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BIOPHYSICS BY THE SEA

INTERNATIONAL CONFERENCE ON FLUORESCENCE SUPER-RESOLUTION MICROSCOPY, SPECTROSCOPY, MOLECULAR CELL MECHANICS AND THEORETICAL NEUROPHYSICS

7 - 12 OCTOBER 2018
POLLENTIA CLUB RESORT, 07400, ALCUDIA, SPAIN

THIS TIME JOINT WITH:

BE-OPTICAL

Sauer Lab

ORGANIZER OF THE EVENT:
GEORG-AUGUST-UNIVERSITY
THIRD INSTITUTE OF PHYSICS
FRIEDRICH-HUND-PLATZ 1
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GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

Scientific program

Monday 08.10.2018

Chair: Jörg Enderlein

9:00 - 9:10 **Jörg Enderlein**: Welcome message

9:10 - 9:40 **Christian Franke,** Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany
„Elucidating the Endosomal Machinery with Correlative Multi-Color Single-Molecule Localization-Microscopy & Electron Tomography“

9:40 - 10:00 **Sebastian Isbaner,** Georg-August-University Göttingen, Germany
“Single Molecule Metal-Induced Energy Transfer for Axial Localization”

10:00 - 10:20 **Felix Wäldchen,** Julius Maximilians University Wuerzburg, Germany
„Lattice Light-Sheet Microscopy for Whole-Cell Single Molecule Imaging“

10:20 - 11:00 

Chair: Ingo Gregor

11:00 - 11:20 **Julian Lehmann,** Julius-Maximilians-University Würzburg, Germany
“PALM stoichiometry of plant anion channels”

11:20 - 11:40 **Steffen Mühle,** Georg-August Universität Göttingen, Germany
“Brownian Dynamics Simulation of (Semi-)Flexible Polymer Chains**”**

11:40 - 12:00 **Amna Abdalla Mohammed Khalid,** Georg-August-University Göttingen, Germany
“MT-MKLp2 cross-linked network studies”

12:00 – 14:00 LUNCH

14:00 - … Discussion session: combination of STORM and MIET techniques

20:00 - … **POSTER SESSION and GET TOGETHER**

Tuesday 09.10.2018

Chair: Ulrich Parlitz

9:00 - 9:20 **Andreas Kurz,** University of Würzburg, Würzburg, Germany
"Correlative live- and fixed cell super resolution imaging"

9:20 - 9:40 **Fabian Zwettler,** Julius-Maximilians-University Würzburg, Würzburg, Germany
"Next generation Expansion Microscopy: combining physical specimen expansion with super-resolution microscopy"

9:40 - 10:00 **Oleksii Nevskyi,** Georg-August-University Göttingen, Germany
"Fluorescent Diarylethenes as Photoswitches for Super-Resolution Microscopy in Material Science"

10:00 - 10:40 

Chair: Andreas Neef

10:40 – 11:00 **Sina Wäldchen,** University of Würzburg, Germany
„Activity-induced ionotropic glutamate receptor dynamics at super-resolution in vivo"

11:00 – 11:20 **Julia Heiby,** Julius-Maximilians-University Würzburg, Germany
"Methionine induces malleability in the N-terminal domain of a spider silk protein"

11:20 – 11:40 **Eugenia Butkevich,** Georg-August-University Göttingen, Germany
„Polymerization of drebrin drives actin network remodeling and sarcomere reorganization"

11:40 - 12:00 **Subhabrata Ghosh,** Georg-August-University Göttingen, Germany
"Heat-treatment Controlled Blue Light Emission in Ternary Glass-ceramic Waveguides"

12:00 – 14:00 LUNCH

14:00 - … Discussion session: PAINT, STORM and Expansion Microscopy

Wednesday 10.10.2018

Chair: Ireneusz Grulkowski

9:00 - 9:30 **Felix Koberling,** PicoQuant, Berlin, Germany
"Mapping Molecules Quantitatively in Confocal Fluorescence Microscopy**"**

9:30 - 9:50 **Akshita Sharma,** Georg-August-University Göttingen, Germany
"Leaflet-dependent diffusion in lipid bilayers using Metal-Induced Energy Transfer Fluorescence Lifetime Correlation Spectroscopy (MIET-FLCS)"

9:50 - 10:10 **Nazar Oleksiievets,** Georg-August-University Göttingen, Germany
"Probing Lipid Diffusion in Curved Membranes with Fluorescence Microscopy"

10:10 - 10:50 

Chair: Felix Koberling

10:50 - 11:10 **Jan Christoph Thiele,** Georg-August-University Göttingen, Germany
"Polymer brushes in motion – measuring flow with nanometre resolution

11:10 - 11:30 **Hongje Jang,** Georg-August-University Göttingen, Germany
"3-dimensional chemotaxis measurement of Dictyostellium Discoideum using multiplane deconvolution imaging setup**"**

11:30 - 11:50 **Franziska Neubert,** University of Wuerzburg, Germany
„Bioorthogonal click labeling of the NMDA receptor subunit NR1"

12:00 – 14:00 LUNCH

14:00 - … Discussion session: Super-Resolution Microscopy and Neurobiology: from Cells to Networks

20:00 - … **POSTER SESSION and GET TOGETHER**

Thursday 11.10.2018

Chair: Dieter Klopfenstein

9:00 – 9:30 **Jörg Enderlein**, Georg-August-University Göttingen, Germany
"Image Scanning Microscopy"

9:30 - 10:00 **Ulrich Parlitz,** Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
"Applications of data analysis and machine learning in life sciences"

10:00 – 10:30 **Andreas Neef,** Max Planck Institute for Dynamics and Self-Organization Göttingen, Germany

10:30 - 11:10 

11:10 - 11:30 **Iryna Kishko,** J. Heyrovsky Institute of Physical Chemistry of the ASCR, Prague, Czech Republic
"Polarity and mobility of the enzyme active cavity as potential factors affecting the enzymatic activity – time dependent fluorescence shift study"

