPs) mediate the selective transport of nutrients in bacteria by interacting with ABC importers. In this contribution we are interested in the coupling of ligand binding and conformational changes in SBPs to understand how this mechanism affects active membrane transport. SBPs are bilobed proteins that are known to exist in distinct open and closed conformations. While the ligand-bound state was often found to be a closed conformation, (semi-)closed conformations were shown to also exist independently. In the simplest case, this allows for two kinetic mechanisms: ligand-binding before conformational change (induced-fit, IF) or conformational change before ligand-binding (conformational selection, CS). Differentiating between these mechanisms has been a challenge for many decades and requires direct observation of structural dynamics and kinetic modelling. To address this, we here employ single-molecule Förster Resonance Energy Transfer (smFRET) to monitor real-time intramolecular conformational changes during ligand interaction. We combine these experiments with stopped-flow bulk kinetics and kinetic rate modelling. This integrated approach enables the comparison of different SBP variants with distinct biochemical properties and energy landscapes. Our results provide mechanistic insights into SBP function and offer a robust experimental framework to distinguish between IF and CS binding paradigms in dynamic proteins.



P37

Programmable heating for fluorescence microscopy using Printed Circuit Board (PCB) technology

Lambert-Paul Jorissen[1,2,3], Laurence Jorissen[1,2,], Jonas Vandevenne[1,2,], Yannick Stulens[4], Jef Hooyberghs[4], Jelle Hendrix[3], Ronald Thoelen[1,2]

[1]UHasselt, Biomedical Device Engineering Group, Institute for Materials Research (IMO), Agoralaan, 3590 Diepenbeek, Belgium
[2]Division IMOMEC, IMEC vzw, Wetenschapspark 1, 3590 Diepenbeek, Belgium
[3]UHasselt, Dynamic Bioimaging Group, Biomedical Research Institute, Agoralaan, 3590 Diepenbeek, Belgium
[4]UHasselt, Data Science Institute, Theory Lab, Agoralaan, 3590 Diepenbeek, Belgium

Precise temperature control is a powerful yet often neglected tool in fluorescence microscopy for studying biomolecular kinetics and thermodynamics. Current heating methods are typically limited by poor temporal resolution, high costs, or technical complexity. To address these limitations, we introduce a low-cost, flexible Printed Circuit Board (PCB) platform that enables rapid and programmable temperature modulation directly at the sample plane. Using software-controlled Joule heating, this system supports both conventional temperature-jump experiments and dynamic thermal perturbations inspired by analog electronics. For example, wave modulation, chirp signals, and frequency filtering enable frequency-domain analysis of molecular relaxation processes.The platform's customizable design and commercial availability make advanced thermal control more accessible for both ensemble and single-molecule experiments.

P38

Click-Proximity Probes Enable Quantitative Single-Molecule and Super-Resolution Imaging

Marvin Jungblut[1], Hannes Keiling[2], Leah Bismin[3], Samira Löw[1], Markus Sauer[1,3], Jürgen Seibel[2] and Gerti Beliu[1,4]

1Rudolf Virchow Center, University of Würzburg, Josef-Schneider-Str.2, 97080 Würzburg, Germany
2Institute of Organic Chemistry, Julius-Maximilians-University Würzburg, Am Hubland, 97074 Würzburg, Germany Germany
3Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
4Regensburg Center for Ultrafast Nanoscopy (RUN), Faculty of Chemistry and Pharmacy, University of Regensburg, 93053 Regensburg, Germany

Single-molecule and super-resolution microscopy require labeling strategies with nanometer precision and minimal linkage error. The quality of quantitative measurements critically depends on placing reactive probes directly at the protein of interest without perturbing its native interactions. Recent advances in proximity labeling have introduced photocatalytic strategies that enable spatially and temporally controlled mapping of protein interactions. Yet, precise and minimally invasive labeling remains a central challenge for single-molecule and super-resolution studies. Most existing strategies rely on bulky affinity-based labeling approaches, such as antibodies or nanobodies, to localize the photocatalyst near the protein of interest (POI). This introduces a significant linkage error and can perturb native protein interactions, which is particularly problematic in confined or crowded environments. To overcome these limitations, we developed a new class of modular Click-Proximity (CP) probes that combine photocatalytic labeling with genetic code expansion (GCE). CPs integrate (i) a tetrazine click handle for site-specific anchoring, (ii) photosensitive modules enabling spatiotemporally controlled cleavage and crosslinking to nearby partners, and (iii) a biotin tag for post-labeling detection and enrichment. In combination with GCE, TCO-functionalized unnatural amino acids can be incorporated site-specifically into the POI, CP probes can be positioned within a few Ångström of functional sites to minimizes spatial offsets, preserves native protein function, and enables accurate nanoscale readouts.We envision this design as particularly well suited for probing dense biological interfaces, such as the immunological synapse, neuronal synaptic clefts, or virus-host contact zones, where nanometer precision is critical. Together, CP probes provide a versatile and minimally invasive labeling concept that directly interfaces with single-molecule spectroscopy and super-resolution microscopy, thereby opening new opportunities for quantitative studies of protein interaction networks.

P39

Probing Biomolecular Condensates: Insights from Fluorescence Studies

Priyanshi Kalra

LION - Biological & Soft Matter, Leiden University, Netherlands

Membrane-less organelles formed through intracellular liquid-liquid phase separation play crucial roles in cellular function and dysfunction, including protein aggregation pathologies. Understanding the rheological characteristics and molecular mobility within these droplets is essential for elucidating how crowding influences biomolecular interactions. Recent studies have shown that phase-separated protein droplets sustain enzymatic activity and exhibit dynamic behavior akin to cellular environments [1]. Additionally, their viscoelastic properties resemble soft glassy materials, where a solid-like network forms within a dynamic fluid phase, affecting molecular diffusion and mechanical stability [2]. Here, we use an artificial system where BSA proteins form droplets via interactions with PEG crowding agents, mimicking cytoplasmic conditions. Using Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Recovery After Photobleaching (FRAP), we investigate the mechanical and dynamic properties of this model system. Our findings suggest that BSA is highly segregated inside the droplet phase, which is significantly more viscous (at least 100 times) or gel-like compared to the fluid supernatant, consistent with observations of viscoelastic behavior in other complex condensed-phase systems [2].

[1] A. Testa, M. Dindo, A. A. Rebane, B. Nasouri, R. W. Style, R. Golestanian, E. R. Dufresne, and P. Laurino, „Sustained enzymatic activity and flow in crowded protein droplets,“ Nature Communications, vol. 12, p. 6293, (2021).

[2] R. Zondervan, T. Xia, H. van der Meer, C. Storm, F. Kulzer, W. van Saarloos, and M. Orrit, „Soft glassy rheology of supercooled molecular liquids,“ Proceedings of the National Academy of Sciences (PNAS), vol. 105, pp. 4993–4998, (2008).

P40

Identification of cancer-induced protein rearrangement in platelets evidenced by STED super-resolution microscopy

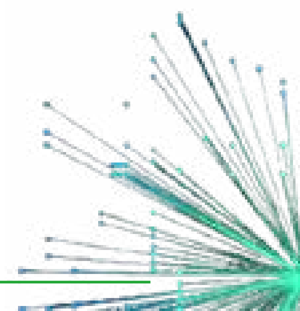
Adam Kenesei[1], Alina Oliferuk[2], Alexandra Rudenscholtz[2], Björn-Christian Ingwersen[1], Hanie Esmaeeli[1], Chinmaya Venugopal Srambickal[1], Marta Lomnytska[2], Jerker Widengren[1]

[1]Department of Applied Physics, Royal Institute of Technology (KTH), Stockholm, Sweden
[2]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

Platelets play a pivotal role in the development and spread of cancer. By interacting with tumor cells, platelets can promote tumor growth by formation of new blood vessels around tumors, and help tumors evade immune responses. So-called „““tumor-educated platelets““" have been identified displaying measurable changes in platelet RNA and protein content. Moreover, our previous studies by stimulated emission depletion microscopy (STED) have also shown changes in protein nano-scale localization patterns in platelets, upon exposure to platelet activators such as thrombin, and when co-cultured with cancer cells in vitro [1,2].
In this work, we investigated if similar changes in protein localization patterns can be found also in clinical platelet samples, from ovarian cancer patients. By STED, we examined platelets from patients with benign adnexal lesions and from patients with stage III-IV ovarian cancer and platelets co-incubated with benign and cancer cells. Our results suggest that multiple proteins show nanoscale redistribution features, in platelets from ovarian cancer patients and in platelets exposed to cancer cells in vitro, reflecting a specific tumor-platelet interplay.
These changes can contribute to a better understanding of this interplay and may serve as future non-invasive biomarkers for early cancer detection, based on minimally invasive liquid biopsies.

[1] Bergstrand J, Xu L, Miao X, Li N, Öktem O, Franzén B, Auer G, Lomnytska M, Widengren J., Nanoscale, 11(20):10023-10033 (2019)

[2] Bergstrand J, Miao X, Srambickal CV, Auer G, Widengren J., J Nanobiotechnology, 20(1):292 (2022)



P41F

Revolutionizing TCSPC Speed: Achieving 400% of the Excitation Rate with Near-Zero Distortion

Giulia Acconcia, Gennaro Fratta, Piergiorgio Daniele, Ivan Labanca, Ivan Rech

Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, 20133, Milan, Italy

Time correlated single photon counting (TCSPC) is the gold standard in fluorescence lifetime imaging. However, it has historically been considered incapable of achieving high speed, as the detector count rate was limited to a few percent of the excitation rate to avoid distortion[1]. In 2023, we revolutionized the paradigm of these measurements by demonstrating a new viable approach to combine high rates with negligible distortion [2]. Our TCSPC methodology involves acquiring, at run time, not only the classic histogram of photon arrival times, but also an additional histogram that tracks the system's status during the measurement. By combining these two histograms, an undistorted data histogram can be obtained under any operating condition.

In this work, we present the experimental results obtained using this new methodology in fluorescence lifetime measurements. We successfully pushed three different single-photon detectors—namely, a Single Photon Avalanche Diode (SPAD)[3], a Hybrid Photodetector (HPD) [4], and a Silicon Photomultiplier (SiPM)—well beyond the traditional TCSPC speed limitations. In particular, we achieved a record count rate as high as 400% of the excitation rate using a custom SiPM-based detection module featuring a dead time of only 1.2ns, ensuring accurate lifetime extraction and high-fidelity reconstruction of the light waveform.

[1] W. Becker, “Advanced time-correlated single photon counting techniques,” Springer Series in Chemical Physics. 2005.

[2] I. Rech, A. Bovolenta, A. Cominelli, and G. Acconcia, “Toward Constraintless Time-Correlated Single-Photon Counting Measurements: A New Method to Remove Pile-Up Distortion,” IEEE J. Sel. Top. Quantum Electron., vol. 30, no. 1, pp. 1–12, Jan. 2024.

[3] G. Fratta, P. Daniele, I. Labanca, I. Rech, and G. Acconcia, “Near-zero distortion in TCSPC at more than one photon per excitation period: experimental validation,” Opt. Lett. Vol. 49, Issue 17, pp. 4958-4961, vol. 49, no. 17, pp. 4958–4961, Sep. 2024.

[4] “PMA Hybrid Series - Hybrid Photomultiplier Detector Assembly | PicoQuant.” [Online]. Available: https://www.picoquant.com/products/category/photon-counting-detectors/pma-hybrid-series-hybrid-photomultiplier-detector-assembly. [Accessed: 10-Mar-2025].

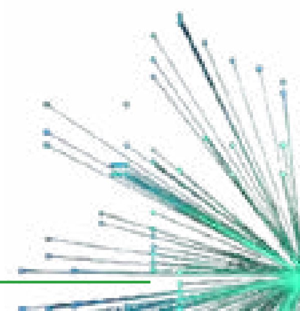
P42

RNA-Quantification in Lipid Nanoparticles with Fluorescence Correlation Spectroscopy

Bernhard Kirchmair[1], Judith Müller[1], Thomas Kellerer[2], Joachim Rädler[1]

[1]Ludwig-Maximilians-Universität München
[2]Hochschule München

Lipid nanoparticles (LNPs) emerged towards the most promising vectors to deliver messenger RNA (mRNA) to mammalian cells. Advanced strategies using multi-component nucleic acid species require a reliable quantification of the stoichiometric ratios. Quantitative knowledge about content and ratios will allow the delivery of genetic programs for regulated gene expression. Therefore, this project seeks to quantify the mRNA-content when varying the LNP size and surface composition. Employing Fluorescence Correlation Spectroscopy (FCS) measurements, assisted by Dynamic Light Scattering (DLS), both size and concentration of LNPs in solution can be estimated, allowing to obtain the average number of RNA molecules per particle. In this work, also mRNA loading dependent on LNP size was determined. Based on this, using Fluorescence Cross Correlation Spectroscopy, the stoichiometric ratio of short interfering RNA (siRNA) and mRNA, both fluorescently labeled, was determined.



P43

Capturing protein-protein interactions in live cells: APC/C and CDC20 in mitosis

Hradini Konthalapalli, Catherine Coates, Jonathon Pines

Institute of Cancer Research, London, UK

Chromosome segregation must be carefully regulated to ensure the fidelity of the distribution of the genome to daughter cells. Unattached kinetochores catalyse the formation of the Mitotic Checkpoint Complex (MCC), the effector of the Spindle Assembly Checkpoint (SAC). The MCC inhibits ubiquitination of mitotic substrates by the active Anaphase Promoting Complex/Cyclosome (APC/CCDC20) until all chromosomes are attached to both spindle poles.
The checkpoint is robust and responsive; one unattached kinetochore can prevent anaphase for hours, but anaphase starts a few minutes after the last kinetochore is attached. Prior work in the field has identified components of the checkpoint and potential mechanisms of interaction. However, there is limited data on the kinetics driving the interactions and the effect of cellular gradients of SAC proteins, kinases, and phosphatases on these interactions.
I am using Fluorescence Cross Correlation Spectroscopy (FCCS) with endogenously tagged SAC and APC/C proteins to quantify protein-protein interactions near chromosomes and in the cytoplasm. Preliminary observations suggest that APC/C and CDC20 interact throughout the cell during mitosis. We also observe that APC/C binds to its activator CDC20 with a higher affinity than APC/CCDC20 and its inhibitor MCC. These experiments serve as a base to build a model of SAC signalling and APC/C activity that includes spatial regulation of these proteins.

P44

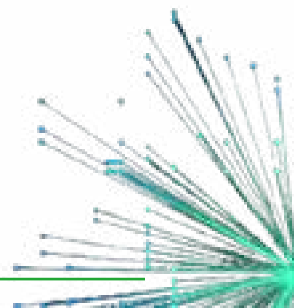
PSF Engineering for Single-Molecule Circularly Polarized Luminescence (CPL) Detection: Sensitivity Analysis and Parameter Estimation

Maximilian Lengauer[1], Isael Herrera[2], Miguel Alonso[3], Sophie Brasselet[4]

[1]lengauer@fresnel.fr
[2]isael.herrera@fresnel.fr
3miguel.alonso@fresnel.fr
[4]sophie.brasselet@fresnel.fr

Circularly Polarized Luminescence (CPL) is gaining increasing attention due to its promising potential in novel optical functions and chemical sensing. Recent advances in chiral molecular design have led to fluorophores with significantly enhanced CPL [1], opening the opportunity for chirality detection at the single-molecule level.
In this work, we investigate the feasibility of single-emitter CPL detection in polarization-sensitive microscopy. We first establish a theoretical framework that models the distribution of polarization states from chiral emitters as captured in a microscope. Based on Fisher Information applied to a formalism inspired from single-molecule Stokes polarimetry [2, 3], we analyze the sensitivity of various point spread function (PSF) engineering techniques in an extended parameter space, including (i) 3D emitter position, (ii) 3D orientation, and (iii) chirality. We identified different PSF engineering techniques which, under typical imaging conditions, reach a Cramér-Rao Lower Bound for chirality factor detection below 0.1 - comparable to values observed in efficient CPL-active molecules - while remaining sufficiently decoupled from the positional and orientational parameters.
Finally, we present an efficient parameter estimation algorithm, which leverages a limited number of pre-computed PSFs to select optimal initial conditions and parameter bounds, significantly accelerating convergence, as compared to unconditioned parameter searches.

[1] Zhang, Y., Yu, S., Han, B., Zhou, Y., Zhang, X., Gao, X., and Tang, Z., Matter, 5, 837 (2022)
[2] Brasselet, S., and Alonso, M. A., Optica, 10, 1486 (2023)
[3] Herrera, I., Alemán Castañeda, L. A., Brasselet, S., Alonso, M. A., J. Opt. Soc. Am. A, 41, 2134 (2024)



P45F

Single-molecule FRET on a minimalistic 3D-printed setup using optimized dyes in the blue-green spectral region

Gabriel Moya Muñoz[1,2]+, Jorge Luna Piedra[2,3+], Pazit Con[2+], Mostofa Rohman1, Siyu Lu[1,2], Thomas Peule[1], Thorben Cordes[1,2*]

[1]Biophysical Chemistry, Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany
[2]Physical and Synthetic Biology, Faculty of Biology, Ludwig-Maximilians-Universität München, Großhadernerstr. 2-4, 82152 Planegg-Martinsried, Germany
[3] PicoQuant Innovations GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
*corresponding author: thorben.cordes@tu-dortmund.de
+these authors contributed equally to this work

Single-molecule Förster Resonance Energy Transfer (smFRET) is a powerful technique for the detection of biomolecules, to probe biomolecular interactions and conformational changes with sub-nanometer spatial resolution. While the technique is widely adopted in structural biology and biophysics, the evolution of smFRET instrumentation has led to an increasing complexity and cost of the setups, often involving multi-laser excitation schemes, time-resolved detection electronics, and sophisticated optical layouts. To provide an accessible alternative, we here present a minimalistic extension of Brick-MIC, a recently introduced 3D-printed micro-spectroscopy platform. Our new Brick-MIC implementation uses continuous-wave excitation at 488 nm and a streamlined set of opto-mechanical components to perform smFRET on dye combinations in the blue-green spectral region. By relying on off-the-shelf optics and a compact design, we were able to significantly reduce both system cost and setup complexity while retaining single-molecule sensitivity by choice of matching dyes for the spectral sensitivity of the detectors. To optimize the photon output of dye combinations such as Alexa488-Cy3B and Alexa488-Atto542, we also introduce ferrocene derivatives as new photostabilizers to increase donor and acceptor brightness via removal of triplet-related dark-states. We demonstrate the capability of the microscopy system with the optimized dye-photostabilizer combinations by resolving distinct FRET efficiencies in static DNA constructs and by detecting conformational changes in bacterial model proteins. This work shows that high-quality smFRET measurements can be achieved using a minimalistic and cost-effective platform.

P46

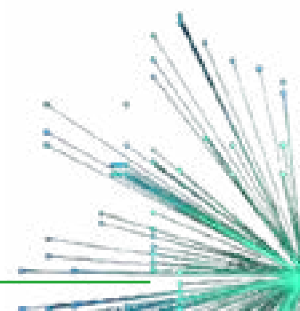
Binding or Unwinding? The role of an RNA binding protein in ribozyme activity

Lennart Lindner, Susann Zelger-Paulus, Roland K.O. Sigel

Winterthurerstrasse 190, 8057 Zürich, Switzerland

Ribozymes are non-coding RNAs folding into a specific, 3-dimensional structure to carry out chemical reactions. For an efficient reaction, some ribozymes rely on protein assistance. While the effect of the protein on the ribozymes activity can easily be assessed by in vitro assays, identifying the underlying mechanism is not. To address this, we combine photoisomerization-related fluorescence enhancement (PIFE) and Förster resonance energy transfer (FRET), two single molecule techniques.
The initial goal is to establish and validate a combined PIFE-FRET methodology, employing the model system of a single-stranded RNA. The Cy3 labelled RNA is immobilized on a cover slip mounted on a TIRF microscope setup. The recorded intensity traces indicate an intensity increase in the presence of the protein hinting at a potential translocase activity.
Subsequently, the interaction of the protein with the ribozyme will be studied using the established PIFE-FRET measurements. Our investigation may provide a model for how primitive RNA systems evolved enhanced catalytic capabilities through the recruitment of protein cofactors.

[1] S. Mohr, M. Matsuura, P. S. Perlman, and A. M. Lambowitz, Proceedings of the National Academy of Sciences of the United States of America, 103, 3569–3574 (2006)
[2] A. M. Pyle, Annual review of biophysics, 45, 183–205 (2016)
[3]] E. Ploetz et al., Sci Rep, 6, 33257 (2016)
[4] H. Hwang and S. Myong, Chemical Society reviews, 43, 1221–1229 (2014)



P47

Toward multiple coordinates: Resolving the intrinsic heterogeneity of a group II intron folding process

Matteo Lisibach, Susann Zelger-Paulus, Roland K.O. Sigel

Departement of Chemistry, University of Zurich, Zurich, CH-8057, Switzerland

In eucaryotic cells, splicing involves over 90 co-factors, while group II introns such as Sc.ai5γ from baker's yeast perform molecular acrobatics through a dynamic and precise folding process without the need for accessory proteins to obtain a proper mature RNA. [1] This folding pathway is based on multiple conformations, which can be illuminated with single-molecule Förster resonance energy transfer.
Most FRET studies rely on a single FRET pair to summarise the overall dynamics, overlooking the conformational changes in other regions of the structure. To address this limitation, we introduced multiple labelling schemes to obtain a comprehensive picture of the folding pathway of this highly dynamic ribozyme. A mutation-guided correlation method was established to map FRET states from one labelling scheme to another, expanding distance into triangular restraints. The goal is to integrate these conformations into a static homology model [2] and due to the state correlations from different labelling schemes, a specific step from the folding pathway could be assigned to the structure and further potential folds proposed. This study unveils interesting insights into a heterogeneously dynamic biomolecule, which was unseen by only one well-established labelling scheme.

[1] Michel F, Ferat JL, Annu Rev Biochem, 64, 435 (1995).
[2] Somarowthu S, Legiewicz M, Keating K.S, Pyle A.M, Nucleic Acids Res, 42, 1947 (2014).

P48

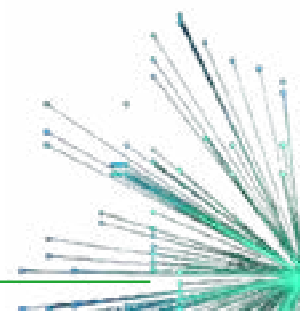
Photophysical structured illumination flowmetry based on the long-lasting emission response of lanthanide luminescent nanoparticles

Haichun Liu[1], Jerker Widengren[1]

[1]Department of Applied Physics, KTH Royal Institute of Technology, S-106 91, Stockholm, Sweden

In this study, we introduce photophysical structured illumination flowmetry (PP-SIF) and explore its merits through comprehensive numerical simulations [1]. PP-SIF makes it possible to capture two-dimensional (2D) flow velocity fields from a single snapshot image of the emission pattern of luminescent probes, by exploiting the intrinsic photodynamics of the imaged probes and taking the applied structured excitation field as a reference. We propose lanthanide-based upconversion nanoparticles (UCNPs)—particularly those operating in the highly biocompatible and transparent NIR-II window (1000–1700 nm)—as suitable probes for implementing PP-SIF.
Unlike established techniques such as particle image velocimetry (PIV) and particle tracking velocimetry (PTV) [2], which rely on tracking individual particles, PP-SIF directly extracts flow information from the emission profile itself. It is also fundamentally distinct from methods that combine structured illumination microscopy (SIM) with flow, where motion merely facilitates imaging rather than being the measurement target [3-5].
By eliminating the need for rastered excitation scans, PP-SIF offers a pathway to significantly faster acquisition of velocity field data, opening new possibilities for rapid, high-resolution flow imaging.

[1] H. Liu, J. Widengren, Research Square preprint, DOI: 10.21203/rs.3.rs-5965275/v1 (2025)



P49

Following ribozyme splicing pathways using escape-time stereometry (ETs)

Katharina Majer[1], Dimitrios Soulias[1], Esra Ahunbay[3], Susann Zelger-Paulus[3], Roland Sigel[3], Madhavi Krishnan[1,2]

[1]Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK
[2]The Kavli Institute for Nanoscience Discovery, Sherrington Road, Oxford, OX1 3QU, UK
[3]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

Ribozymes are catalytically active RNAs. Since their discovery[1,2], the active field of ribozyme research has provided extensive insights into the wide range of essential to life functions that ribozymes perform[3]. This understanding also fuelled research into applying ribozymes for therapeutic purposes. Ribozyme splicing, which involves the removal of non-coding regions and the joining of coding regions, is a crucial mechanism in gene expression and can be critically dependent on the molecular 3D conformation[4]. A variety of techniques has thus been applied to study ribozyme folding, including single-molecule techniques like fluorescence correlation spectroscopy or single-molecule Förster resonance energy transfer[5].
Here, we are using escape-time stereometry (ETs)[6] to follow the self-splicing of a ribozyme (group II intron) in two different buffers, that have previously been found to promote different intron conformations. ETs is a fast, versatile, high-throughput nanofluidic single-molecule method recently developed in our group. It enables real-time characterisation of the size and shape of molecules and molecular complexes in native solutions without the application of external fields or immobilisation. We are monitoring the self-splicing of a group II intron observing a multitude of conformational states of molecules in a wide size range, demonstrating the application of ETs to study complex reactions.

[1] Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE, Cech TR, Cell, 31, 147-57 (1982).
[2] Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S, Cell, 35, 849-57 (1983).
[3] Yang M, Xie Y, Zhu L, Li X, Xu W, ACS Catalysis, 14, 16392-422 (2024).
[4] Lilley DMJ, Curr Opin Struc Biol, 15, 313-23 (2005).
[5] Cardo L, Karunatilaka KS, Rueda D, Sigel RKO, Methods Mol Biol, 848, 227-51, (2012).
[6] Zhu1 X, Bennett TJD, Zouboulis KC, Soulias D, Grzybek M, Benesch JLP, El-Sagheer AH, Coskun Ü, Krishnan M, Science, to appear (2025).

P50

Rotational Dynamics of Single Molecules at the Interfaces of Thin Polymer Films

David Malsbenden[1], Daniel Marx[2], Oleksii Nevskyi[2], Jörg Enderlein[2], Dominik Wöll1

[1]Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, 52074 Aachen, Germany
[2]III. Institute of Physics – Biophysics, Georg-August-University Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Thin polymer films are used as protective coatings in advanced nanotechnological applications. The progressive miniaturization of these devices leads to an increasing surface-to-volume ratio of the applied polymer films. Consequently, interfacial effects, i.e. the macromolecular mobility at the polymer-air and polymer-substrate interface, are critical for the physical properties and durability of thin polymer films [1]. In order to probe the structural heterogeneities [2], the rotational diffusion of single fluorescent probe molecules embedded in thin polymer films is measured using defocused imaging techniques. The emitters display pronounced anisotropy in their angular emission distribution, resulting in specific orientation-dependent patterns. Such patterns can be modeled using classical Maxwell electrodynamics, providing valuable information regarding the three-dimensional orientation of the imaged molecules [3]. By simultaneously employing fluorescence lifetime imaging microscopy (FLIM), we combine metal-induced energy transfer (MIET) imaging with defocused imaging, allowing for the correlative measurement of both nanometer-resolved axial position and rotational mobility of a fluorophore [4]. In particular, this methodology enables the determination of rotational diffusion profiles directly at the interfaces of polymer films. Furthermore, we analyze the rotational dynamics across varying temperature ranges in the vicinity of the glass transition temperature.

[1] E. U. Mapesa, N. Shahidi, F. Kremer, M. Doxastakis, J. Sangoro, J. Phys. Chem. Lett., 12, 117-125 (2021).
[2] B. M. I. Flier, M. C. Baier, J. Huber, K. Müllen, S. Mecking, A. Zumbusch, D. Wöll, J. Am. Chem. Soc., 134, 480-488 (2012).
[3] M. Böhmer, J. Enderlein, J. Opt. Soc. Am. B, 20, 554-559 (2003).
[4] Deres, G. A. Floudas, K. Müllen, M. Van der Auweraer, F. De Schryver, J. Enderlein, H. Uji-i, J. Hofkens, Macromolecules, 44, 9703-9709 (2011).

P51F

Evaluating MINFLUX experimental performance <em>in silico

Zach Marin[1,2], Jonas Ries[1,2,3]

[1]Max Perutz Labs, Vienna Biocenter Campus (VBC), Vienna, Austria
[2]University of Vienna, Center for Molecular Biology, Department of Structural and Computational Biology, Vienna, Austria
[3]University of Vienna, Faculty of Physics, Vienna, Austria

MINFLUX is an emerging super-resolution microscopy technique for imaging and tracking with record resolution for a given number of detected photons. The performance of MINFLUX is well understood under idealized conditions. Yet, obtaining high-quality data remains challenging because of the complexity of performing a measurement and because in non-ideal biological samples numerous imperfections can cause unforeseen artifacts. How these experimental challenges impact MINFLUX quality and how they can be mitigated is still not well understood.
Here, we present SimuFLUX, a comprehensive simulator for MINFLUX experiments. It includes realistic models of point-spread functions, fluorophores, microscope mechanics and estimators. It allowed us to answer longstanding questions on how imperfections such as misalignments, fluorophore blinking and bleaching, background or vibrations affect MINFLUX accuracy. Of note, we found that resolution of MINFLUX DNA-PAINT imaging is severely limited by diffusive imaging strands, and that MINFLUX tracking has a strong bias when measuring diffusion coefficients of fast diffusing molecules.
We demonstrate how SimuFLUX can be used to optimize experimental parameters, and to simulate entire experiments to judge feasibility of a project.

P52

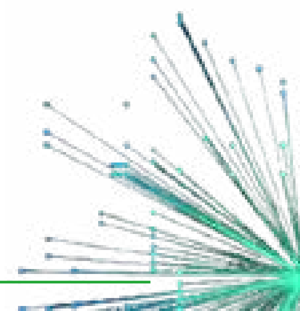
Unraveling CAR-T Cell Activation and Synapse Dynamics Using DNA-PAINT Single-Particle Tracking

Luciana Martinez[1], Tony Monteza Cabrejos[1], Miruna Tanase[1], Olivia Dalby[1,2], Efstratios Kirtsios[3], Alice Giustacchini[3], Sabrina Simoncelli[1,2]

[1]1London Centre for Nanotechnology, University College London, London, UK
[2]Department of Chemistry, University College London, London, UK
[3]Molecular and Cellular Immunology Unit, UCL Great Ormond Street Institute of Child Health, University College London, London, UK

Chimeric Antigen Receptor (CAR)-T cells offer a promising cancer therapy [1], but the molecular mechanisms behind CAR activation remain poorly understood. Unlike native T cell receptors (TCRs), CARs are believed to form structurally distinct synapses [2], and the dynamics of their activation remain largely unexplored.
In this study, we employ DNA-PAINT-based single-particle tracking (DNA-PAINT-SPT) [3] to investigate the nanoscale organization and dynamics of anti-CD19 CARs in live CAR-T cells. DNA-conjugated anti-ALFA nanobodies provide high-contrast, long-term single-molecule tracking, enabling precise spatial and temporal resolution to monitor CAR behavior.
To study synapse formation, lipid bilayers functionalized with CD19 and ICAM-1 are used to mimic cell-cell interaction interfaces and CAR T-cells activation. This system allows us to examine CAR clustering and diffusion, capturing early activation events and tracking changes in receptor behavior over time using sub-20 nm resolution.
Through trajectory classification and diffusion modelling, we quantitatively analyze receptor movement and clustering patterns. This technique offers powerful insights into the real-time behaviour of synthetic immune receptors and opens new avenues for studying their nanoscale architecture and functional dynamics.

[1] C. H. June et al., Science, 359, 1361–1365 (2018).
[2] A. J. Davenport et al., Proc. Natl. Acad. Sci. U.S.A., 115, E2068–E2076 (2018).
[3] C. Niederauer et al., Nat. Commun., 14, 4345 (2023).



P53

smFRET-guided integrative modelling of RNA - from single structures to a structural ensemble

Richard Börner[1], Felix Erichson[1], Mirko Weber[1], Fabio D. Steffen[2]

[1]Laserinstitut Hochschule Mittweida, Mittweida University of Applied Sciences, Technikumplatz 17, 09648 Mittweida, Germany
[2]University of Zurich, Zurich, Switzerland

FRET-assisted integrative modelling of RNA requires both a structural ensemble and reliable experimental FRET data that capture binding and folding trajectories [1]. Such ensembles can be generated either through 3D structure prediction tools or all-atom molecular dynamics (MD) simulations. However, MD simulations are computationally expensive and limited in their ability to sample the full conformational space of biomolecular ensembles. To address this, we use three tools RNAComposer, FARFAR2, and AlphaFold3 for the 3D structure prediction of a ribosomal RNA tertiary contact composed of a kissing loop and a GAAA tetraloop motif [2,3]. Our goal is to generate a highly diverse structure collection, which is then validated and filtered using base-pairing analysis and eRMSD. We compute the multi-accessible contact volume (mACV) for the FRET pair sCy3 and sCy5 using FRETraj, enabling prediction and comparison of the FRET distributions for each filtered structure collection [1,4]. These predicted distributions are then analyzed and weighted against an experimental FRET distribution. Our results show that RNAComposer and AlphaFold3 generate only a limited subset of possible conformations and fail to reproduce the full experimental FRET distribution characteristic of the unbound (low FRET) state of the GAAA tetraloop motif. In contrast, FARFAR2 produces a much more diverse ensemble, covering a broad range of conformations that resemble those sampled in MD simulations initialized from multiple unbound-state seed structures. The diversity enables FARFAR2 to replicate the in-solution smFRET experiment in silico. We demonstrate that FARFAR2 can complement MD simulations with knowledge-based structure initialization to reproduce FRET measurements accurately, even for an unbound, thus structurally heterogeneous state.