11:30 - 11:50 **Tao Chen,** Georg-August-University Göttingen, Germany
"Single Molecule Study Reveals the Kinetics and Dynamics of Single Nanoparticle"

12:00 - 14:00 LUNCH

18:00 - … BE-OPTICAL Be-Optical Project PIs meeting

Friday 12.10.2018, Be-Optical Session

Chair: Cristina Masoller

BE-OPTICAL

9:00 – 9:15 **Soheil Mojiri,** Georg August University Göttingen, Germany
„Multi-plane Phase Contrast (MPC) imaging“

9:15 – 9:30 **Shun Qin,** Georg-August-University Göttingen, Germany
“The Image Reconstruction of Spinning Disk Confocal-Image Scanning Microscopy**”**

9:30 – 9:45 **Tommaso Alterini,** Universitat Politècnica de Catalunya, Terrassa, Spain
“One-shot hyperspectral fundus camera with visible and infrared image sensors**”**

9:45 – 10:00 **Donatus Halpaap,** Universitat Politècnica de Catalunya, Barcelona, Spain
„Studying interference patterns of laser diode light under different pump current and feedback conditions for speckle reduction in double pass imaging“

10:00 – 10:15 **Raúl Quiñonez,** Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
“Defibrillating the heart with light”

10:15 – 10:30 **Antu Gortari,** Centre national de la recherche scientifique (CNRS), Paris, France
“Metasurface-based total internal reflection bioimaging”

10:30 – 11: 00 

11:00 – 11:15 **Vineesh Kappadan,** Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
“Motion artifact compensation in optical mapping studies with motion by combining marker-free tracking and ratiometric imaging”

11:15 – 11:30 **Adrià Escobet Montalbàn,** SUPA, School of Physics and Astronomy, University of St Andrews, UK
“TRAFIX: Wide-Field Multiphoton Imaging Through Scattering Media”

11:30 – 11:45 **Alfonso Jiménez Villar,** Nicolaus Copernicus University, Toruń, Poland
“Active Optical Components in Scanning Laser Ophthalmoscope to Visualize Ocular Structures”

11:45 – 12:00 **Ana Rodríguez Aramendía,** Institut de Microcirurgia Ocular (IMO), Barcelona, Spain
“Anterior and posterior segment SS-OCT system integrated into an instrument for autonomous evaluation of the visual function”

12:00 – 12:15 **Mariano Gonzalez Pisfil,** PicoQuant GmbH, Berlin, Humboldt-Universität zu Berlin, Berlin, Germany
“Multy-Species Diffusion Studies in Membranes Utilizing Scanning FCS and Super-Resolution Microscopy”

12:15 – 12:30 **Pablo Amil Marletti,** Universitat Politècnica de Catalunya, Barcelona, Spain
“Novel network-based methods for retinal fundus image analysis and classification**”**

14:00 – 16:00 **Jose Javier Ramasco**, Instituto de Fisica Interdisciplinar y Sistemas Complejos, IFISC Mallorca, Spain
"Scientific presentation and visualization"

Abstracts

(Listed alphabetically by last name)

One-shot hyperspectral fundus camera with visible and infrared image sensors

Tommaso Alterini, Fernando Díaz-Doutón, Meritxell Vilaseca
Centre for Sensors, Instruments and Systems Development (CD6), Universitat Politècnica de Catalunya, Terrassa, Spain.

In the last years, several attempts have been made in order to use hyperspectral technology for imaging the eye fundus. Hyperspectral imaging systems use more than three spectral bands allowing spectral information of the retina to be obtained that otherwise might remain hidden, i. e., using color conventional fundus photography.

The currently existing hyperspectral retinographs have limited spectral sensitivity in the visible range of the spectrum (from 400 nm and 700 nm) since they commonly employ digital CCD or CMOS cameras together with liquid crystal tunable filters or LED-based illumination systems. In both cases, only few spectral bands are available since spectral images are acquired sequentially and this, together with the fact that the eye moves very fast (especially because of microsaccades that are a kind of fixational eye movements that typically occur during prolonged visual fixation) make the wavelength scanning process linked to hyperspectral fundus cameras too slow.

In order to overcome these limitations, in this study we present a new practical hyperspectral fundus setup that permits spectral images to be acquired with one single shot exposure. Furthermore, the system includes a CMOS camera (Orca Flash 4.0, Hamamatsu, Japan, 2048x2048 pixels, 16 bits) and an InGaAs one (C12741-03, Hamamatsu, Japan, 640x512 pixels, 12 bits) with sensitivity from 400 nm to 1000 nm and from 950 nm to 1700 nm, respectively. This allows spectral analysis of biological tissue from 400 nm to 1200 nm, that is, over all the entire retinal investigable optical window if the absorption of the ocular media in front of the retina is taken into account (cornea, lens, aqueous humor and vitreous). In particular, the system sequentially illuminates the retina with 13 clusters of LEDs with the following peak wavelengths: 420 nm, 460 nm, 490 nm, 535 nm, 50 nm, 620 nm, 670 n, 740 nm, 850 nm, 940 nm, 1050 nm, 1140 nm, and 1200 nm. By means of electronic synchronization between cameras and LEDs, which allows reducing dead time between spectral frames avoiding light contamination from other LEDs, precise and fast illumination and acquisition are granted. The acquisition of all wavelengths lasts 522 ms in total: 242 ms for the wavelengths comprised between 420 nm to 940 nm acquired with the CMOS, and 280 ms for those from 1050 nm to 1200 nm acquired with the InGaAs. Moreover, a custom-made acquisition software (MATLAB, MathWorks, Inc., United States) permits to easily switch between the fixation target view, which permits the stable alignment of the eye, and the entire spectral acquisition.

The performance of the system was firstly evaluated on an artificial eye (OEMI-7, Ocular Instruments, USA). Besides a complete optical characterization, tests of repeatability and safety were carried out following ISO standards. Finally, the system was tested on some volunteer's eyes.