[1] Steffen FD, Cunha RA, Sigel RKO, Börner R, Nucleic Acids Research (2024).
[2] Gerhardy S, Oborská-Oplová M, Gillet L, Börner R et al., Nature Communications (2021).
[3] Weber M, Erichson F, Antczak M, Zok T, Steffen FD, Szachniuk M, Börner R, to be submitted (2025).
[4] Steffen FD, Sigel RKO, Börner R, Bioinformatics (2021).

P54

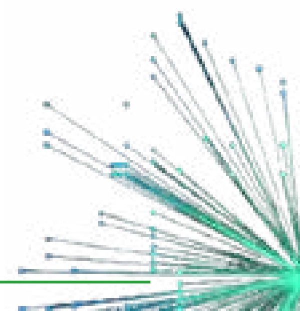
Towards a single-molecule approach to capture the delicate interplay between translation and misfolding on the ribosome

Ivana V. Bukvin[1], Juliana Abramovic[1], Judith Frydman[1]

[1]Department of Biology, Stanford University, Stanford, CA 94305, USA

The ribosome is increasingly recognised as an active participant in de novo protein folding, not only through the vectorial nature of synthesis but by modulating co-translational folding energetics, stabilising partially folded intermediates, and acting as a hub for chaperone engagement. While intrinsically disordered proteins (IDPs) underlie many neurodegenerative diseases, all proteins face their earliest decision between folding and misfolding during biosynthesis. Yet, our ability to capture these events remains limited, particularly at the resolution needed to disentangle kinetics and structural transitions in real time.
We are developing a single-molecule Förster resonance energy transfer (smFRET) approach to investigate how translation kinetics and nascent chain conformational dynamics intersect during biosynthesis on the ribosome. As a model system, we focus on huntingtin exon 1 (Httex1), an IDP linked to Huntington's disease. Its expanded polyglutamine tract (>35 CAG repeats) is flanked by a polyproline region associated with stalling, and an amphipathic N-terminal segment thought to initiate misfolding.
With this approach, we are progressing towards understanding how the ribosome modulates both translation kinetics and the conformational landscape of huntingtin during biosynthesis, offering structural and mechanistic insight into co-translational misfolding and its relevance to disease.

[1] Streit, J.O., Bukvin, I.V., Chan, S.H.S. et al. The ribosome lowers the entropic penalty of protein folding. Nature 633, 232–239 (2024)
[2] Aviner, R., Lee, TT., Masto, V.B. et al. Polyglutamine-mediated ribotoxicity disrupts proteostasis and stress responses in Huntington's disease. Nat Cell Biol 26, 892–902 (2024).



P55

Spectral characterization of terrylene photoproducts

Rana Mhanna, Julia Berger, Gregor Jung

Saarland University , Biophysical chemistry , Campus B2.2 - 66123 Saarbrücken

Single-molecule chemistry (SMC) by means of fluorescence microscopy allows us to study a reactive system at the molecular scale, thus 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 reaction1, and in the current case, we study the fluorescence colour change during the photooxidation of terrylene2. 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 matrices3. An effect of the excitation wavelength on promoting a specific reaction pathway over another has been observed and was attributed to formation of terrylene/oxygen complexes4. However, regardless of the pathway, the reaction yields luminescent photoproducts with a spectral shift relative to terrylene. We present herein our experimental findings on the actual spectral signatures of the reaction products by use of spectrally resolved, highly parallelized TIRF microscopy.

[1] A. Rybina, C. Lang, M. Wirtz, K. Grußmayer, A. Kurz, F. Maier, A. Schmitt, O. Trapp, G. Jung, D. P. Herten, Angew. Chem. Int. Ed. 52, 6322–6325 (2013).
[2] T. Christ, F. Kulzer, P. Bordat, T. Basché, Angew. Chem. Int. Ed. 40, 4192-4195 (2001).
[3] S. Kummer, F. Kulzer, R. Kettner, T. Basché, C. Tietz, C. Glowatz, C. Kryschi, J. Chem. Phys. 107, 7673-7684 (1997).
[4] R. Mhanna, J. Berger, M. Jourdain, S. Muth, R. J. Kutta, G. Jung, ChemPhysChem, 2025, e202400996.

P56

Mapping heterodimerization and domain interplay controlling pioneer activity of Ascl1 with single-molecule FRET

Shrutarshi Mitra[1], Kateryna Nitsenko[2], Sarah F. Ruidiaz[3], Ásdís Laufeyjardóttir[4], Davide Mercadante[5], Pétur O. Heidarsson[1,4]

[1]Section for Biomolecular Sciences, The Kaj Ulrik Linderstrøm-Lang Centre for Protein Science, Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen
[2]School of Life Science, The University of Warwick
[3]Department of Cellular and Molecular Medicine, University of Copenhagen
[4]Department of Biochemistry, University of Iceland
[5]School of Chemical Sciences, The University of Auckland

Pioneer transcription factors (pTFs) possess unique abilities to target DNA in nucleosome-rich, condensed chromatin and initiate cell-fate changes1. Achaete-scute homolog 1 (Ascl1) is a pTF that can reprogram fibroblasts to induced neuronal cells (iN)2

Using smFRET and simulations, we mapped the structure and dynamics of Ascl1 both in its monomeric form and in functional complexes. We generated several doubly fluorescently labelled cysteine variants of full-length and isolated N- and C-domains of Ascl1 to probe discrete regions within the polypeptide sequence. Our results showed novel crosstalk between the N-IDR and the bHLH domain contributing to Ascl1 compaction. Ascl1 undergoes significant conformational and dynamic changes upon forming heterodimers with its partner protein E12α and DNA/Ascl1/E12α ternary complexes. Strikingly, we observed that Ascl1 can bind both DNA and nucleosomes in a monomeric form but with reduced specificity. The molecular mechanism of Ascl1 dimerization and chromatin interactions is likely applicable to the broader bHLH protein family and forms a foundation for enhancing Ascl1-based neuronal reprogramming strategies.

1. Iwafuchi-Doi, M. & Zaret, K. S. Cell fate control by pioneer transcription factors. Development, 143, 1833-1837 (2016).
2. Ali, F. R. et al. The phosphorylation status of Ascl1 is a key determinant of neuronal differentiation and maturation in vivo and in vitro. Development 141, 2216-2224 (2014).
3. Ide, M. et al. Genetic association analyses of PHOX2B and ASCL1 in neuropsychiatric disorders: evidence for association of ASCL1 with Parkinson's disease. Hum Genet, 117, 520-527 (2005).

P57

Super-Resolution Mapping of T Cell Receptor Forces via tension-PAINT

Anthony Monteza Cabrejos[1], Rohan O'Donnell[1], Cecilia Zaza[1], Sabrina Simoncelli[2]

[1]University College London, London Centre for Nanotechnology, 19 Gordon St, London WC1H 0AH, United Kingdom
2University College London, Department of Chemistry, 20 Gordon St, London WC1H 0AJ, United Kingdom

T-cells are crucial for human immunity as they eliminate pathogens and surveil tumours. In adaptive immune responses, T-cell receptors (TCRs) recognise ligands displayed on major histocompatibility complexes, forming an immunological synapse that initiates signalling cascades leading to proliferation, differentiation, or cell death. Despite the critical nature of this process, the mechanism by which extracellular ligand binding transmits signals across the membrane remains poorly understood.
Emerging evidence suggests that mechanical forces play a key role in regulating immunoreceptor–ligand interactions, influencing TCR mechanotransduction —where force-sensitive conformational shifts modulate downstream signalling and ultimately determine cell fate. Here, we employ tension-PAINT, a recently developed method that integrates DNA-based molecular force sensors with DNA-PAINT super-resolution imaging, to map the forces acting on individual TCRs with pN resolution and sub-30 nm precision.
By simultaneously sensing mechanical forces and visualising their spatial organisation over time, this approach offers an unprecedented platform for dissecting the mechanotransduction pathways that govern T-cell activation. Ultimately, the insights gained from this imaging technique may illuminate fundamental aspects of TCR signalling and pave the way for novel immunotherapeutic targeting of force-dependent mechanisms in T-cells.

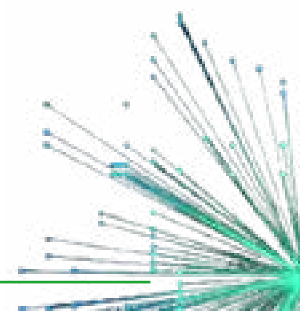
P58

Dissecting the TATA box sequence using smFRET

Fabio Morella; Pooyeh Asadiatouei; Evelyn Ploetz; Don C. Lamb

Ludwig Maximilian University of Munich

TATA binding protein (TBP) is a key component of the eukaryotic transcription initiation machinery. The interaction of TBP with a TATA box triggers the assembly of the preinitiation complex, marking the first step in gene transcription. Structural and biochemical studies have shown that, upon binding of TBP to the TATA box, the DNA strand bends sharply. In this study, we used single-molecule fluorescence resonance energy transfer (smFRET) to study the TBP-induced DNA bending of the double-stranded DNA (dsDNA) of the human H2B promoter. We labeled the dsDNA strands with donor-acceptor dye pairs and probed the binding of yeast TBP to the TATA box sequence via the FRET efficiency changes. When the consensus H2B promoter sequence is investigated in the presence of TBP, two distinct FRET populations are observed: a low FRET peak corresponding to DNA alone and a high FRET peak indicating TBP binding and DNA bending. To test whether TBP's high affinity for the H2B promoter TATA box arises from multiple potential binding positions, we introduced different point mutations to the consensus sequence, potentially blocking potential binding sites. In conclusion, we detected a distribution of populations in the high FRET efficiency state due to TBP binding despite the presence of the point mutations, pointing out the high stability of the TBP-DNA complex. Nevertheless, some differences exist in the position of the FRET efficiency peaks of the mutated DNA sequences, indicating that the TBP binds at different positions along the TATA box sequence. By comparing the frame-wise and molecular-wise analyses of the traces, we could determine that the FRET values are static on the timescale of the measurement, suggest that TBP does not move along the TATA box sequence during this time. Additionally, the mutations decrease the binding affinity of TBP to the TATA box sequence, indicated by a decrease in the high FRET population. Overall, our observations reveal new insights on the mechanism of interaction between yeast TBP and the TATA box sequence of the promoter for H2B on a molecular level.



P60F

Modulated illumination with an optical nano-chip for in-depth SMLM

Maxime Meghnagi[1], Abigail Illand[1], Pierre Jouchet[1], Guillaume Dupuis[1], Emmanuel Fort[2], Sandrine Lévêque-Fort[1]

[1]Intsitut des Sciences Moléculaires d'Orsay, 598 Rue André Rivière, 91400 Orsay, France
[2]Institut Langevin, 1 Rue Jussieu, 75005 Paris, France

Improving in-depth imaging while observing a large field of view compatible with spatial biology applications remains a challenge in single molecule localization microscopy. To improve axial resolution but also improve depth of observation within the sample, a time modulated illumination was proposed as an alternative, which encodes the axial position within the phase of their modulated emission. This technique called ModLoc [1] allows to reach a sub-7-nm axial precision and to image complex samples such as tissues and spheroids. To further improve the efficiency, an alternative compact and stable excitation set-up based on an engraved nano-chip is currently being implemented, which permits to directly generate the two beams needed to create the interference pattern, as well as the phase shift that displaces the pattern within the sample. On the detection side, the aim is to also increase the volume observed in a single shot, therefore a new demodulation module has been designed to extend the observed field of view and to simplify the extraction of the phase of the modulation for all emitters. A full characterization of the system will be presented along with results on biological samples.

[1] Jouchet, P.; Cabriel, C.; Bourg N.; et al.: Nanometric axial localization of single fluorescent molecules with modulated excitation. Nature Photonics, Vol. 15, 2021, pp. 297-304. DOI: 10.1038/s41566-020-00749-9.

P61

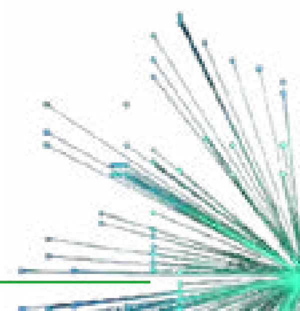
Exploring the Conformational Dynamics with Metal Induced Energy Transfer

Atanu Nandy, Tao Chen, Jörg Enderlein

Third Institute of Physics - Biophysics, Georg August University, Friedrich-Hund-Platz 1, 37077 Göttingen

Gaining insight into the complex dynamical behavior of biomolecules is essential for understanding their function and interactions. One of the most powerful and sensitive approaches developed to date is single-molecule Förster resonance energy transfer (smFRET), which has become a key technique for investigating conformational dynamics and molecular interactions at the level of individual biomolecules. Despite its versatility, smFRET faces several limitations, including labeling efficiency, restricted spatial range, and challenges in translating FRET efficiencies into precise distance measurements.
In this work, we introduce a novel method that combines Metal-Induced Energy Transfer (MIET) with Fluorescence Correlation Spectroscopy (FCS) to study the conformational dynamics of biomolecules across a wide temporal range—from nanoseconds to seconds—and over distances up to 150 nanometers. Unlike smFRET, MIET requires labeling of the biomolecule with only a single fluorophore. It enables nanometer-precision determination of the vertical distance between the fluorophore and a metal-coated substrate.1
We demonstrate the capabilities of this approach by investigating the conformational dynamics of DNA constructs, including DNA hairpins and Holliday junctions, highlighting its potential as a complementary or alternative technique to smFRET for probing biomolecular structure and dynamics over extended temporal and spatial regimes.1,2

Chizhik, A. I.; Rother, J.; Gregor, I.; Janshoff, A.; Enderlein, J. Nat. Photonics 8, 124 (2014)Chen, T., Karedla, N.; Enderlein, J. Nat. Commun. 15, 1789 (2024)



P62F

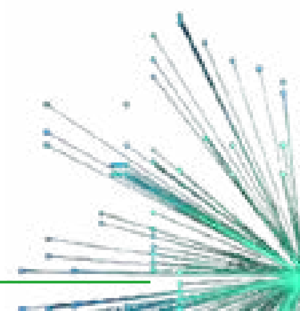
Fluorescence-Lifetime SMLM as a Versatile Tool for Multiplexed, Environment-Sensitive and 3D Super-Resolution Imaging

Oleksii Nevskyi[1], Jan Christoph Thiele[1], Dominic A. Helmerich[2], Marvin Jungblut[2], Niels Radmacher[1], Sankar Jana[3], Alexandre Fürstenberg[4], Dominik Wöll[3], Markus Sauer[2], Jörg Enderlein[1]

[1]III. Institute of Physics – Biophysics, Georg-August-University Göttingen, Göttingen, Germany
[2]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany
[3]Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, 52074 Aachen, Germany
[4]Department of Physical Chemistry and Department of Inorganic and Analytical Chemistry, University of Geneva, 1211 Geneva, Switzerland

Fluorescence-lifetime single-molecule localization microscopy (FL-SMLM) adds a powerful dimension to conventional super-resolution techniques by combining precise spatial localization with lifetime-based contrast.[1] We present a versatile FL-SMLM platform that enables multiplexed imaging of spectrally overlapping fluorophores, local environmental sensing and isotropic 3D super-resolution.
By integrating a single-photon detector array into a confocal laser scanning microscope, we combine FL-SMLM with image scanning microscopy (ISM), achieving a twofold improvement in lateral localization accuracy while preserving a straightforward implementation.[2] This approach also eliminates chromatic aberrations and enables robust multicolor imaging based solely on lifetime differences.
Furthermore, we demonstrate the application of FL-SMLM for probing local water content in thermo-responsive microgels, exploiting the differential quenching of red-emitting fluorophores by H_2O versus D_2O.[3] This allows for nanoscale mapping of hydration dynamics during temperature-induced phase transitions. Finally, by combining FL-SMLM with metal-induced energy transfer (MIET) imaging, we achieve isotropic 3D super-resolution maps of subcellular structures.[4]
Altogether, FL-SMLM emerges as a powerful and adaptable platform for multidimensional nanoscale imaging—bridging molecular specificity, environmental sensitivity, and high-precision 3D localization in both biological and materials science applications.

[1] J. C. Thiele, D. A. Helmerich, N. Oleksiievets, R. Tsukanov, E. Butkevich, M. Sauer, O. Nevskyi, J. Enderlein, ACS Nano, 14, 14190 (2020).
[2] N. Radmacher, O. Nevskyi, J. I. Gallea, J. C. Thiele, I. Gregor, S. O. Rizzoli, J. Enderlein, Nat. Photonics, 18, 1059 (2024).
[3] S. Jana, O. Nevskyi, H. Höche, L. Trottenberg, E. Siemes, J. Enderlein, A. Fürstenberg, D. Wöll, Angew.