Optimal viewing of superficial retinal structures was achieved with short wavelengths, while images in the infrared range, especially those beyond 950 nm, allowed obtaining information from deeper layers of the choroid thanks to the lower absorption of melanin and water. The developed system seems to be useful for differentiating arteries and veins due to the differences in the hemoglobin concentration and thus, absorption. The system is now being tested as a means of improving diagnosis of ocular diseases.

Novel network-based methods for retinal fundus image analysis and classification

Pablo Amil, Fabián Reyes-Manzano, Lev Guzmán-Vargas, Irene Sendiña, and Cristina Masoller

Retinal fundus imaging is a non-invasive method that allows visualizing the structure of the blood vessels in the retina, the characteristics of these structures can show the presence of diseases such as diabetic retinopathy (DR) and glaucoma. Here we present a novel method to analyze and quantify changes in the retinal blood vessel structure in patients diagnosed with glaucoma or with DR. First, we extract a tree-like graph from the blood vessel structure using segmentation and filtering techniques. Then, we apply the concept of node-distance distribution (NDD) from the center node (that represents the optical disc) to quantify graphs' structural differences between the groups of healthy patients and non-healthy patients. We also use fractal analysis to characterize the extracted graph. Applying these techniques to three image databases we find some significant differences between the healthy and non-healthy groups (p-values lower than 0.005 or 0.001 depending on the method and on the database). However, the results are very sensitive to the segmentation and to the resolution of the image database.

Bioorthogonal labeling with tetrazine-dyes for live cell labeling and super-resolution microscopy

Gerti Beliu, Andreas Kurz, Lisa Behringer-Pliess, Markus Sauer

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The expansion of the genetic code (GCE) via incorporation of non-canonical amino acids (ncAAs) into proteins of mammalian cells or whole organisms enables site-specific and tag-free labeling. The combination of biorthogonal click chemistry with small, bright and photostable organic tetrazine-functionalized fluorophores substantially advances biological imaging especially in super-resolution microscopy.

Since most organic dyes are commercially available as tetrazine-derivatives this method can be easily applied for efficient labeling of mammalian cells. Furthermore, many tetrazine-dyes show fluorogenic behaviour, i.e. the fluorescence intensity increases considerably upon reaction with a strained dienophile, e.g. TCO, which makes the method in particular interesting for wash-free live-cell labeling. We studied the quenching mechanisms and identified an electron transfer reaction from the electron donor tetrazine to the dye's excited singlet state as underlying fluorescence quenching mechanism. Using steady-state and time-resolved fluorescence spectroscopy as well as fluorescence correlation spectroscopy (FCS) we demonstrate that tetrazine forms ground- and non-fluorescent excited state complexes with fluorescent dyes belonging to the class of rhodamine and oxazine dyes such as ATTO 488, and ATTO 655, respectively, besides dynamic collisional quenching.

To test the applicability of the dyes for super-resolution imaging, we labeled actin with TCO-functionalized phalloidin and imaged the cells by laser-scanning microscopy (LSM), re-scan confocal microscopy (RCM), structured illumination microscopy (SIM) and single-molecule localization microscopy by direct stochastic optical reconstruction microscopy (dSTORM). Furthermore, we show how GCE with ncAAs and tetrazine-dyes can be successfully applied for visualization of membrane receptors by dSTORM.

Actin-remodeling properties of Drebrin in vitro and in vivo

Eugenia Butkevich, Arindam Ghosh, Christina Jayachandran, Christoph F. Schmidt

Drittes Physikalisches Institut, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Actin filament organization in striated muscle cells is crucial for the sarcomere function. Here, we identified drebrin as a sarcomere component localizing in the actin/myosin overlap region. A native gel electrophoresis revealed that in human cardiomyocytes drebrin occurs as monomer as well as dimer and oligomer containing 3-5 molecules. Recombinantly expressed drebrin monomers, however, readily aggregate into polymers. In *in vitro* experiments, excess of drebrin added to solution of actin filaments, resulted in a network structure composed of long curved bundles. Overexpression of drebrin in cardiomyocytes led to displacement of □-actinin and tropomyosin and disorganization of the sarcomere lattice into non-striated filamentous bundles. In contrast, down-regulation of drebrin using shRNA did not cause significant changes in sarcomere architecture. Our results indicate that within the sarcomere, drebrin plays regulatory, rather than structural role, controlling the assembly of actin filaments and access of other actin-binding proteins required for efficient actin-myosin interaction.

Single Molecule Study Reveals the Kinetics and Dynamics of Single Nanoparticle

Tao Chen

III. Institute of Physics, University of Goettingen, Friedrich-Hund-Platz 1, 37077 Göttingen

Nanoscale particles play the most important role in energy catalytic process. They can be of diverse material compositions, such as metals, oxides, and sulfides, and they catalyze a wide range of transformations including oxidation, reduction, (de)hydrogenation, carbon–carbon or carbon–heteroatom bond coupling and cleavage reactions.[1] Their catalytic versatility makes them widely applicable in petroleum processing, photochemistry, fine chemical synthesis and fuel cell. It is thus important to characterize the catalytic behavior of nanoparticles, as it's meaningful for rationally guiding the catalyst's design, improving the reaction condition and industrial application.