P63

Hexagonal Boron Nitride for Single-Molecule Biophysics

Daria Orekhova, Sabina Caneva

Delft University of Technology, Delft, Netherlands

Hexagonal boron nitride (hBN) is emerging as a promising platform for single-molecule biophysics studies due to its favourable combination of structural, chemical and optical properties. It is an atomically smooth and inert 2D material that is optically transparent, and which enables studies of biomolecule dynamics in 2D confinements at the single-molecule level [1]. In this work we show that ATTO647N-labelled ssDNA structures immobilized on a coverslip underneath hBN flakes can be imaged with TIRF microscopy with single-molecule resolution and retain their emissive properties. We compare the photophysics of the fluorophores in buffer, in air and under hBN coverage, and find that the hBN coverage decreases the photo switching rate compared to Atto647N exposed to ambient conditions. The ON-time and intensity before bleaching of the fluorophores shows a decrease compared to those in buffer and varies as a function of the hBN thickness. We ascribe this to the presence of lattice defects in the hBN, which can exchange energy with the fluorophores. The arrangement of the dyes can be accurately imaged, showing promise of this platform as a single-molecule platform for surface-based biosensing.

[1] D. H. Shin, et.al., bioRxiv 2023.11.01.565159; https://doi.org/10.1101/2023.11.01.565159

P64

Integrative Design of Fluorescence Experiments: From Labeling Strategy to Coarse-Grained Prediction of Fluorescence Observables

Thomas-Otavio Peulen, Thorben Cordes

Faculty for Chemistry and Chemical Biology, Physical Chemistry, TU Dortmund University, 44227 Dortmund, Germany

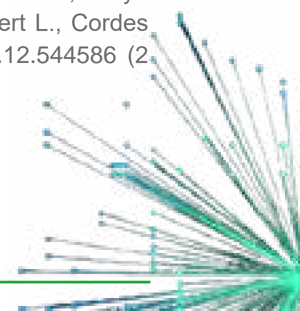
The design of fluorescence-based experiments—such as Förster Resonance Energy Transfer (FRET) and Protein-Induced Fluorescence Enhancement (PIFE)—requires a careful balance between structural accuracy, experimental feasibility, and photophysical interpretation. We present a coarse-grained simulation framework to support experimental planning from labeling site selection to signal interpretation. This approach incorporates simplified representations of proteins and fluorophores to model dye positioning (1), linker flexibility, steric accessibility (2), and potential local quenching effects (3). Different fluorophore classes, including fluorescent proteins, xanthene dyes, and cyanine dyes, are evaluated for their spatial footprint, environmental sensitivity, and likelihood of perturbing the native protein structure. Fluorescent proteins, while genetically encoded and structurally bulky, offer stable labeling with minimal local quenching, whereas small-molecule dyes provide higher spatial resolution at the cost of increased perturbation risk. Our simulations help predict FRET efficiency distributions and PIFE sensitivity by sampling possible dye positions and protein conformations. This integrative strategy allows researchers to design fluorescence experiments (2, 4) with improved accuracy and robustness, especially when combining FRET and PIFE to track conformational dynamics and transient interactions. The framework demonstrates the power of coarse-grained, structure-informed modeling to optimize fluorophore placement and signal interpretation in complex biological systems.

(1) Peulen T.O., Sali A., bioRxiv, https://doi.org/10.1101/2023.10.26.564048 (2023)

(2) Dimura M., Peulen T.O., Sanabria H., Rodnin D., Hemmen K., Hanke C.A., Seidel C. A. M., Gohlke H., Nat. Comm, 11, 5394 (2020)

(3) Peulen T.O., Opanasyuk O., Seidel C.A.M., J. Phys. Chem. B , 121, 35, 8211–8241 (2017)

(4) Gebhardt C., Bawidamann P., Schuetze K., Moya Munoz G. G., Spring A.-K., Griffith D., Lipfert L., Cordes T., bioRxiv, https://doi.org/10.1101/2023.06.12.544586 (2023)



P65

A unified computational strategy for multi-target super-resolution imaging with SPAD array detector

Francesco Del Bufalo[1], Giacomo Garrè[2], Mattia Donato[3], Michele Oneto[4], Alberto Diaspro[5], Lisa Cuneo[6], Alessandro Zunino[7], Giuseppe Vicidomini[8]

[1]francesco.delbufalo@iit.it
[2]giacomo.garre@iit.it
[3]mattia.donato@iit.it
[4]michele.oneto@iit.it
[5]alberto.diaspro@iit.it
[6]lisa.cuneo@iit.it
[7]alessandro.zunino@iit.it
[8]giuseppe.vicidomini@iit.it

Single-photon avalanche diode (SPAD) array detectors are increasingly replacing single-element detectors in laser-scanning microscopy (LSM)
Here, we propose a class of algorithms based on maximum-likelihood estimation that jointly reconstructs ISM images and performs lifetime analysis. As example,
These algorithms can potentially become the standard framework for FLISM, enabling accessible and accurate functional imaging.

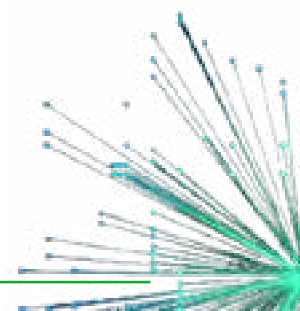
P66

T4 Bacteriophage as a Nature-Crafted 3D Nanoruler for Super-Resolution Microscopy

Jose I. Gallea[1], Oleksii Nevskyi[1], Zuzanna Kaźmierczak[2], Tao Chen1, Paulina Miernikiewicz[2], Ivan Gligonov[1], Anna Chizhik [1], Lenny Reinkensmeier[3], Krystyna Dąbrowska[2], Mark Bates[3], Joerg Enderlein[1]

[1]Georg August University of Göttingen, Göttingen, Germany.

In the rapidly advancing field of super-resolution fluorescence microscopy, significant efforts are being made to extend its applications into the three-dimensional domain. Recent advances have made it possible to achieve isotropic resolution at the nanometer scale, allowing the visualization of three-dimensional subcellular structures with unprecedented clarity. A critical aspect of this progress is the need for biologically compatible 3D structures, also known as standards or rulers, to validate resolution capabilities and accuracy. Selecting the ideal standard is a challenging task, requiring well-defined geometry, stability in a variety of environments, and the ability to specifically label at distances below the diffraction limit. In response to this need, we introduce the non-human infecting bacteriophageT4 as a versatile bio-ruler for 3D super-resolution microscopy. This naturally occurring DNA-protein complex has a unique icosahedral capsid with dimensions of 120 nm in length and 86 nm in width, paired with a cylindrical hollow viral tail, forming a distinctive 260 nm geometric rocket-shaped structure. Using DNA point accumulation for imaging in nanoscale topography (DNA-PAINT) in conjunction with optical astigmatism, we have achieved detailed visualization of T4's 3D structure with unprecedented clarity using light microscopy. In addition, we demonstrate the utility of T4 for assessing microscope performance, where the phage provides an easy-to-prepare rigid three-dimensional structure for calibrating microscopes with nanometer accuracy.



P70F

Temperature-dependent conformational signatures of membrane protein, BmrA using single-molecule FRET

Kémil Belhadji, Alicia Damm, John Manzi, Daniel Lévy, Raju Regmi, Patricia Bassereau

Institute Curie, CNRS, Physics of Cells and Cancer, Paris, France

Membrane proteins are essential for exchanges across the impermeable lipid bilayer. Such proteins work via adopting various conformations depending on stimuli such as ATP hydrolysis or ligand binding. In addition, the influence of temperature and membrane's mechanical properties have a critical role on conformational states of membrane proteins.

We focus on BmrA, a bacterial ABC transporter, that undergoes conformational changes upon ATP hydrolysis alternating between open or close conformations. Previously, performing ensemble measurements, we observed that the evolution of the ATPase activity of BmrA followed an exponential increase with temperature. Our work aims at characterizing the equilibrium between the two conformational states of the protein based on the rise in activity observed at higher temperature. Therefore, we investigate the dynamical remodeling of temperature-dependent BmrA conformations at the single-molecule resolution with different liposomes sizes: 29 nm and 125 nm. Using single-molecule fluorescence resonance energy transfer (FRET) with these reconstituted in-vitro membranes we investigate the conformational switches depending on the temperature as well as on the membrane curvature, influencing the nanoscale conformations, and thus the protein activity. Our work will provide fundamental insights to the biophysics of membrane-protein interactions, and the role of temperature in activity of bacterial membrane transporters

P68

Pushing the Limits of FLIM: Ultrafast Photon Detection with LINCam, PhotonPix, and LINTag

Yury Prokazov, André Weber, Evgeny Turbin, Werner Zuschratter

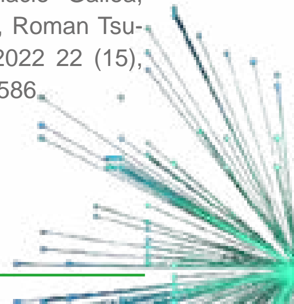
Klosterwuhne 42, 39124 Magdeburg

We present a high-performance photon detection and data acquisition system for advanced fluorescence lifetime imaging (FLIM) and time-resolved applications, offering the LINCam, PhotonPix, and LINTag technologies. LINCam is a wide-field TCSPC-based FLIM camera featuring high quantum yield photocathodes, sub-50 ps temporal resolution, and ultra-high sensitivity under low excitation power (<30 mW/cm²). Among others it enables label-free metabolic imaging, FLIM-FRET, single-molecule detection, and time-resolved spectroscopy with minimal photodamage. PhotonPix complements this setup with ultra-fast single-photon counting capabilities, utilizing a microchannel plate photomultiplier for <2 ns dead time, timing resolution below 35 ps, and sustainable count rates >100 MHz, with burst capabilities up to 1 GHz. For high-throughput data acquisition, LINTag delivers over 400 million time-stamped photon events per second across eight configurable channels, with <8.5 ps timing precision via 10GbE TCP/IP or FPGA-based access. This integrated system has demonstrated exceptional performance in tracking NAD(P)H/FAD dynamics during brain cell activity under physiological conditions, offering deep insights into neuronal metabolism without staining. Its modularity, precision, and speed make it ideally suited for biomedical imaging, spectroscopy [1], advanced photon correlation studies and single molecule detection [2, 3].

[1] Weber, André, Hartig, Roland and Zuschratter, Werner. „FRET-analysis in living cells by fluorescence lifetime imaging microscopy: experimental workflow and methodology“ Methods in Microscopy, vol. 2, no. 1, 2025, pp. 73-84. https://doi.org/10.1515/mim-2024-0027

[2] Basak, Samrat and Tsukanov, Roman. „Advanced fluorescence lifetime-enhanced multiplexed nanoscopy of cells“ Methods in Microscopy, vol. 2, no. 1, 2025, pp. 23-32. https://doi.org/10.1515/mim-2024-0029

[3] Single-Molecule Fluorescence Lifetime Imaging Using Wide-Field and Confocal-Laser Scanning Microscopy: A Comparative Analysis, Nazar Oleksiievets, Christeena Mathew, Jan Christoph Thiele, José Ignacio Gallea, Oleksii Nevskyi, Ingo Gregor, André Weber, Roman Tsukanov, and Jörg Enderlein, Nano Letters 2022 22 (15), 6454-6461, DOI: 10.1021/acs.nanolett.2c01586



P69

Integrating Coarse-Grained Molecular Dynamics and Fluorescence Spectroscopy to Unravel Tau Protein

Eva Punter[1,2], Enrico Carlon[2], Jelle Hendrix[1]

[1]Dynamic Bioimaging Lab, Biomedical Research Institute, Universiteit Hasselt, Gebouw C - Agoralaan, 3590 Diepenbeek, Belgium
[2]Soft Matter and Biophysics, KU Leuven, Celestijnenlaan 200 D, 3001 Leuven, Belgium

The intrinsically disordered protein Tau, which stabilises microtubules in neurons, has been shown to undergo liquid-liquid phase separation in physiological conditions, forming dynamic biomolecular condensates. These reversible droplets are believed to be part of the pathological aggregation pathway of Tau, which has been associated with neurodegenerative diseases such as Alzheimer's disease and frontotemporal dementia. Understanding the structural and dynamic properties of Tau in both diffuse and phase-separated states has therefore become a high priority. Here, I present an integrative approach combining coarse-grained molecular dynamics (MD) simulations with in vitro fluorescence spectroscopy techniques to probe Tau's conformational ensemble and, ultimately, the interactions and dynamics within the droplets. By using experimental data from single-molecule Förster resonance energy transfer (smFRET) and fluorescence correlation spectroscopy to guide and restrain MD simulations, we generate models that reflect biologically relevant conformational ensembles. Not only can they provide new insights on the molecular level, exploring disease-relevant protein dynamics, but they also allow for in silico experiments and analyses prior to in vitro experiments.

P71F

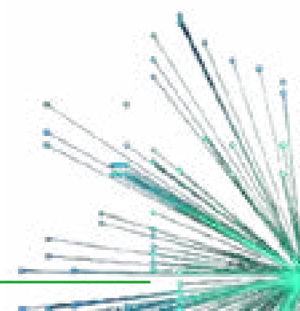
Tether-free single-molecule FRET uncovers hidden hairpin dynamics of CRISPR RNA

Bok-Eum Choi[1], Hugh Wilson[2], Quan Wang[1]

[1]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA
[2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, USA

The CRISPR-Cas9 gene editing system functions as a programmable nuclease by CRISPR RNA (crRNA) which forms guide RNA (gRNA) in a partial duplex with tracrRNA. The 20-nucleotide 'spacer' at the 5' end of crRNA is a user-defined sequence that directs Cas9 to a target DNA for cleavage. Nevertheless, programmability of spacer sequence potentially leads to unintended secondary structure formation in gRNA. Previous studies propose secondary structure formation in gRNAs as a plausible mechanism for decreased editing efficiency for certain sequences, but experimental evidence is lacking1,2. Due to the inherent structural heterogeneity and dynamics of RNA molecules, high resolution structural analysis of RNAs using traditional structural methods is challenging. Herein, we use recently developed tether-free single-molecule FRET (ABEL-FRET) to probe the structure and dynamics of crRNA. Our measurements reveal the previously unknown sequence-dependent hairpin folding-unfolding dynamics of 5' end crRNA. As the 5'-end hairpin forming tendency increases, crRNA tends to spend more time in the folded state and slightly reduce Cas9 activity. Strikingly, even crRNA with low hairpin formation tendency ($\Delta G < 1$ kcal/mol) can still be trapped in the hairpin state with Mg^{2+}. Our results indicate that the sequence-dependent hairpin formation is common in crRNA and can affect editing efficiency.

[1] Nathan Wong, Weijun Liu & Xiaowei Wang, Genome Biology, 16:218 (2015)
[2] Summer B. Thyme, Laila Akhmetova, Tessa G. Montague, Eivind Valen & Alexander F. Schier, Nature Communications, 7:11750 (2016)



P72

New eyes on medicines and vaccines: seeing how they work one molecule at a time

Sabrina Leslie

2128 East Mall, Vancouver, BC, V6T 1Z4

This talk highlights advances in single-molecule microscopy of DNA, RNA, and lipid nanoparticles with applications in drug discovery and development. Traditional bulk measurements often miss rare events and complex reactions, prompting the need for more detailed approaches. The Leslie Lab at UBC has developed a powerful technique—Convex Lens-induced Confinement (CLiC)—which confines and controls molecules in cell-like conditions without tethering them to a surface. This allows for real-time, high-resolution observation of molecular interactions.

The lab investigates how complex environments, such as molecular crowding inside cells, affect DNA and RNA interactions. CLiC microscopy also enables studies in genomic mapping, CRISPR gene editing, protein interactions, and nanoparticle behaviour—key areas that drive new drug development. These insights help fill crucial knowledge gaps, such as understanding binding kinetics and sequence- or structure-dependent events, which are essential for improving how medicines are designed.

In addition to its biological applications, the Leslie Lab is advancing real-time control of reaction environments and high-throughput microfluidic platforms. These innovations not only offer deeper biophysical insights, but also provide practical tools to support large-scale precision medicine efforts in both public research and industry partnerships. Ultimately, this work contributes to the creation of more targeted and effective therapeutic strategies.