The performance of a catalytic particle is determined by its chemical composition, the surface structures (size, shape and porosity) and the accessibility. In order to better predict and understand the influence of these parameters on the catalytic performance, a board range of physicochemical characterization techniques has been developed. Among these techniques, microscopic techniques that combine an excellent spatial and temporal resolution have been attracting extensive attention. Until now, the application of single molecule fluorescence microscopy (SMFM) and super resolution techniques/microscopy (SRM) has achieved great success and obtained a lot of new information which hardly get from average-ensemble experiment.[2] Here, we apply SMFM and SRM to study the catalysis of single nanoparticles, that is, study the catalytic behavior (kinetics and dynamic) of single nanoparticles by SMFM and SRM:

(1) Catalytic kinetic and dynamic of single Pd nanoparticles: By characterized the catalytic rate of a series of well-defined Pd nanocubes with different sizes (5 ~ 23 nm) and Pd nanooctahedrons with SMFM, we revealed the size effect of Pd nanocube on catalytic kinetic and dynamics. Then the catalytic kinetics and dynamics of the different types of surface atoms (plane and edge) were revealed by a statistical quantitative deconvolution of observables obtained from traditional single-molecule nanocatalysis of Pd nanocrystals. Lastly, we comprehensively study the shape effect on the catalytic kinetics and dynamics of Pd nanoparticles.

(2) Surface diffusion mechanism of product on Pd nanosheet: Based on the analysis of the size-dependent product formation and dissociation processes on different types of surface atoms at single-particle level, we predicted that the product molecules formed on edge sites tend to translocate to plane sites for further dissociation. The prediction was confirmed by direct observing the surface translocation or diffusion of individual product molecules formed on different types of surface atoms on single Pd nanosheets through super-resolution imaging.

(3) Catalytic activation energy of single Au nanoparticles: By monitoring the temperature-dependent catalytic activity of single Au nanocatalysts for a fluorogenic reaction, we derive the activation energies via multiple methods for two sequential catalytic steps (product formation and

dissociation) on single nanocatalysts. The wide distributions of activation energies across multiple individual nanocatalysts indicate a huge static heterogeneity among the individual nanocatalysts. The compensation effect and isokinetic relationship of catalytic reactions are observed at single particle level.

1. Schlögl, R., Heterogeneous Catalysis. *Angew. Chem. Int. Ed.* **2015,** *54* (11), 3465-3520.
2. Chen, T.; Dong, B.; Chen, K.; Zhao, F.; Cheng, X.; Ma, C.; Lee, S.; Zhang, P.; Kang, S. H.; Ha, J. W.; Xu, W.; Fang, N., Optical Super-Resolution Imaging of Surface Reactions. *Chem. Rev.* **2017,** *117* (11), 7510-7537.

Elucidating the Endosomal Machinery with Correlative Multi-Color Single-Molecule Localization-Microscopy & Electron Tomography

Christian Franke[1], Urska Repnik[1], Sandra Segeletz[1], Nicolas Brouilly[2], Yannis Kalaidzidis[1,3], Jean-Marc Verbavatz[4], Marino Zerial[1]

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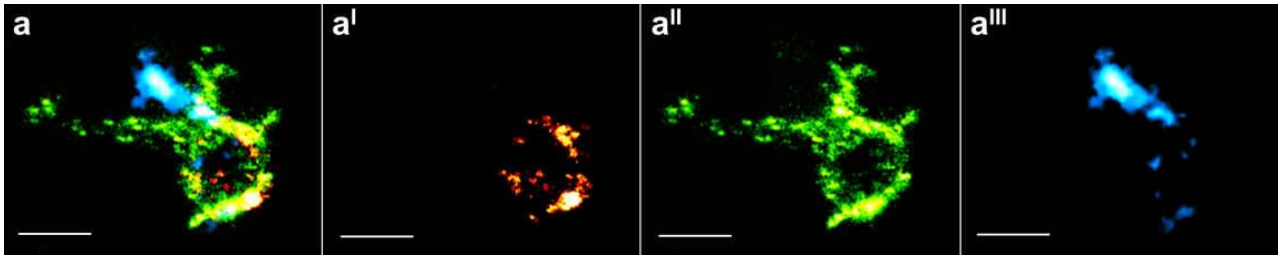
[2]Electron Microscopy Facility at the Developmental Biology Institute of Marseille, Aix-Marseille University, Marseille, France.
[3]Faculty of Bioengineering and Bioinformatics, Moscow State University, Moscow, Russia.
[4]Institut Jacques Monod, CNRS, Université Paris-Diderot, Sorbonne Paris Cité, 75013 Paris, France.

Single-Molecule Localization-Microscopy (SMLM) has emerged as a powerful and versatile tool to study cellular organelles with near-molecular resolution. Multi-color SMLM combines the broad range of conventional fluorescent markers with superior spatial resolution. Although several dye pairs have been deployed succesfully in recent years, the vast majority of approaches rely on cell permeabilization, e.g. ‘‘immonu-labelling’’, and therefor destroying the cellular ultrastructure.
Since many membrane-bound organelles are compartmentalized into functional sub-domains it is often impossible to assess whether two particular structures are part of the same organelle from SMLM signal alone. We therefor aimed to perform super-resolution correlative light and electron microscopy (CLEM).
So far, super-resolution CLEM approaches have been mostly limited to single fluorophores, either a fluorescent protein or an organic dye and there has been no report of super-resolution CLEM allowing the simultaneous visualization of multiple labelled molecules of interest in cells by SMLM.
Here, we report a three-color correlative single-molecule localization-microscopy & electron tomography workflow that utilizes the combination of the fluorescent protein Dronpa (PALM) and commonly used organic dyes (*d*STORM), with electron tomography yielding high-resolution 3D ultra-structur overviews, on semi-thin *tokuyasu* – cryosections. We applied this three-color correlative workflow to examine the sub-compartmental distribution of Transferrin (Tfn), epidermal growth factor (EGF) and the small GTPase Rab5 on early endosomes.
We show for the first time, that Rab5c, labelled with Dronpa at near-endogenous levels (HeLa BAC), is organized in few distinct nano-domains on the endosomal membrane, mostly exclusive to the directly labelled EGF. Our data illustrates different stages of early endocytosis and cargo sorting, as we resolve heterotypic fusion of 50 – 100 nm small EGF-positive vesicles with early endosomes, nano-domains of EGF on the endosomal membrane and internalized EGF as bulk and intra-luminar vesicles. We further show that directly labelled Tfn is both present on the bulk of early endosomes and forms diverse tubular structures of < 100 nm up to micrometer size.