P73F

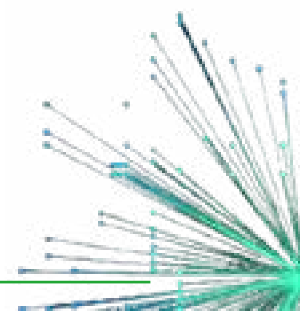
Tunable linker systems for broad use of functional dyes in single-molecule imaging

Mostofa Ataur Rohman[1], Marcus Lantzius-Beninga[2], Thomas Otavio-Peulen[1], Andreas Herrmann[2] and Thorben Cordes[1]

[1]Biophysical Chemistry, Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany
[2]Institute of Technical and Macromolecular Chemistry, (RWTH) Aachen University, Worringerweg 2, 52074 Aachen, Germany

Commercial fluorophores are vital in the life sciences, supporting imaging, DNA sequencing, single-molecule studies, and biomedical assays. However, their utility is often limited by fixed photophysical properties, fast photobleaching, limited functional versatility, and lack of modular bioconjugation options. Here, we introduce a modular chemical biology approach using a 'linker' system that connects biological targets, commercial dyes, and functional moieties, e.g., photostabilizers. These linkers are synthesized via a one-pot Ugi four-component reaction, enabling rapid and diverse customization. Each linker features a bioconjugation handle, a click-compatible unit for fluorophore attachment, and a functional moeity to tune dye behavior. This strategy converts conventional fluorophores into adaptable probes with improved photostability, controlled blinking or environmental responsiveness.[1] This contribution describes the incorporation of new triplet-state quenchers and dye-attachment moeities into the linker structure to further enhances probe performance by reduction of photobleaching and blinking. We evaluate the performance of various linker-dye combinations using fluorescence correlation spectroscopy (FCS), single-molecule FRET (smFRET) and TIRF to assess photobleaching and power-dependent assays to quantify blinking duration, triplet-state lifetimes, dark-state recovery. The modular linker system offers a flexible toolkit for customization of fluorophores in the final step of biolabelling, expanding applicability of commercially available dyes in advanced imaging techniques such as super-resolution microscopy and functional single-molecule studies in vitro and in vivo.

[1] https://doi.org/10.1002/ange.202112959



P74

RNA-Inhibitor Interactions using Multi-site smFRET

Abdul Rahman Sadiq, Metteo Lisibach, Susann Zelger-Paulus, Roland K.O. Sigel

Department of Chemistry, University of Zurich, Zurich, CH-8057, Switzerland

For around fifty years, structure determination methods have provided static snapshots of biomolecule–small molecule interactions with ever-increasing resolution. However, these techniques fall short in capturing how such interactions influence the dynamic structural rearrangements within biomolecules, such as domain-specific movements critical to their function[1].
To address this limitation, we employ two-colour single-molecule Förster Resonance Energy Transfer (smFRET) with multiple labeling positions, enabling the measurement of multiple distances within a biomolecule[2]. As a model system, we investigate group II introns—large self-cleaving RNA molecules that serve as paradigms to study mRNA splicing. These introns are absent in humans but are present in essential housekeeping genes of human-pathogenic fungi, making them promising antifungal targets with minimal side-effects[3].
We focus on Intronistat B, a small-molecule inhibitor that binds to the evolutionarily conserved catalytic core of group II introns, disrupting their splicing activity[4,5]. Using smFRET, we track RNA structural rearrangements in the presence of the inhibitor, revealing interesting results that shed light on the binding mode of the RNA-small molecule interaction. Furthermore, the study highlights smFRET techniques as powerful tools for exploring RNA-inhibitor interactions and provides valuable insights that can guide rational design of novel antifungals.
Financial support from UZH is gratefully acknowledged.

[1] H. M. Al-Hashimi, N. G. Walter, Curr Opin Struct Biol, 18, 321, 2008.
[2] R. Roy, S. Hohng, T. Ha, Nat Methods, 5, 507, 2008.
[3] J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus, L. Tomaska, Molecular Genetics and Genomics, 272, 173, 2004.
[4] O. Fedorova, G. E. Jagdmann, R. L. Adams, L. Yuan, M. C. Van Zandt, A. M. Pyle, Nat Chem Biol, 14, 1073, 2018.
[5] I. Silvestri, J. Manigrasso, A. Andreani, N. Brindani, C. Mas, J.-B. Reiser, P. Vidossich, G. Martino, A. A. McCarthy, M. De Vivo, M. Marcia, Nat Commun, 15, 4980, 2024

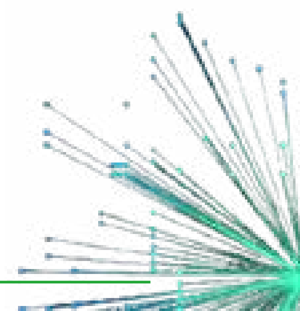
P75

Exciton annihilation evident in TCSPC-FCS study of aggregating photosynthetic antenna complexes from plants

F. Conradie1, B. van Heerden1, T. P. J. Krüger[1]

[1]Department of Physics, University of Pretoria, Pretoria, 0028, South Africa

The automatic photoprotection of photosynthetic antenna complexes that initiate light-harvesting have been a subject of great interest for potentially improved solar energy technology, enhanced crop efficiencies, and biosensing. Light-Harvesting Complex II (LHCII) is the main pigment-protein antenna in green plants and exhibits the remarkable capability to switch between a light-harvesting and a photoprotective state when exposed to fluctuating sunlight intensities. The in-vivo conditions that activate this switch can be mimicked by in-vitro aggregation. Despite more than three decades of research, the molecular mechanism responsible for the strong energy quenching in these aggregates is still unknown. We investigated LHCII aggregation in a stepwise manner and performed fluorescence correlation spectroscopy (FCS) along with time-correlated single-photon counting (TCSPC) on a home-built setup to correlate the aggregate composition with their excited-state lifetimes. We discovered a non-linear relationship between the steady-state fluorescence and average lifetimes, which is explained well by increased annihilation of diffusing singlets due to an accumulation of triplets in aggregates. An approximated model of exciton annihilation showed excellent correspondence with the TCSPC-FCS data. These results demonstrate the importance of distinguishing non-linear annihilation from quenching in photoprotective studies of LHCII.



P76

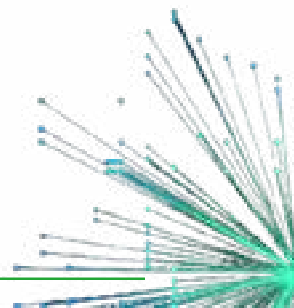
Nanofluidic-Assisted Single-Molecule Fluorescence Burst Size Distribution Analysis

Subhartha Sarkar[1], Damir Sakhapov[2], Jörg Enderlein[1]

[1]Drittes Physikalisches Institut - Biophysik, Georg-August-Universität, Göttingen, Germany
[2]Delft University of Technology, The Netherlands

Fluidic transport through nanochannels enables precise manipulation and real-time observation of single biomolecules, offering a robust platform for studying nanoscale dynamics and quantifying molecular fluorescence properties. In this work, we determine Burst Size Distributions (BSDs) employing a nanofluidic system designed for single-molecule fluorescence spectroscopy. The platform consists of nanochannel arrays fabricated on silicon wafers, through which biomolecules are driven via either pressure or electroosmotic flow.
We applied this system to analyze short (35–50 base pair) double-stranded DNA fragments labelled with a defined number of ATTO 647N fluorophores. A continuous electroosmotic flow transports the labelled DNA molecules through the confocal excitation and detection volume in a highly controlled manner, so that every molecule transition produces discrete and well-defined photon bursts which depends on the intrinsic brightness of the molecule. This brightness values is proportional to the number of fluorophores attached. By constructing burst size distribution histograms, we can resolve distinct populations corresponding to different labelling stoichiometries.
Generally, our technique enables precise, label-specific quantification of fluorophore stoichiometry at the single-molecule level under solution-phase conditions. Our method provides a scalable and efficient tool for quality control of molecular labelling and can be broadly applied to single-molecule studies of complex molecular mixtures.

Lesoine, J. F., Venkataraman, P. A., Maloney, P. C., Dumont, M. E., & Novotny, L. (2012). Nanochannel-based single molecule recycling. Nano letters, 12(6), 3273-3278.



P78

Technical Challenges in Single-Complex Fluorescence-Excitation CD Spectroscopy at Cryogenic Temperatures

Stefan Goppelt, Bayreuth, Germany

New York University, New York, USA

Circular dichroism (CD) spectroscopy is a widely used technique for probing the structural properties of chiral biological systems, including light-harvesting complexes. Conventional ensemble CD measurements average signals over many complexes, masking individual heterogeneity. To address this, we developed a fluorescence-excitation CD spectroscopy setup capable of measuring single light-harvesting complexes under cryogenic conditions. However, these samples often exhibit strong linear dichroism (LD) signals, which can be orders of magnitude larger than the CD signal and do not average out at the single-complex level. This makes robust suppression of intensity and polarization artifacts critical. In particular, precise control of the polarization of the excitation light is essential to minimize LD-induced artifacts. Here, we present the technical challenges associated with single-complex fluorescence-excitation CD spectroscopy and outline our solutions for minimizing measurement artifacts.

P84F

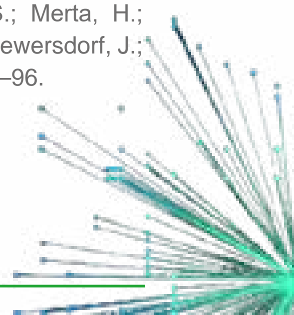
Fast and long-term super-resolution STED microscopy of nanostructural organellar dynamics using a neural network

Ashwin Balakrishnan[1], Johanna Rahm[1], Alexandra Kaminer[1], Maren Wehrheim[2,3], Marius Glogger[4], Laurell F. Kessler[1], Mathias Kaschube[2,3], Hans-Dieter Barth[1], Mike Heilemann[1]

1Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, Frankfurt, Germany
[2]Department of Computer Science and Mathematics, Goethe University Frankfurt, Frankfurt, Germany
[3]Frankfurt Institute for Advanced Studies (FIAS), Frankfurt, Germany
[4]Optical Imaging Competence Centre, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

The advent of super-resolution microscopy has made it possible to visualise nanostructural architecture and its dynamics in cells. However, understanding fast nanostructural rearrangements and following these dynamics over long periods of time is commonly limited by photobleaching and phototoxicity. This either allows for studies focussing on fast (minutes) structural rearrangements that are limited to a short observation time [1] or those focussing on long periods of time (hours) yet are limited to intermittent imaging every few minutes [2].
Various strategies have been devised to tackle either photobleaching [3] or phototoxicity [4]. In this work, we apply a neural network based denoising to overcome both limitations simultaneously to enable long-term and fast STED microscopy in live cells. We trained a UNet-RCAN network [5] with experimental data and used this model to denoise images recorded with stimulated emission depletion (STED) microscopy and ultra-low irradiation intensities (~70x reduction). Using this model, we visualised the dynamics of the endoplasmic reticulum (ER) in live cells over hours with sub-second time resolution in planar imaging and over few minutes with seconds time resolution in volumetric imaging [6]. We extended our work towards two-colour imaging and visualised the nanostructural dynamics of ER and mitochondria in living cells simultaneously over hours with s time resolution. Our work lays the foundation for studies involving fast intra- and inter-organellar dynamics with nanostructural detail in living cells over long term.

[1] Schroeder, L. K.; Barentine, A. E. S.; Merta, H.; Schweighofer, S.; Zhang, Y.; Baddeley, D.; Bewersdorf, J.; Bahmanyar, S. J. Cell Biol. 2019, 218(1), 83–96.



[2] Zheng, S.; Dadina, N.; Mozumdar, D.; Lesiak, L.; Martinez, K. N.; Miller, E. W.; Schepartz, A. Nat. Chem. Biol. 2023. https://doi.org/10.1038/s41589-023-01450-y.
[3] Glogger, M.; Wang, D.; Kompa, J.; Balakrishnan, A.; Hiblot, J.; Barth, H.-D.; Johnsson, K.; Heilemann, M. ACS Nano 2022, 16 (11), 17991–17997.
[4] Henriques, R.; Rosario, M. del; Gómez-de-Mariscal, E.; Morgado, L.; Portela, R.; Jacquemet, G.; Pereira, P. Research Square, 2024. https://doi.org/10.21203/rs.3.rs-4809905/v1.
[5] Ebrahimi, V.; Stephan, T.; Kim, J.; Carravilla, P.; Eggeling, C.; Jakobs, S.; Han, K. Y. Commun Biol 2023, 6 (1), 674.
[6] Rahm, J. V.; Balakrishnan, A.; Wehrheim, M.; Kaminer, A.; Glogger, M.; Kessler, L. F.; Kaschube, M.; Barth, H.-D.; Heilemann, M. Small Sci. 2024. https://doi.org/10.1002/smsc.202400385.

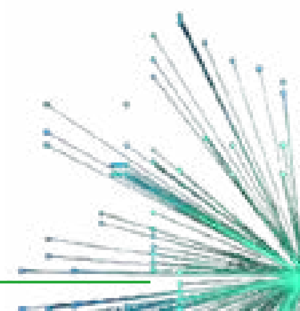
P80

Using changes in photophysical properties to detect ligand binding at moderate concentrations

Ralf Schmauder, Maik Otte, Andrea Schweinitz, Christian Sattler, Klaus Benndorf

Institute of Physiology II, Jena University Hospital, Friedrich Schiller University, Jena 07743, Germany

Many physiologically relevant processes involve ligands at moderate concentrations (KD~µM). However most single-molecule studies on receptor-ligand interactions are performed with high affinity ligands (KD ~nM and below), as otherwise background signal from free ligand becomes prohibitively large.
Here we show for cyanine –based fluorescent ligands, that ligand binding alters the fluorophores photophysics, leading to increased fluorescence lifetimes and molecular brightness. Additionally, the increased lifetime leads to a reduced anisotropy of the fluorescence of the bound ligands, calling for caution in the interpretation of anisotropy-based binding assays using cyanines.
In contrast to other ligands with state-dependent brightness were additionally interactions between ligands and fluorophore or fluorophore and receptor are required, this change in photophysics does not represent an additional coupled reaction in the kinetic scheme of the system, thus not altering the apparent system dynamics
As examples, we show data from fluorescently labeled cGMP for cyclic nucleotide gated- channels and fluorescently labeled ATP for P2X channels. The altered fluorescence lifetimes allows for direct detection of bound fluorescent ligands by FLIM microscopy or gated-detection in the presence of unbound free fluorescent ligands or fluorophores. We further evaluate the transfer of this approach to single-molecule binding-measurements.



P81

A DNA-based exciton collider to monitor one-dimensional exciton diffusion

Tim Schröder, Philipp Wutz, Sebastian Bange, John Lupton, Philip Tinnefeld, Jan Vogelsang

Ludwig-Maximilians-University Munich, Germany

Photonic devices play a major role in modern communication and quantum computing technology. These devices are mainly based on lithographic semiconductor technology, and their manufacturing is limited by the wavelength of light and serial processing. To build smaller and biocompatible photonic devices, DNA nanotechnology offers precise control over the modifications and stoichiometry. Additionally, DNA nanotechnology provides parallel processing of multiple devices. We harness these advantages to map exciton diffusion in a DNA origami-based one-dimensional wire containing up to nine organic dyes.Excitons are injected via a FRET pair at the outermost positions. As the excitons move towards each other, the degree of single-photon emission rises due to singlet-singlet annihilation, as analyzed with picosecond time-resolved photon antibunching.[1] Importantly, we find that the fingerprint of one-dimensional diffusive annihilation in this construct is convoluted with spectral crosstalk—mainly caused by the direct excitation of the acceptor dyes and by the photophysics of the donor dyes.[2] Taking these effects into account, we observe good agreement between simulations and experiments, demonstrating a robust understanding of excitonic processes and a reliable design of DNA-based excitonic wires. Overall, we lay the foundation for DNA-based excitonic devices and shed light on the potential of DNA-based photonic technology.

P82F

Single Molecule 3D Orientation and Localization Microscopy (SMOLM) via ratiometric 4-polarization projection microscopy on dense actin structures

Charitra Sree Senthil Kumar[1], Cesar Augusto Valades Cruz[1,2], Luis Arturo Alemán-Castañeda[1], Manos Mavrakis1, Sophie Brasselet[1]

[1]Institut Fresnel, Aix Marseille Univ, CNRS, Centrale Med, Marseille, France

[2]Institute of Hydrobiology, Chinese Academy of Sciences

The dynamics of intracellular processes rely on both spatial arrangement and orientation of biomolecules. While Single-Molecule Localization Microscopy (SMLM) has revolutionized nanoscale imaging, a deeper understanding of cellular processes requires access to molecular orientation. Single-Molecule Orientation and Localization Microscopy (SMOLM) addresses this by determining both the position and orientation of fluorescent molecules, which are intrinsically encoded in the point spread function (PSF). PSF engineering and fitting techniques have been developed to extract this information [1], but they often involve complex setups and computationally intensive analyses, limiting their applicability in dense biological environments.

Previously, we introduced a polarization-splitting technique that enables the retrieval of the 2D orientation and wobbling extent of fluorescent molecules through ratiometric analysis across four polarization channels, with minimal PSF deformation [2]. Here, we extend this approach to full 3D orientation determination, based on a simple back focal plane filtering [3]. We image dense, complex 3D actin architectures such as tumor cell lamellipodia, macrophage podosomes and T-cell receptor cluster zones [4]. We finally show that this approach, combined with controlled excitation polarization, gives also access to molecular rotational mobility at single-molecule level. We show that information about such dynamics is highly dependent on the fluorophore's nature and environment.