Besides the potential to investigate the sub-domain organization in other membrane compartments, like the Golgi apparatus or ER, expanding the current correlative multi-color SMLM & electron tomography workflow with e.g. Tag-Fusion-Proteins in Knock-In systems will open up an even broader range of addressable endosomal proteins in the future.



Three-color SMLM on semi-thin *tokuyasu* – cryosections of an early endosome. (a) Aligned image of three endogenous-style labelled endosomal proteins, revealing the intricate organization in nano- domains and tubules. (a^{I}) Directly labelled EGF (AlexaFluor647) is organized in nano-domains on the endosomal membrane and intra-luminar vesicles & reveals heterotypic fusion events of 50 – 100 nm small EGF vesicles to the membrane. (a^{II}) Directly labelled Tfn (AlexaFluor568) forms the globular part of the endosome, featuring various tubular structures. (a^{III}) Rab5c-Dronpa is organized in nano-domains on the endosomal membrane.

Heat-treatment Controlled Blue Light Emission in Ternary Glass-ceramic Waveguides

Subhabrata Ghosh

Georg-August-University Göttingen, Germany

Here we present 1 mol% Eu-doped 70 SiO_2-23 HfO_2-7 ZnO (mol%) glass-ceramic waveguides as a function of heat-treatments for on-chip blue-light emitting source applications in optoelectronics as well as integrated optics. The structural evolution of ZnO/HfO_2 nanocrystals and its effect on Eu^{2+}/Eu^{3+} emission as a function of heat-treatments has been demonstrated. It is observed that HfO_2 evolves as both spherical and rod-like nanocrystals whereas ZnO evolves as spherical nanocrystals with higher heat-treatment temperatures. This nanocrystalline environment is found to play a significant role to enhance the photoluminescence (PL) of Eu-ions within the ternary glass-ceramic matrix in blue region of the electromagnetic spectrum. The reduction of Eu^{3+} ions to Eu^{2+} ions within the glass-ceramic matrix takes place in the highly crystalline environment with heat-treatment temperatures and hence the blue-light emission characteristics are attributed to the $4f^6 5d \rightarrow 4f^7$ energy level transition of Eu^{2+} ions. Therefore, 1 mol% Eu-doped 70 SiO_2-23 HfO_2-7 ZnO (mol%) glass-ceramic waveguide can be considered as a promising functional optical material for on-chip blue-light emitting source applications.

Metasurface-based total internal reflection bioimaging

Antu Gortari

Centre national de la recherche scientifique (CNRS), Paris, France

Research into artificial 2D materials with the ability to abruptly change light properties on subwavelength distances, also known as electromagnetic metasurfaces have been -in recent years- gaining more and more traction as both nanofabrication technologies and applications push them into visible wavelengths. These advancements, out of reach until very recently due to technical limitations, have opened a whole new range of possibilities to reshape reflected and transmitted light out of ultra-thin optical devices. Yet, there is one field of application that is just now starting to gather attention: bioimaging techniques.

One of these techniques, particularly well-suited for the study of the localization and dynamics of molecules and events near a cell plasma membrane, is Total Internal Reflection Fluorescence (TIRF) microscopy. This technique relies on the generation of a evanescence field, typically on a glass-water interface, with a light beam being totally internally reflected within the glass substrate due to its high incidence angle. This field, around 100nm in thickness, is the one responsible for the uniquely confined fluorophore excitation that TIRF provides, improving image contrast while reducing photodamage and photobleaching.

Despite being first conceived as a microscopy technique more than 50 years ago, TIRF microscopy could only cement its unique place as high numerical aperture objectives capable of outputting those high incidence angles started to become available. While these objectives managed to offer an easier implementation than previous prism-based alternatives, they also came along with a new set of limitations, namely and mainly the need for an immersion media (oil), large and bulky objectives, small field of view and elevated costs. Although high horizontal resolution at the expense of field of view, experimental complexity and costs is a trade-off that is sometimes acceptable, this is not always the case.

Here we introduce the realization of metasurface-based TIRF microscopy substrates consisting of periodic arrays of asymmetric grooves fabricated in titanium dioxide on borosilicate glass substrates. These grooves, of 48nm to 212nm in width, were optimized through rigorous coupled-wave analysis (RCWA) to couple up to 89% of the incoming normally incident light into the first diffraction order, which outputs at an angle that suffices total internal reflection for glass-air and glass-water interfaces. This eliminates the requirement for high NA objectives or prisms to achieve the evanescence field on the sample's surface, as well as the need for physical contact of the optical system with the sample. Our optical analysis show that we have an intensity ratio as high as 5:1 between the ±1 orders, and 5:2 between the 1st and the main 0th order, meaning that most of the light is being redirected asymmetrically and propagates throughout the length of the glass substrate.

By having both the ability to utilize lower-magnification air objectives and a large evanescence field surface, this approach provides unique TIRF conditions not accessible by traditional methods. Additionally, while these structures are currently fabricated by e-beam lithography, their simplicity makes them compatible with soft UV nanoimprint, suitable for cost-effective scale production, which could bring TIRF capabilities to inexpensive wide-field microscopes.

Studying interference patterns of laser diode light under different pump current and feedback conditions for speckle reduction in double pass imaging

Donatus Halpaap
Universitat Politècnica de Catalunya, Barcelona, Spain

The Double pass (DP) imaging technique is a diagnostic method used to obtain an overall estimation of the optical quality of an eye, containing information on scattering as well as higher order aberrations. This is achieved by measuring the point spread function of the eye by recording the image of a point source on the retina, i.e. a collimated light beam enters the eye, passes through the ocular media, is reflected at the retina, passes the ocular media again in reverse direction and is recorded after exiting the pupil.
We study speckle formation in DP images with the goal of reducing speckle in a cost-effective way. To achieve this, we use different semiconductor light sources, e.g. a semiconductor laser diode which is subject to different amounts of optical feedback and pump current modulation, or a superluminescent light emitting diode, based on amplified spontaneous emission, all aiming at reducing the coherence length of the sources.
Here, we study the effect of the number of the emitted modes in dependence of the pump current on the contrast of the light's interference pattern. We also check how optical feedback and current modulation changes the spectral properties of a laser diode and to which degree speckle contrast can be reduced by using that light. We plan to apply the results from this study to DP imaging.
Furthermore, we study the influence of camera settings (gain, black level, exposure time) on the speckle contrast measured in DP images, and compare the results of a simple model with real measurements.