[1] Brasselet S. and Alonso M.A., Optica 10 (11), 1486-1510 (2023).

[2] Rimoli, C.V. et al. Nat Commun 13, 301 (2022).

[3] Hohlbein, J. and Hübner, C.G., J. Chem. Phys. 129, 094703 (2008).

[4] Charitra S. Senthil Kumar, et al. in prep. Single molecule 3D orientation and localization imaging using simple ratiometric polarization splitting.

P83F

Imaging Functional Microstructures to Understand the Working Mechanism of Perovskite Solar Cells in Operation

Sudipta Seth[1], Boris Louis[1], Koki Asano[3], Ran Ji[2], Martin Vacha[3], Yana Vaynzof[2], Ivan Scheblykin[4], Johan Hofkens[1]

[1]Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Belgium
[2]TU Dresden, Germany
[3]Tokyo Institute of Technology, Japan
[4]Division of Chemical Physics, Lund University, Sweden

The performance and stability of solar cells are typically assessed through macroscopic photophysical and electrical measurements. These observed bulk properties result from the convolution of microscopic structural, chemical, and functional properties, which are influenced by defects, carrier transport, and chemical reactions under external stimuli such as light, bias, and ambient conditions. This is especially true for soft and dynamic light-harvesting materials like halide perovskites. Therefore, understanding the functionality of the microstructures in a device, particularly under operational conditions, is crucial for accurately interpreting and enhancing device performance. Conventional techniques such as scanning electron microscopy, and electron/ x-ray-based analytical methods can provide high spatial resolution but are mostly limited to structural characterization. Moreover, these surface-sensitive or invasive techniques often alter the material properties.
To address these challenges, we have implemented a microscale functional imaging method (CLIM) that utilizes photoluminescence fluctuations to reveal contrasts associated with defect dynamics in semiconductor materials. CLIM images correlated with SEM reveal crucial information about the structure-function relationship in the bare thin films. Particularly noteworthy is the large amplitude fluctuation of photoluminescence of these films when incorporated in a solar cell. The local functional regions in a solar cell are much larger as compared to the bare film. Moreover, the fluctuation amplitude and functional regions strongly depend on the device's operational regime. From the statistical analysis of intensity fluctuations, we provide insights into the type of metastable defects responsible for fluctuating non-radiative recombination processes in thin film and operational solar cells.
The insights gained from microscale functional imaging contribute to a deeper understanding of device efficiency, structure, and durability, which are crucial for the rational engineering of the next generation of devices.

B. Louis¥, S. Seth¥*, Q. An, J. Ran, Y. Vaynzof, J. Hofkens, I. G. Scheblykin, Advanced Materials 2024, 2413126

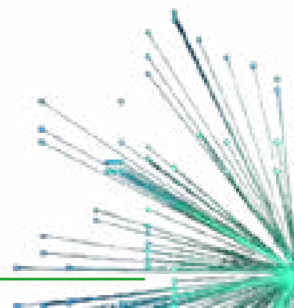
P79F

Fourier-limited electronic transitions in surface-adsorbed quantum emitters

Alexey Shkarin[1], Masoud Mirzaei[1,2], Burak Gurlek[1,2], Ashley J. Shin1, Johannes Zirkelbach[1,2], Irena Deperasinska[3], Boleslaw Kozankiewicz[3], Tobias Utikal[1], Stephan Goetzinger[1,2,4], Vahid Sandoghdar[1,2]

[1]Max Planck Institute for the Science of Light, D-91058 Erlangen, Germany
[2]Department of Physics, Friedrich Alexander University Erlangen-Nuremberg, D-91058 Erlangen, Germany
[3]Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland
[4]Graduate School in Advanced Optical Technologies (SAOT), Friedrich Alexander University Erlangen-Nuremberg, D-91052 Erlangen, Germany

Organic dye molecules doped in organic host crystal provide an exquisite platform for quantum optics because they can reach Fourier-limited spectra at low temperatures. This is in large part due to the highly ordered and stable crystal structures, which provide a low-noise environment, thus minimizing spectral diffusion and dephasing. Many important fundamental studies and technological applications, however, require quantum emitters to be exposed on surfaces. To date, Fourier-limited spectra have remained elusive for quantum emitters on surfaces. In fact, the general wisdom expects surfaces to be intrinsically unsuitable for such studies because they contain defects and contaminants.
In our recent work, we show that it is possible to achieve Fourier-limited electronic transitions for molecules on pristine surfaces of an organic crystal. We have developed a novel sample preparation method, where molecules are sublimated onto the pristine surface of an organic crystal at cryogenic temperatures. We provide detailed quantitative studies on the resulting inhomogeneous broadening at the ensemble level and the behavior of the homogeneous linewidth at the single-molecule level. By comparing the spectral properties of the same molecular species in the gas phase with its properties on the surface and bulk, we shed light on several fundamental aspects of guest-host interactions. This study constitutes an important step in combining high-resolution spectroscopy and quantum optical studies with techniques as AFM and STM, which provide direct access to individual molecules.



P85

Escape-time stereometry (ETs) for measuring mRNA poly(A) tail length

Dimitrios Soulias[1], Ayaka Hogg[2], Sebastian Golojuch[1], Alexander Welch[2], Andre Furger[2], Madhavi Krishnan[1,3]

[1]Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK
[2]Department of Biochemistry, University of Oxford, Oxford, South Parks Road, Oxford, OX1 3QZ, UK
[3]The Kavli Institute for Nanoscience Discovery, University of Oxford, Sherrington Road, Oxford OX1 3QU, UK

Poly(A) tail length plays a key role in regulating mRNA stability and translation, making its accurate profiling essential for understanding post-transcriptional gene regulation. A variety of methods—from early enzymatic and microarray-based techniques to advanced high-throughput sequencing—have been developed to measure poly(A) tail length, each bringing new insights while facing limitations in accuracy, bias, or read length. A recently developed technique, called escape-time stereometry (ETs), leverages nanoscale confinement and thermal motion to sensitively resolve molecular size and conformation under physiological solution conditions. By engineering entropic traps that amplify size- and shape-dependent residence times, this approach enables high-throughput, microchip-based wide-field imaging of molecular interactions with exceptional sensitivity. Here, we demonstrate the application of ETs for profiling mRNA poly(A) tail length by hybridizing short fluorescently labelled oligonucleotides (12-mer poly(dT)) to the poly(A) tail, rendering mRNA fluorescent only upon successful probe binding. ETs distinguishes unbound probes from mRNA–probe hybrids based on their vastly different molecular sizes, which result in distinct escape times. Measuring the signal-to-noise ratio of such hybrids allows us to infer the number of annealed probes on the mRNA, providing a quantitative readout of poly(A) tail length. This amplification-free approach enables precise, high-throughput tail-length measurements under native conditions.

P86

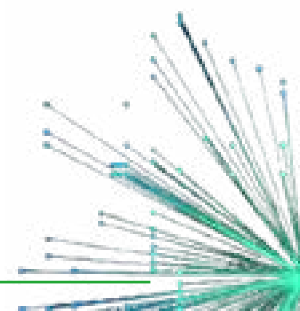
Single-molecule spectroscopy of GPCR oligomers in lipid nanodiscs using an Anti-Brownian Electrokinetic Trap (ABELtrap)

L. Spantzel[1], I. Pérez1, T. Heitkamp[1], A. Westphal[2], S. Reuter[2], R. Mrowka[2], M. Börsch[1]

[1]Single-Molecule Microscopy Group, Jena University Hospital, Friedrich Schiller University Jena

The human neurotensin receptor 1 (NTSR1), part of class A G protein-coupled receptors (GPCRs), exists in a dynamic equilibrium between monomers and dimers. To study its oligomerization in live mammalian cells, fluorescently tagged NTSR1 variants (mNeonGreen or mRuby3 fusions at the C-terminus) were encapsulated in 10-nm-sized soluble lipid nanodiscs using styrene-maleic acid copolymer (SMALPs). NTSR1 was stably expressed in HEK293T. The SMALP technique disrupts the plasma membrane of living HEK293T cells instantaneously and yields isolated NTSR1 within its native lipid environment for single-particle analysis.A custom-built confocal microscope enabled single-molecule spectroscopy of NTSR1-SMALPs using an Anti-Brownian Electrokinetic trap (ABELtrap, invented by A. E. Cohen and W. E. Moerner) to extend the recording time of individual nanodiscs up to 1 second. Fluorescence brightness distributions and analysis of homoFRET using fluorescence anisotropy as the read-out from thousands of SMALPs revealed shifts in the monomer-to-dimer ratio after ligand addition to the living HEK293T cells. The ABELtrap's active positioning and feedback control improved photon count rates and quantification of NTSR1's oligomerization behavior. The SMALP/ABELtrap approach provided valuable insights into the NTSR1 dynamics and interactions within native lipid membrane environments.

L. Spantzel, I. Pérez, T. Heitkamp, A. Westphal, S. Reuter, R. Rrowka, M. Börsch, „Monitoring oligomerization dynamics of individual human neurotensin receptors 1 in living cells and in SMALP nanodiscs," Proc. SPIE 12384, 1238407 (2023); doi: 10.1117/12.2648222



P87

Exploring the resolution limits of single-molecule FRET

Lukas Whaley-Mayda, Yonglei Sun, Quan Wang

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

Single-molecule Förster resonance energy transfer (smFRET) has established itself as a popular tool in biophysics by leveraging the classic single-molecule abilities to directly observe dynamics and distributions of structures. However, smFRET is still typically considered a "low-resolution" method in a structural biology context. One aspect of this is characterized by the resolving power—the ability to distinguish structures through differences in FRET efficiency—manifested in smFRET histogram peak widths. While fundamentally limited by photon shot noise, smFRET experiments are often subject to additional broadening that can drastically decrease resolution, especially when surface-immobilization is used. We previously introduced the ABEL-FRET platform for smFRET in an anti-Brownian electrokinetic (ABEL) trap, which facilitates the extended observation of molecules in free solution, thereby removing the need for surface tethering. Here we explore multiple experimental factors influencing smFRET resolution through comparative measurements on DNA rulers in ABEL trap and surface-immobilized modalities using both prism-TIRF and confocal imaging. We find that the act of surface-immobilization introduces excess heterogeneity to varying degree depending on the tethering/passivation strategy, while ABEL-FRET can restore photon-limited performance yielding ultra-high precision with theoretical sub-angstrom-resolution. We further characterize the performance of different dye-pairs, which can introduce additional photophysical broadening and heterogeneity. We apply ultra-precision ABEL-FRET to investigate distortions in DNA duplex structure. Overall, our results suggest that new classes of higher structural-resolution biophysical questions are within reach of smFRET.

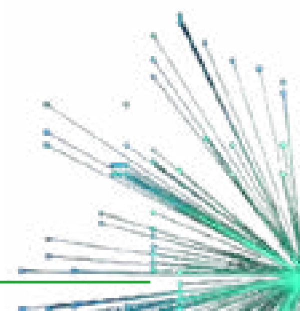
P88

Model-free photon analysis of diffusion-based single-molecule FRET experiments

Ivan Terterov[1], Daniel Nettels[2], Tanya Lastiza-Male[1], Kim Bartels[3], Christian Loew[3], Renee Vancraenenbroeck[3], Itay Carmel[1], Gabriel Rosenblum[1], Hagen Hofmann[1]

[1]Department of Chemical and Structural Biology, Weizmann Institute of Science, Herzl St. 234, 76100 Rehovot, Israel
[2]Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[3]Centre for Structural Systems Biology (CSSB), DESY and European Molecular Biology Laboratory Hamburg, Notkestrasse 85, 22607 Hamburg, Germany

Photon-by-photon analysis tools for diffusion-based single-molecule Förster resonance energy transfer (smFRET) experiments often describe protein dynamics with Markov models. However, FRET efficiencies are only projections of the conformational space such that the measured dynamics can appear non-Markovian. Model-free methods to quantify FRET efficiency fluctuations would be desirable in this case. Here, we present such an approach. We determine FRET efficiency correlation functions free of artifacts from the finite length of photon trajectories or the diffusion of molecules through the confocal volume. We show that these functions capture the dynamics of proteins from micro- to milliseconds both in simulation and experiment, which provides a rigorous validation of current model-based analysis approaches.



P89

ClearFinder: a Python GUI for annotating cells in cleared mouse brain

Margarita Tevosian[1,2,3], Stefan Pastore[4,5], Philipp Hillenbrand[2], Beat Lutz[2,3] and Susanne Gerber[4]

[1]UHasselt, Dynamic Bioimaging Lab (Prof. Jelle Hendrix), Advanced Optical Microscopy Centre, Biomedical Research Institute, Agoralaan C (BIOMED), B3590 Diepenbeek, Belgium
[2]Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University Mainz, Germany
[3]Leibniz Institute for Resilience Research (LIR), Mainz, Germany
[4]Computational Systems Genomics Group, Institute of Human Genetics, University Medical Center Mainz, Germany

Tissue clearing combined with light-sheet microscopy is gaining popularity among neuroscientists interested in unbiased assessment of their samples in 3D volume.
ClearMap and CellFinder are tools for analysing neuronal activity maps in an intact volume of cleared mouse brains. However, these tools lack a user interface, restricting accessibility primarily to scientists proficient in advanced Python programming.
We developed ClearFinder1: an easy-to-adopt graphical user interface (GUI) for cell quantification and group analysis of whole cleared mouse brains. Basic statistical analysis and additional visualization features allow a quick evaluation of the data and establishment of quality checks.
ClearFinder1 offers a comprehensive solution tailored for scientists from various disciplines to process whole-brain light-sheet microscopy imaging data efficiently. Key features include:

- user-friendly interface: ClearFinder provides an intuitive platform for detecting and assigning cells throughout the entire mouse brain volume using the capabilities of the ClearMap and CellFinder tools
- robust installation process: by maintaining independent virtual environments, ClearFinder ensures a robust and standardized installation process, simplifying the setup for users
- enhanced functionality: ClearFinder extends basic processing capabilities by incorporating additional features such as result visualization through heatmap plotting and basic statistical analysis including PCA and box plots.

Pastore, S., Hillenbrand, P., Molnar, N. et al. ClearFinder: a Python GUI for annotating cells in cleared mouse brain. BMC Bioinformatics 26, 24 (2025). https://doi.org/10.1186/s12859-025-06039-x

P90

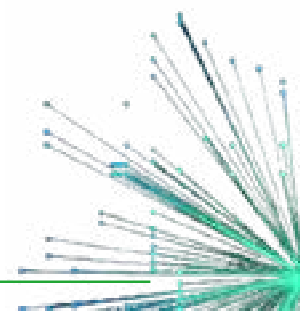
Combined spectral and lifetime measurements of Nile Red in pNIPAM microgels

Leon Trottenberg, Dominik Wöll

Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, 52074 Aachen

Microgels are three-dimensional cross-linked polymer networks. In good solvents, microgels are swollen, and significant amounts of solvent are taken up into the polymer network. This leads to interesting properties for a variety of applications.[1] These properties depend on the local structure of the microgels. In order to gain insight into the local structure of microgels, we previously developed a Nile Red PAINT based method for the super-resolved determination of the local polarity inside microgels.[2] This work aims to deepen the understanding of the interaction between Nile Red and pNIPAM. Therefore, we combined confocal spectral and lifetime measurements of Nile Red inside single microgels. From these measurements, we conclude that the Nile Red probe has a non-negligible influence on the local structure of the polymer system. Additionally, we found a preferential solvation in binary solvent mixtures around the pNIPAM chains, which has also been recently shown in a different work.[3] We plan to further extend these findings by simplifying the polymer system to more basic architectures.

[1] F. A. Plamper and W. Richtering, Accounts of Chemical Research, 50, 131–140 (2017).
[2] A. Purohit, S. P. Centeno, S. K. Wypysek, W. Richtering, and D. Wöll, Chemical Science, 10, 10336–10342 (2019).
[3] S.P. Centeno, K. Nothdurft, A.S. Klymchenko, A. Pich, W. Richtering and D. Wöll, Journal of Colloid and Interface Science, 678, 210-220 (2025).



P91

FCHO homologous disordered proteins explore different conformational landscape to initiate Endocytosis

Carlos A Elena-Real, Antonia Körber, Mihai Bodescu, Katrhin Motzny, Sigrid Milles

Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125, Berlin, Germany.