Methionine induces malleability in the N-terminal domain of a spider silk protein

Julia C. Heiby[1] and Hannes Neuweiler[1]

[1]Department of Biotechnology & Biophysics, Julius-Maximilians-University Würzburg, Germany

Spidroin N-terminal domains assemble upon pH change from 7 to 6 in a spider's spinning duct via an elusive mechanism involving electrostatically assisted association and conformational change. The domain has an unusually high number of methionine residues. Methionine mediates a unique side chain flexibility - a generic phenomenon proposed by Samuel Gellman (1).
We hypothesized that methionine may play a role in the dynamics of the dimerization process. To investigate this question we replaced by site directed mutagenesis all core methionines of an NTD protein with leucine, a structurally similar amino acid, which confers less flexibility (1).
The mutant protein expressed surprisingly well and showed a strong increase of stability: the denaturation midpoint temperature increased by 20 K. At the same time the association of the dimer was weakened to a low µM range, in comparison to a low nM range for the wild type protein, i.e. function was impaired. Our findings suggest that core Methionine have a major role in conferring malleability in the spidroin NTD. Evolution probably selected proteins with increased dynamics and structural plasticity required for function at the expense of higher stability (2).

References

(1) Gellman et al.. On the Role of Methionine Residues in the Sequence-Independent Recognition of Non-Polar Protein Surfaces. Biochemistry. 1991;
(2) Heiby JC et al.. manuscript in preparation. 2018;

Single Molecule Metal-Induced Energy Transfer for Axial Localization

Sebastian Isbaner, Roman Tsukanov, Narain Karedla,
Arindam Ghosh, Ingo Gregor, and Jörg Enderlein

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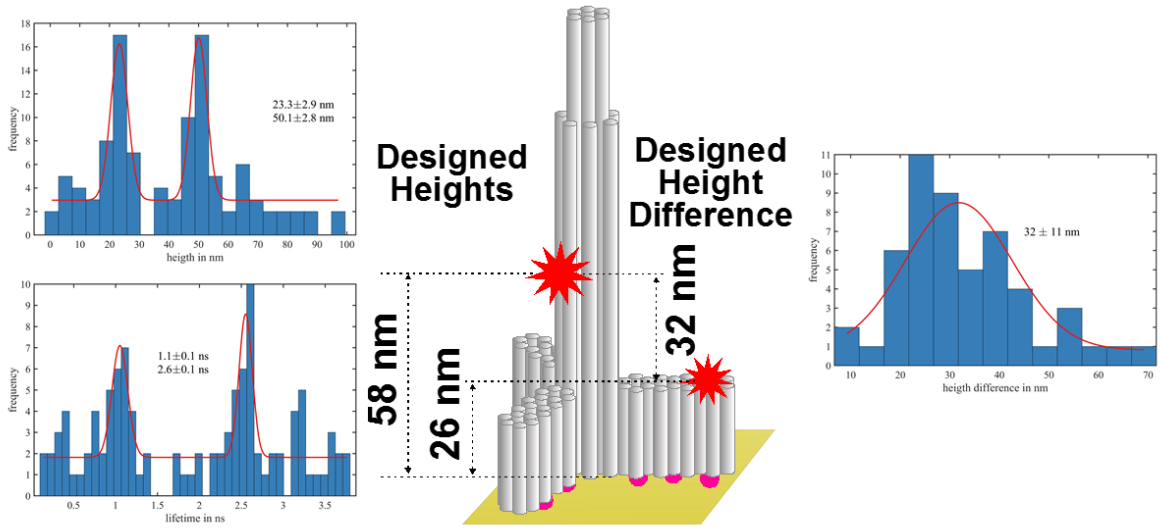

Figure 1: Axial co-localization of two emitters on a DNA origami structure. The origami structure is immobilized to a thin gold film and labeled with fluorescent molecules at defined heights. The axial position of the dye molecules was estimated with a precision of <5 nm and the distance between the two emitters with 11 nm precision.

Superresolution microscopy today is able to resolve structures on the order of a few nanometers, far below the optical diffraction limit. In particular, single-molecule localization methods are used routinely to resolve intricate biomolecular structures by making use of sparse turn-on events of fluorescent molecules. However, most of these techniques are limited to the sample plane and offer no or limited resolution along the axial direction. Here, we present a

new method to localize several emitters along the optical axis with nanometer precision. The core principle behind this is the distance dependent fluorescence quenching of an emitter close to a metal surface, which we term Metal-Induced Energy Transfer (MIET). The measured fluorescence lifetime can be converted into the molecule's distance from the surface using a theoretical model [1]. We apply this method to co-localize multiple emitters on a DNA origami structure labeled with dyes at known heights using step-wise bleaching [2]. Alternatively, DNA PAINT allows to collect an in principle unlimited amount of photons from a single origami structure. The combination with existing lateral localization methods would allow an isotropic nanometer localization accuracy.