Clathrin-mediated endocytosis (CME) is the cellular mechanism used to uptake multiple cargoes, executed with remarkable spatiotemporal precision and high cargo selectivity. This selectivity is encoded in the early phases of endocytosis, when multiple proteins, containing both folded domains and disordered regions, are working together in a coordinated manner. Among them, homologous FCHO 1 and 2 proteins stand out as the first to arrive at the endocytic pits. Despite having a similar domain architecture with conserved folded regions, their long disordered stretches differ significantly, suggesting they are responsible for conferring tissue-specific functions.
Here, we study the structural dynamics and interaction landscapes of the intrinsically disordered regions in both FCHO1 and 2. Using NMR spectroscopy, we identified local transient secondary structure elements with varying levels of structure and different dynamic regimes in both proteins, suggesting evolutionary differences. In addition, we used fluorescence correlation spectroscopy (FCS) to examine the global dynamics of both FCHO IDRs by grafting florescent dyes at different positions with distinct local structure. This combination of NMR and fluorescent spectroscopy provides crucial molecular insights into the selectivity exerted by these endocytic initiators.

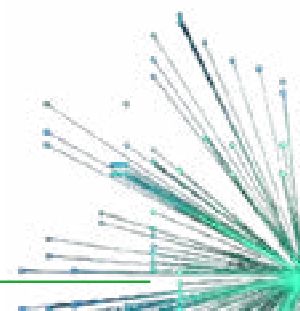
P92

Raster Image Correlation Spectroscopy in the Presence of Frame-to-frame Diffusing Artifacts

Yarne Vankerkhoven[1], Veerle Lemmens[1], Irene Gialdini[2], Don Lamb[2], Joshua Simpson[3], David Alsteens[3,4], Jelle Hendrix[1]

[1]Dynamic Bioimaging Lab, Advanced Optical Microscopy Centre and Biomedical Research Institute, Hasselt University, B3590 Diepenbeek, Belgium
[2]Department Chemie and Center for NanoScience, Ludwig-Maximilians-Universität München, D80539, Germany
[3]Louvain Institute of Biomolecular Science and Technology, Université catholique de Louvain, 1348 Louvain-la-Neuve, Belgium
[4]WELBIO department, WEL Research Institute, 1300 Wavre, Belgium

Studying biomolecules in realtime in their cellular context is vital to understanding their function. A popular method for quantifying the dynamics of fluorescently labeled molecules from confocal imaging data is raster image correlation spectroscopy (RICS). Critically however, current image detrending and segmentation methods fail with samples that contain slowly-diffusing sparse bright (or dark) features that only displace between consecutive frames. We present a simple, yet robust way to prevent such artifacts from corrupting RICS analyses by intensity segmentation of detrended data. We first applied the new method on simulated mixtures of excessive fast-diffusing particles and sparser, 10-fold brighter slow-diffusing particles. We show that compared to normal correction methods, only the new method manages to recover the correct diffusion properties of the fast-diffusing species. Then, we applied the new method to real data, specifically, mixtures of pentamers and clusters of pentamers of the transmembrane Glycine receptor and clusters of the epidermal growth factor receptor. In all cases, processing imaging data to remove sparse artifacts resulted in RICS correlations that could be very well described by simple diffusion models. Taken together, we believe our new segmentation method increases the robustness of RICS significantly for samples containing slowly-diffusing bright or dark fluorescent outliers.



P93

Combined MINFLUX – SRS – TPE FLIM imaging of bacteria and their targeted host cells

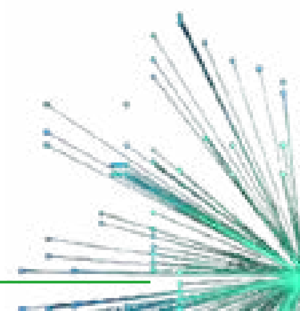
Chinmaya Venugopal Srambickal [1], Maryam Sanaee[1], Lenny Reinkensmeier[2], Hanie Esmaeeli1,René Sigmund[2], Mark Bates[2], Joachim Piguet[1], Gero Stibenz[3], Anandi Narayana Moorthy[4], SarpBamyaci[4], Andreas Schönle[5], Ingo Rimke[3], Alexander Egner[2], Birgitta Henriques-Normark[4],Jerker Widengren[1]

[1]Department of Applied Physics, Royal Institute of Technology, SE -10691 Stockholm, Sweden.
[2]Department ofOptical Nanoscopy, Institute for Nanophotonics, Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany
[3]APEAngewandte Physik & Elektronik GmbH, Plauener Str. 163-165, 13053 Berlin, Germany
[4]Department of Microbiology,Tumour and Cell Biology, Karolinska Institutet, Stockholm, Sweden
[5]Abberior Instruments GMBH, Göttingen,Germany

Since its inception, MINFLUX has rapidly delivered unprecedented tracking and imagingcapabilities with nanometre precision. It has been employed to visualize the motion of kinesin[1] and dynein [2] in live cells, monitor protein transport through nuclear pore complexes [3], andresolve intracellular protein distributions at the nanoscale [4,5]. While imaging individual proteindistributions provides essential intermolecular context, integrating additional imagingmodalities enables more holistic insights, encompassing cellular morphology, environmentsand metabolic activity.In this work, we integrated a MINFLUX prototype with a laser prototype for stimulated Ramanscattering (SRS) [6], capable of switching between femtosecond and picosecond pulse-widthoperation within minutes. This flexibility enabled cellular SRS imaging alongsideautofluorescence imaging of metabolic cofactors such as NADH and FAD via two-photonexcitation (TPE). Furthermore, a time-correlated single-photon counting (TCSPC) system wasincorporated to facilitate fluorescence lifetime imaging microscopy (FLIM) under TPE conditions.Using this multimodal setup, we acquired SRS and TPE-FLIM images of both bacterial and hostcells, complemented by high-precision MINFLUX imaging within selected regions of interest.These proof-of-concept measurements lay the groundwork for future studies on bacterial infection and host-cell interactions.

[1] Deguchi, Takahiro, et al. „Direct observation of motor protein stepping in living cells using MINFLUX." Science379.6636 (2023): 1010-1015.
eabl7560.

[2] Schleske, Jonas M., et al. „MINFLUX reveals dynein stepping in live neurons." Proceedings of the National Academyof Sciences 121.38 (2024): e2412241121.
[3] Sau, Abhishek, et al. „Overlapping nuclear import and export paths unveiled by two -colour MINFLUX." Nature640.8059 (2025): 821-827.
[4] Pape, Jasmin K., et al. „Multicolor 3D MINFLUX nanoscopy of mitochondrial MICOS proteins." Proceedings of theNational Academy of Sciences 117.34 (2020): 20607-20614.
[5] Grabner, Chad P., et al. „Resolving the molecular architecture of the photoreceptor active zone with 3D-MINFLUX."Science Advances 8.28 (2022): eabl7560.
[6] Reinkensmeier, Lenny, et al. „Novel Laser Technology Enables 10x Faster SRS Imaging and Rapid Tuning inBiological Samples." bioRxiv (2025): 2025-01.



P94

Monitoring Dynamic Conformations of a Single Fluorescent Molecule Inside a Protein Cavity

Alan M. Szalai[1,2], Santiago Sosa [1,3], Lucía F. Lopez[1], Juan Manuel Prieto[4], Cecilia Zaza[1], Aleksandra K. Adamczyk2, Hernán R. Bonomi3, Marcelo A. Marti[4], Guillermo P. Acuna[2], Fernando A. Goldbaum[5], Fernando D. Stefani[1,6]

[1]Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Department of Physics, University of Fribourg, Fribourg, Switzerland
[3]Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina
[4]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN)- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[5]Centro de Rediseño e Ingeniería de Proteínas and Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (CRIP-IIB-UNSAM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[6]Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Fluorescence nanoscopy and single-molecule methods are entering the realm of structural biology, breaking new ground for dynamic structural measurements at room temperature and liquid environments. In this work, DNA-PAINT, polarization-dependent single-molecule excitation, and protein engineering are combined to determine the orientation of a fluorophore forming hydrogen bonds inside a protein cavity. This method, that we applied in a previous work to study the orientation of a dye bound to a DNA origami, allowed us to observe multiple conformations of a fluorophore inside a Brucella Lumazine Synthase (BLS) decamer. The observed conformations are in good agreement with molecular dynamics simulations, enabling a new, more realistic interplay between experiments and simulations to identify stable conformations and the key interactions involved. Furthermore, jumps between conformations were monitored with a precision of 3° and a time resolution of a few seconds, confirming the potential of this methodology for retrieving dynamic structural information of nanoscopic biological systems under physiologically compatible conditions.

P95

New analysis options push the limits of FLIM imaging modalities

Klaudia Fillipek, Ellen Schmeyer, Kamil Bobowski, Marcelle König, Marcus Sackrow, Markus Götz, Stefan Eilers, Fabian Jolmes, Evangelos Sisamakis, Felix Koberling, Matthias Patting, Rainer Erdmann

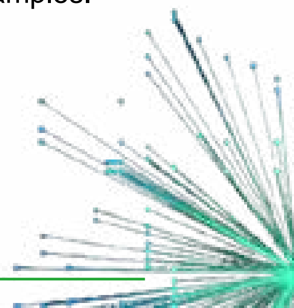
PicoQuant, Rudower Chaussee 29, 12489 Berlin

Keywords: Fluorescence Lifetime IMaging (FLIM), Image Scanning Microscopy (ISM), Single-Photon Avalanche Diode (SPAD), Array Detector, Confocal Microscopy

The newly released software NovaFLIM brings a huge boost of efficiency in FLIM, FLIM-FRET and anisotropy analysis of z-stacks, time-lapse series and tiled and stitched images acquired with a Luminosa single photon counting confocal microscope from PicoQuant. In addition to the seamless integration into the Luminosa microscope, NovaFLIM can work with data acquired with the MicroTime 200 and the various LSM upgrade kits from PicoQuant for Nikon, Olympus and Zeiss LSMs.

The efficiently implemented batch analysis based on GPU accelerated algorithms saves users a lot of time, as do advanced export options. A new aspect is that one can easily create 1D and 2D histograms of fitted parameters and use them for a quantitative, robust and reproducible ROI definition as well as for comparing results from images which show structures with differing morphology. New routines for advanced and flexible ROI handling based on such histograms as well as phasor plots open new analysis possibilities.

Moreover, the newly released software NovaISM allows for the analysis of ISM-FLIM images acquired with the PDA-23 add-On of the Luminosa microscope. Image scanning microscopy (ISM) with a SPAD array detector achieves resolution enhancements of about 1.5 to 1.7 times in comparison to normal confocal images, in combination with spatial deconvolution. Even for 2d-recordings/data the contrast of the ISM-FLIM images is enhanced significantly by rejecting out-of focus light. Such rejection enhances not only the signal-to-noise-ratio, but also the lifetime contrast in the FLIM images. These benefits enable either faster image acquisition or gentler imaging of live samples.



P96

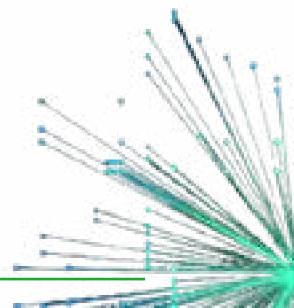
Super-Resolution Imaging in Whole Cells and Tissues via DNA-PAINT on a Spinning Disk Confocal with Optical Photon Reassignment

Cecilia Zaza[1], Megan D. Joseph[1], Olivia P. L. Dalby[1,2], Rhian F. Walther[3], Karol Kołątaj4, Germán Chiarelli[4], Franck Pichaud[3,6], Guillermo P. Acuna[4,5], Sabrina Simoncelli[1,2]

[1]London Centre for Nanotechnology, University College London, 19 Gordon Street, WC1H 0AH London, United Kingdom.
[2]Department of Chemistry, University College London, 20 Gordon Street, WC1H 0AJ London, United Kingdom.
[3]Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, UK
[4]Department of Physics, University of Fribourg, Chemin du Musée 3, Fribourg CH 1700, Switzerland
[5]Swiss National Center for Competence in Research (NCCR) Bio-inspired Materials, University of Fribourg, Chemin des Verdiers 4, CH-1700 Fribourg, Switzerland
6Institute for the Physics of Living Systems, University College London, London WC1E 6BT, UK

The implementation of single-molecule localization microscopy (SMLM) has often been constrained by selective illumination configurations required to achieve the high signal-to-noise ratio (SNR) necessary for single-molecule detection. This results in a trade-off between penetration depth, field-of-view, and spatial resolution. The most widely used implementation of SMLM is in combination with wide-field illumination, particularly through total internal reflection (TIR) or highly inclined and laminated optical sheets (HILO) excitation, which routinely achieve lateral localization precision below 10 nm. However, this comes at the expense of limited penetration depth of less than 250 nm for TIR and a small field-of-view of approximately 20 μm in diameter for HILO.
Recently, confocal-based configurations, including spinning disk confocal (SDC), have also been combined with SMLM techniques. However, the spatial resolution achievable with SMLM in a SDC microscope, is limited due to the reduction of both the excitation intensity and the detection efficiency, as emission light is partially blocked by the disks.
To enhance photon collection efficiency, microlensing the emission pinhole through optical photon reassignment (OPR) has proven effective in increasing SNR. In this talk, we will explore the extent to which an SDC-OPR configuration can surpass current optical setups, mitigating the trade-offs between penetration depth, field-of-view, and spatial resolution for SMLM.

We will benchmark the resolving power of the SDC-OPR by visualizing reference standards for super-resolution microscopy, including DNA origami, structural proteins of the nuclear pore complex, and microtubules. Furthermore, we will demonstrate the capability of the SDC-OPR by examining the spatial organization of proteins associated with a novel pathway involved in aberrant T cell activation.



P98

Plasmonic Nanotaper Meta-surfaces for High-Contrast Live-Cell Imaging

Cheng ZHANG, Susobhan Choudhury, Katrin G. Heinze

Rudolf Virchow Center for Integrative and Translational Bioimaging, Julius-Maximilians-Universität Würzburg (JMU), Würzburg, Germany

Artificially engineered plasmonic nanostructures enable the generation of three-dimensional confined optical hotspots that exceed the diffraction limit of light [1]. In this study, we employ a metasurface substrate comprising a plasmonic nanotaper array to achieve enhanced brightness and contrast in live-cell imaging. To mitigate cytotoxicity induced by direct contact between cell membranes and sharp nanotaper features, a biocompatible dielectric (PMMA) planarization layer is integrated at the cell-substrate interface. This layer preserves proximity to the plasmonic hotspots while shielding cells from mechanical or chemical degradation. Highly ordered nanotaper array, manufactured via scalable nanosphere lithography [2], exhibits precise geometric order, enabling robust confinement of electromagnetic field within a confined hotspot volume and a localized electric field intensity enhancement exceeding 10^3-fold at nanotaper apices. This configuration facilitates high-contrast imaging with long-term stability. When implemented in conventional confocal microscopy for live-cell imaging, we observe remarkable brightness and contrast enhancement over 2 orders of magnitude compared to the case using a planar control substrate. Our practical approach establishes a framework for advancing high spatiotemporal resolution microscopy techniques, particularly for the studies of cellular dynamics where minimizing phototoxicity and preserving viability are paramount.

[1] Lukas Novotny, Bert Hecht, Principle of nano-optics, 2nd edition, Cambridge University Press (2012).

[2] Christy L. Haynes, et al, Nanosphere Lithography: A Versatile Nanofabrication Tool for Studies of Size-Dependent Nanoparticle Optics. J. Phys. Chem. B, 105, 5599-5611 (2001).

P99F

Revisiting multichannel processing with in-depth multitarget 3D ModLoc imaging

Lancelot Pincet[1], Abigail Illand[1], Pierre Jouchet[1], Emmanuel Fort[2], Sandrine Lévêque-Fort[1]

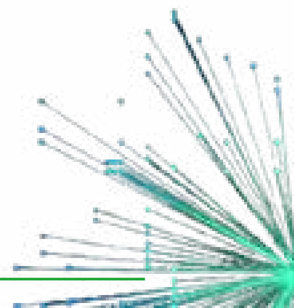
[1]Institut des Sciences Moléculaires d'Orsay, Université Paris Saclay, CNRS, Orsay France

[2]Institut Langevin, CNRS, Paris France

Single Molecule Localization Microscopy (SMLM) has become a widely adopted technique for achieving nanometric resolution by extracting the precise positions of individual fluorophores from temporal image stacks, often with a localization precision of ~10 nm. Beyond transverse super-resolution, other molecular parameters—such as axial position or fluorophore identity—can also be retrieved, leveraging the single-molecule nature of the data. These additional parameters are typically extracted using multichannel acquisitions, where each channel corresponds to a different optical condition. For instance, in spectral demixing for multitarget imaging, a dichroic mirror splits the emission into two spectrally distinct detection paths. Similarly, in ModLoc [1] in-depth 3D imaging, axial information is encoded in the phase of a structured excitation pattern and decoded via four demodulated channels.

However, multichannel SMLM imaging introduces considerable complexity in data processing, especially due to large raw data volumes and the diversity of processing strategies. To address this, we present a modular Python package called ModPro designed for SMLM processing, offering building blocks to create custom multichannel workflows. We demonstrate the capabilities of this toolkit on a challenging dataset combining 3D ModLoc imaging with spectral demixing, highlighting its adaptability and performance in complex acquisition schemes.