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3-dimensional chemotaxis measurement of Dictyostellium Discoideum using multiplane deconvolution imaging setup

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We have already investigated the chemotactic behavior and signal pathway of Dictyostellium Discoideum (D. d.) using a fast multi-plane 3D Imaging setup. Chemotactic behavior of this cell has been studied for several years. The migration event of D. d. is observed when they are starved. They secrete cyclic adenosine monophosphate (cAMP) molecules, and the cheomoattractants make the cells gather in one position. 10,000~100,000 neighboring cells develop into a mature fruiting body containing spores that germinate into amoebae when food becomes available again. Most of our understanding of the general chemotactic mechanism is based on polymerization and depolymerization process of actin filaments, and the actin molecules are generally distributed and connected on cell membrane surface. However, sometimes the molecules make a big flow in the cells and the role of the flow is not clear yet. In this study, we studied this phenomenon using fast wide-field imaging of eight distinguished planes of sample simultaneously. This technique has been developed and reported several years ago. For this experiment, we imaged 3-dimensional movement of the GFP labeled actin molecules. Fluorescently labeled actin binding molecule (Lim-E-mGFP) were used for this measurement. To confine single cells in limited area, microfluidic channel system was also applied in this study. For the final analysis of the movies, we applied deconvolution algorithm for 3-dimensional condition. The results of this work shed more light in finding the role of actin molecules and their flow in chemotaxis of dictyostellium.

Motion artifact compensation in optical mapping studies with motion by combining marker-free tracking and ratiometric imaging

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Fluorescence imaging or optical mapping provides highly detailed visualizations of cardiac electrophysiology in isolated, intact hearts. Recent developments in optical mapping have opened the path for being able to perform imaging with beating and moving hearts. Here, we show that marker-free motion tracking and ratiometric imaging can be combined effectively to reduce motion artifacts when filming a beating Langendorff-perfused isolated rabbit heart. We also show that marker-free motion tracking with simultaneous imaging of action potential and calcium transient waves provides a novel tool for investigating the electromechanical dynamics of the heart. We find that combining motion tracking and ratiometry can significantly enhance motion artifact reduction and allows the comparison and cross-validation of the two techniques with respect to each other.

MT-MKLp2 cross-linked network studies

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The kinesin 6 MKLP2 motor is N-terminal Kinesin, with unique features. It plays critical roles in cell division. Scientists know little about MKLP2; however, few earlier findings suggested that MKlp2 is a good candidate for new cancer therapies. To gain insight into the motor regulation and mechanism, we are studying truncated MKLP2. Here, I will present the studies of a dimeric truncated MKlp2 (55-665), *in vitro*. Our data confirm that the dimeric truncated MKlp2 motors are active and they display strong bundling activity. An astonishing finding, we observed the formation of novel three-dimensional microtubule-MKLP2 construct cross-linked network with unique properties.

Polarity and mobility of the enzyme active cavity as potential factors affecting the enzymatic activity – time dependent fluorescence shift study

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Enzymes are effective biological catalysts with exceptional specificity and selectivity. Enzymatic reactions take place in the active sites, often hidden inside the protein core. The active sites are connected to the bulk solvent through one or more transport tunnels. Beyond the complementarity between the substrate and the binding site, tunnels offer another level of resolution for the molecules entering and leaving the enzyme active cavity. The tunnels discriminate preferred substrates or cofactors, reduce the access of solvents which may disturb chemical reactions, or prevent the escape of reactive intermediates, potentially harmful to the cells [1]. The hydration and mobility of these access pathways towards the active sites are believed to profoundly affect their function [2]. However, only a few approaches for monitoring these characteristics within the relevant protein regions are available. Here we apply a fluorescence method for the site-specific analysis of the extent of hydration in enzyme Haloalkane Dehalogenase. This approach is based on recording „time dependent fluorescence shift“ (TDFS) [3] placing the dye in the various positions along the tunnel of this enzyme [4,5]. The hydration monitored within the biologically relevant regions of the dehalogenase enzymes is then compared with their enzyme kinetics of various mutants, which can bring the deeper insight into the functioning of these enzymes.

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Mapping Molecules Quantitatively in Confocal Fluorescence Microscopy

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Single molecule fluorescence microscopy has been established in the Life Sciences as an essential tool to study the characteristics and dynamics of individual fluorescent emitters both *in vitro* as well as *in vivo*. Still, acquiring quantitative information from the confocal observation volume is a challenging task, whereas knowing the absolute number or concentration of proteins in, e.g., cellular structures can significantly improve our understanding of cell biology being an important step towards quantitative microscopy.
In this talk, a new quantitative analytical tool will be presented that is based on recording coincident photons. The approach, *Counting by Photon Statistics* (CoPS), relies on a statistical analysis of detected photon coincidences to estimate the number of independent fluorescent labels in the observation volume [1]. Hereby, CoPS exploits the photon antibunching effect: a single photon emitter can only generate one photon at a time.
Originally developed for point measurements, CoPS recently has been extended to an imaging scheme [2]. Both a molecular brightness and the spatial density of fluorophores per image pixel are determined. By summing over all corresponding pixels, the number of molecules in an object can be calculated.
Using a confocal fluorescence microscopy setup with pulsed excitation, four single-photon detectors and a parallel time-correlated single photon counting electronics (MicroTime 200, PicoQuant) we proof the applicability of the method with artificial model systems (immobilized DNA origami) and present first steps towards biological samples.

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Correlative live- and fixed cell super resolution imaging

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In the past years many imaging techniques have been developed to face various demands regarding the biological question. Imaging speed, reduced phototoxicity and resolution beyond the diffraction limit were the keyplayers driving the development. Unfortunately, so far no technique could combine all these attributes with maximum efficiency.

We therefore set up a microscope, with correlative imaging techniques. The Rescanning Confocal Microscope (RCM) based on the image scanning principle, provides fast (up to 1 frame per second) and super-resolved (170 nm @488nm excitation) imaging [1]. Additionally, we installed 3D-*d*STORM detection capable to resolve structures down to a spatial resolution of ~ 20 nm.

The system is equipped with a microfluidic pump system, that allows fixation of samples on stage, staining and exchange of buffers on the fly. In combination with the elution of already stained labels (madSTORM [2]), there is no limitation concerning number of labels and photophysical properties.