[1] Pierre Jouchet, Clément Cabriel, Nicolas Bourg, Marion Bardou, Christian Poüs, Emmanuel Fort, Sandrine Lévêque-Fort, Nature Photonics, 15, 297–304 (2021)



P100

Extending volumetric imaging in single molecule localization microscopy

Jimeng Zhou, Sandrine Lévêque-Fort

Institut des Sciences Moléculaires d'Orsay, Université Paris Saclay, CNRS, Orsay France

Single molecule localization microscopy provides high-resolution imaging in the lateral plane. Further developments are still required to improve the axial precision but also the capacity to image in depth. Typically, a high numerical aperture objective is used to benefit of the smallest point spread function (PSF) and optimal lateral precision, but it also restricts the depth of field to typically less than ~800 nm. A common approach to extend the volumetric observation is z-stacking—acquiring sequential images at different focal depths. While effective, this method is time-consuming and reduces temporal resolution, limiting its applicability to dynamic biological processes, which are now accessible thanks to self blinking dyes.
We aim to develop an optical strategy that enables fast volumetric imaging in SMLM without compromising lateral resolution. Specifically, we explore the use of phase masks in the detection path to engineer the point spread function (PSF), thereby extending the volume of observation between a factor 2 to 5. I will present the principles and performances of the phase mask implementation, discuss its combination with a localization approach in 3D, and highlight its potential for real-time volumetric imaging in biological samples.

P101

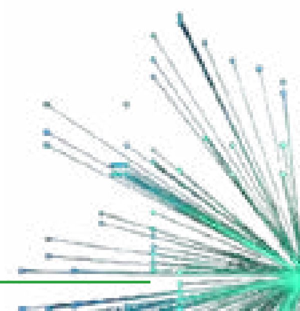
Examining protein multimerization using Escape-time Stereometry

Xin Zhu, Dimitrios Soulias, Madhavi Krishnan

12 Mansfield Road, Chemistry Research Lab, Oxford,UK, OX1 3TA

Protein multimerization plays an important role in biology, yet direct, high-resolution characterization of multimeric states in the solution phase remains a significant challenge. Here, we demonstrate that Escape Time Stereometry (ETs)—a high-throughput, and field-free single-molecule method capable of distinguishing between molecules based on their size, shape – can resolve oligomeric states of individual protein complexes in solution under physiological conditions. By measuring the distribution of escape times as labelled proteins exit a landscape of nanofluidic trap arrays, we extract two key parameters characterizing molecular complexes, namely size and shape –within seconds of measurement. We demonstrate the power of ETs using two highly distinct biomolecular systems. First, we examine insulin, a small therapeutic protein known to undergo concentration-dependent multimerization. ETs resolves distinct populations corresponding to monomeric, dimeric, and hexametric states, enabling precise quantification of oligomer distributions under formulation-relevant conditions. We also monitor in real-time the dynamic transition of multimeric states during dilution from formulation (~100 µM) to physiological levels (~1 nM) and measure dissociation constants characterizing the underlying interactions. Next, we apply ETs to citrate synthase (CS), a protein known for its fractal-like multimerization properties. We demonstrate the ability to discriminate between various oligomeric states, detecting dimers, hexamers and 18-mers. We also observe the elusive 36-mer and 54-mer states of CS for the first time in solution phase, previously detected solely in electron microscopy.

[1] Sendker, F.L., Lo, Y.K., Heimerl, T. et al. Emergence of fractal geometries in the evolution of a metabolic enzyme. Nature 628, 894–900 (2024). https://doi.org/10.1038/s41586-024-07287-2.



P102

Expanding the Horizon of FCS with SPAD Arrays: A Promising Outlook for New Applications

Marcelle Koenig[1], Anders Barth[1], Evangelos Sisamakis[1], Fabian Barachati[1], Johan Hummert[1], Felix Koberling[1], Ivan Michel Antolovic[2], Rainer Erdmann[1]

[1]PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
[2]Pi Imaging Technology SA, EPFL Innovation Park, 1015 Lausanne, Switzerland

Fluorescence Correlation Spectroscopy (FCS) is a well-established tool for studying molecular interactions and dynamics at the single-molecule level. The recent integration of single-photon avalanche diode (SPAD) arrays combined with time-resolved instrumentation in confocal microscopy provides new possibilities for FCS that provide new insights for live cell investigations.
Here, we evaluate enhanced FCS applications which are enabled by the integration of a cooled high-performance 23-pixel SPAD-array that was developed jointly with Pi Imaging Technologies as an add-on to the confocal microscope Luminosa. The SPAD array allows for the simultaneous detection of multiple fluorescence signals based on single photon counting with high temporal resolution. Any pixel combination within the SPAD array can selected for advanced FCS analyses. Thus, compared to point detectors, spatially resolved information about molecular diffusion and dynamics becomes available. This enables e.g. spot-variation FCS for the identification of potentially hindered diffusion in live cell investigations. Spatial pixel cross-correlations can be used to uncover directional diffusion. The integration of Time-Correlated Single-Photon Counting (TCSPC) provides further information about the fluorescence lifetimes. These can be utilized for an even more comprehensive understanding of complex biological mechanisms.

The integration of SPAD-arrays with time-resolved detection represents a significant advancement for confocal microscopes. Apart from the improved optical resolution for imaging purposes via image scanning microscopy (ISM), SPAD array based detection allows for a multitude of new FCS modalities for studying complex biological processes in both temporal and spatial domains.

P103

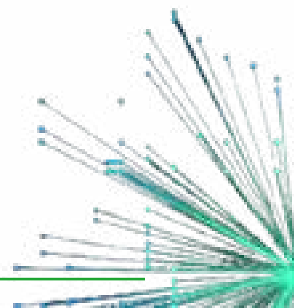
Tunable linker systems for broad use of functional dyes in single-molecule imaging

Mostofa Ataur Rohman[1], Marcus Lantzius-Beninga[2], Thomas Otavio-Peulen[1], Andreas Herrmann[2] and Thorben Cordes[1]

[1]Biophysical Chemistry, Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany
[2]Institute of Technical and Macromolecular Chemistry, (RWTH) Aachen University, Worringerweg 2, 52074 Aachen, Germany

Commercial fluorophores are vital in the life sciences, supporting imaging, DNA sequencing, single-molecule studies, and biomedical assays. However, their utility is often limited by fixed photophysical properties, fast photobleaching, limited functional versatility, and lack of modular bioconjugation options. Here, we introduce a modular chemical biology approach using a ‘linker’ system that connects biological targets, commercial dyes, and functional moieties, e.g., photostabilizers. These linkers are synthesized via a one-pot Ugi four-component reaction, enabling rapid and diverse customization. Each linker features a bioconjugation handle, a click-compatible unit for fluorophore attachment, and a functional moeity to tune dye behavior. This strategy converts conventional fluorophores into adaptable probes with improved photostability, controlled blinking or environmental responsiveness.[1] This contribution describes the incorporation of new triplet-state quenchers and dye-attachment moeities into the linker structure to further enhances probe performance by reduction of photobleaching and blinking. We evaluate the performance of various linker-dye combinations using fluorescence correlation spectroscopy (FCS), single-molecule FRET (smFRET) and TIRF to assess photobleaching and power-dependent assays to quantify blinking duration, triplet-state lifetimes, dark-state recovery. The modular linker system offers a flexible toolkit for customization of fluorophores in the final step of biolabelling, expanding applicability of commercially available dyes in advanced imaging techniques such as super-resolution microscopy and functional single-molecule studies in vitro and in vivo.

[1] https://doi.org/10.1002/ange.202112959



P104

Single-molecule and time-resolved fluorescence microscopy studies of the interaction between synapsin-1/α-synuclein condensates and membranes

Maria Loidolt-Krüger, Berlin, Germany

P105

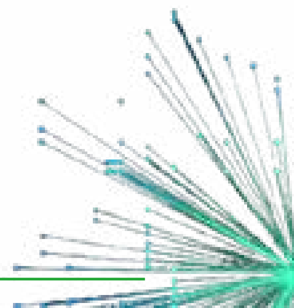
Temperature-dependence and Crowding-Induced Modulation in PFK1-Driven Metabolic Regulation

Harshita Rastogi, Simon Ebbinghaus

1) Lehrstuhl für Biophysikalische Chemie, Ruhr-Universität Bochum, 44780 Bochum, Germany. 2) Research Center Chemical Sciences and Sustainability, Research Alliance Ruhr, 44780 Bochum, Germany

This work elucidates how phosphofructokinase-1 (PFK1) directs metabolic regulation through dynamic, phase-separated condensates called glucosomes—transient compartments that spatially organize glycolytic enzymes.1 By integrating fluorescence live-cell imaging, Fast Relaxation Imaging (temperature-induced), and enzymatic assays, we aim to understand the biophysical principles driving PFK1's liquid-liquid phase separation and its functional consequences. We have employed wide-field and confocal fluorescence microscopy to visualize the cytoplasmic localization of metabolic enzyme controlling glucose flux under varying conditions in human cells.2 Our in vitro analyses demonstrate macromolecular crowding enhanced substrate binding cooperativity of PFK1 while diffusion-limited catalytic turnover rates, revealing how crowded environments modulate enzyme function.3 We investigated the temperature dependence of enzymatic rates contributing significantly to the temperature dependence of metabolic processes inside living cells. The study also highlights the existence and divergence of optimal activity temperature from the global stability temperature of the enzyme.4 By correlating in vitro results with in-cell measurements, we demonstrate how cellular crowding fine-tune microenvironment of glycolytic enzymes by modulating activity and physicochemical mechanisms.

[1] Kohnhorst, C. L., Kyoung, M., Jeon, M., Schmitt, D. L., Kennedy, E. L., Ramirez, J., Bracey, S. M., Luu, B. T., Russell, S. J., An, S. J Biol Chem., 292, 9191-9203 (2017).
[2] Kyoung, M., Kennedy, E. L., Jeon, M., Augustine, F., Chauhan, K. M., An, S. Biophys J., 123, 5A (2024).
[3] Webb, B. A., Forouhar, F., Szu, F. E., Seetharaman, J., Tong, L., Barber, D. L. Nature, 523, 111-114 (2015).
[4] Walker, E. J., Hamill, C. J., Crean, R., et al. ACS Catal.,14, 4379-4394 (2024).



P106

Using Light Field Microscopy to measure the orientation of Single Molecules in 3D

Luis Aleman-Castaneda 1, Arturo Vesga2, Hannah Heil3, Sam Daly4, Ezra Bruggeman4, Steven Lee4, Sophie Brasselet1, Ricardo Henriques2,5

1Institut Fresnel, Aix Marseille Univ, CNRS, Centrale Med, Marseille, France
2Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal
3Division of Infection Medicine, Department of Clinical Sciences, Faculty of Medicine, Lund University, Lund, Sweden
4Yusuf Hamied Department of Chemistry, University of Cambridge, UK
5Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal

Single-molecule orientation and localization microscopy (SMOLM) allows measuring the orientation and wobbling of individual fluorophores as well as determine their 3D position. However, most SMOLM techniques rely on complex point spread function (PSF) fitting [1], which makes these approaches complex to implement and calibrate and their data hard to analyse. A possibility to overcome this is to exploit the intensity distribution at the back focal plane (BFP) either in scanning [2] or wide-field configurations. For the latter, although it has been widely used in 2D [3], its extension to 3D still poses few challenges regarding the correct evaluation of orientation parameters in different z-planes.
In this work, we present a new methodology for single molecule light field microscopy (SMLFM) [4], a technique that has been used to determine the 3D position of individual emitters with great success by probing the BFP phase distribution (related ultimately to defocus, i.e. axial position). What we propose is to exploit additionally the BFP instensity distribution (dictated by orientation and wobble), to simulatenously estimate the orientation of the emitters. We present some preliminary results using beads and single fluorphores.

[1] V. Curcio, et al, “Birefringent Fourier filtering for single molecule coordinate and height super-resolution imaging with dithering and orientation,” Nat Commun, 11, 5307 (2020).
[2] M. Lieb et al., „Single-molecule orientations determined by direct emission pattern imaging,“ J. Opt. Soc. Am. B, 21, 1210-1215 (2004).
[3] C. Rimoli et al., “4polar-STORM polarized super-resolution imaging of actin filament organization in cells,” Nat Commun, 13, 301 (2022).
[4] R. Sims et al., „Single molecule light field microscopy,“ Optica, 7, 1065-1072 (2020).

P108

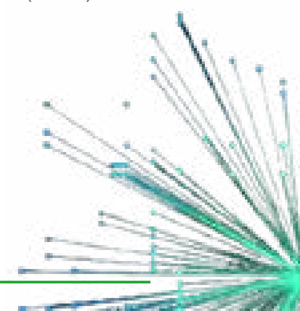
Imaging, Quantifying and Mapping Human Chromatin Remodeler Dynamics: From Phase-separation-mediated Intranuclear Organization to Cancer-mutant-specific Regulatory Landscape

Ziqing Winston Zhao

Department of Chemistry, Centre for BioImaging Sciences and Mechanobiology Institute, National University of Singapore

SWI/SNF chromatin remodelers are a key family of multi-subunit complexes that regulate genome access via nucleosome translocation/ejection. Despite their prevalent implications in cancers, their intranuclear dynamics in vivo and how misregulation of such dynamics could underpin cancers remain poorly understood. Herein, using single-molecule tracking (SMT), we quantified the live-cell diffusion and chromatin-binding dynamics of the fully assembled SWI/SNF remodeler complexes. Leveraging a novel super-resolved density mapping strategy, we further revealed heterogenous, nanoscale remodeler binding “hotspots” across the nucleoplasm. To elucidate the mechanism driving such intranuclear organization, we showed that BRG1, the core ATPase/translocase subunit common to major subtypes of the SWI/SNF family, undergoes phase separation both in vitro and in live cells, mediated by its IDR-rich C-terminus (BRG1C). Condensates of BRG1C form across a wide range of (including endogenous) expression levels, are highly dynamic and spatially colocalize with nucleolus, with their formation, localization and liquid-like properties governed by a specific molecular grammar. Moreover, live-cell SMT revealed differential diffusional and chromatin-binding dynamics of BRG1C in a condensate-specific and chromatin-acetylation-dependent manner. These findings shed insights into a multi-modal, phase-separation-mediated landscape for organizing remodeler dynamics in space and time, and establish the biophysical basis for aberrant remodeler–chromatin interactions underpinning diverse cancer-associated remodeler mutations.

Engl, W., Kunstar-Thomas, A., Chen, S., Ng, W. S., Sielaff, H., Zhao, Z. W. Single-molecule imaging of SWI/SNF chromatin remodelers reveals bromodomain-mediated and cancer-mutants-specific landscape of multi-modal DNA-binding dynamics. Nature Commun. 15, 7646 (2024).



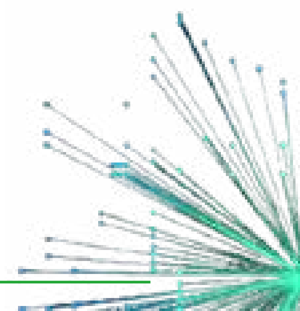
P110

INTRACRINE SIGNALING OF THE EGF RECEPTOR

Noémi Bilakovics[1], Julianna Volkó[1], Jeppesen Chinzorig[1], István Csomós[1] , Katalin Tóth[1], Krisztina Németh[2], Gábor Mocsár[1],György Vereb[1], Péter Nagy[1], György Vámosi[1]

[1]University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology
[2]MS Metabolomic Research Laboratory, Centre for Structural Science, Research Centre for Natural Sciences, Budapest, Hungary

The epidermal growth factor receptor (EGFR) is a transmembrane protein. Its activation starts with binding its ligand, epidermal growth factor (EGF), which precursor form is the preEGF. EGFR is involved in cancer development hence it is an important target in anticancer therapies. Monoclonal antibodies targeting the extracellular domain of EGFR are used as inhibitors. We described the intracrine signaling mechanism of the IL-2 receptor, where the receptor and the ligand could form a complex and start signaling already in the Golgi. We were interested whether a similar intracrine signaling mechanism can also take place in cells expressing both EGFR and the soluble EGF or the preEGF ligand. We studied the steps of the signaling process with various methods. We applied an anti-phospho-EGFR antibody to detect receptor phosphorylation in the Golgi visualized by a TagBFP-labeled giantin. To directly test whether the different forms of the EGF ligand can bind to the EGFR in the Golgi we used FLIM-FRET and FCS measurements. The receptor dimerization in the Golgi was also detected by FLIM-FRET. Our results show that the mature EGF ligand, contrary to preEGF, binds to EGFR and causes its oligomerization and phosphorylation in the Golgi. If such intracrine signaling occurs, antibody therapies against EGFR may be inefficient in cancer cells expressing both EGFR and its mature EGF ligand.



30th International Workshop – Made memorable by you.

PicoQuant