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PALM stoichiometry of plant anion channels

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Anion channels from the SLAC/SLAH family are general master switches of plant stress responses. In addition, in roots a subgroup of channels load the vascular tissue with nitrate and chloride. The activity of the main nitrate and chloride loading anion channel, SLAH3, is controlled by heteromerization with the electrical silent subunits SLAH1 and 4 or alternatively by cytosolic acidification. Although the crystal structure of a bacterial homologue (HiTeha) of plant SLAC/SLAH anion channels is available and suggests a trimeric structure, the stoichiometry and the multimerization level of the plant anion channel counterparts is still scant.

Single-molecule localization microscopy has brought molecular counting within the native environment [3] and as protein stoichiometry is often equated with cellular function we use PALM applications and single molecule counting strategies to analyse the stoichiometric distribution of anion channel complexes. We determine stoichiometric changes upon heteromerization of SLAH3 with SLAH1 and 4 and monitor the multimerization level of SLAH3 upon cytosolic acidification. Our data reveals new insights in the regulation mechanism of plant anion channels.

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Multi-plane Phase Contrast (MPC) imaging

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Phase contrast microscopy is a label-free technique, which exploits phase shifts in white-light traversing through a transparent specimen. The core idea is to convert these phase shifts to changes in intensity in the final image. The technique, capable of resolving sub-cellular organelles in living cells, has found significant applications in cell biology. However, most of the volume reconstruction analysis used by three-dimensional imaging methods compromise temporal resolution and are complex in nature. Here, we propose the combination of phase contrast apparatus in illumination and a customized prism in detection path rendering simultaneous acquisition of eight planes through the specimen depth. Using this approach, we recorded 3D images of living cells (axonemes) up to 200 Hz. Most importantly, the method does not demand any iteration, reconstruction, or post-processing analysis**.**

Key words: multi-plane imaging, phase contrast microscopy.

Brownian Dynamics Simulation of (Semi-)Flexible Polymer Chains

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Brownian Dynamics provides a powerful tool into which hydrodynamics can easily be adopted. We embed a discretized elastic rod into this framework and find ourselves wielding a versatile, generic tool with very few parameters. This allows us to investigate the nano-scale properties of single molecules by translating experimental data into model parameters such as the persistence length of peptides/DNA or the hydrodynamic radius of amino acids/nucleotides. In particular, we have studied the diffusion coefficients (2fFCS) and contact rates (PET-FCS) of short, flexible peptides in solution, as well as the stiffness and reconfiguration time (dynaMIET) of ssDNA bound to a surface in a shear flow.

TRAFIX: WIDE-FIELD MULTIPHOTON IMAGING THROUGH SCATTERING MEDIA

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The field of optical imaging has seen exceptional advances over the last decade. Super-resolution and wide-field low photo-damage imaging are now key tools for biological and medical sciences. Whilst these advances have been startling, the unmet challenge to date in all optical imaging is to penetrate deeper. Here we present TRAFIX as an approach to address this challenge. It uses a judicious combination of temporal focusing [1] with single-pixel detection [2] to obtain wide-field images within or through biological tissue without aberration correction or characterization of the turbid media.

The outstanding ability of temporal focusing beams to propagate in scattering medium with minimal speckle formation [3] is used to project orthonormal light patterns (in a Hadamard basis) onto fluorescent samples located inside or behind a turbid medium. Fluorescent light emitted by the sample is collected in an epi-fluorescence configuration and the total intensity is measured in a single-pixel detection scheme. As there is no need to have any spatial resolution in the imaging system, TRAFIX tolerates 'scrambling' of emitted light achieving remarkable imaging depths. We demonstrate the potential of TRAFIX by imaging a fluorescent micropattern through rat brain slices reaching a maximum imaging depth of 7 scattering mean free path lengths. We prove that TRAFIX achieves higher signal-to-background ratio and lower photo-bleaching than standard point-scanning two-photon microscopy for equivalent laser intensities. Finally, we show that TRAFIX works well under typical biological research conditions by obtaining images of fluorescent beads and human embryonic kidney cells through scattering phantoms and unfixed human colon tissue (Fig. 1).

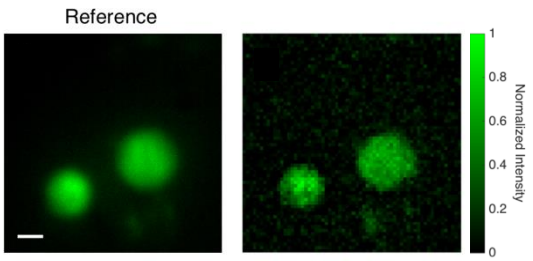

Figure 1: Human embryonic kidney cells imaged through a scattering phantom. (**a**) Reference image. (**b**) Retrieved image through 540 µm of scattering phantom.

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Bioorthogonal click labeling of the NMDA receptor subunit NR1

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N-Methyl-*D*-aspartate receptors (NMDARs) are heterodimer ion channels of two NR1 subunits and two NR2 (NR2A or NR2B) domains. They play an important role in synaptic plasticity, memory function and activation of secondary intracellular signal cascades and are also involved in synaptic autoimmune disorders. We employ super-resolution microscopy, in particular *direct* stochastic optical reconstruction microscopy (*d*STORM), which requires small fluorescent labels. Therefore, a new labeling method, called bioorthogonal click-chemistry by genetic code expansion was applied to achieve a very specific NMDAR-NR1 membrane staining in different cell lines. We generated several mutants incorporating an unnatural amino acid at various extracellular positions in order to covalently attach small organic fluorophores including Cy5-tetrazine. After optimizing mutants in transfected human cell lines with regard to labeling efficiency and receptor functionality, they were tested by fluorescence microscopy and whole-cell patch clamp. The results show that this labeling approach is superior to available immunocytochemistry protocols and show great promise also for live cell experiments.

FLUORESCENT DIARYLETHENES AS PHOTOSWITCHES FOR SUPER-RESOLUTION MICROSCOPY IN MATERIAL SCIENCE

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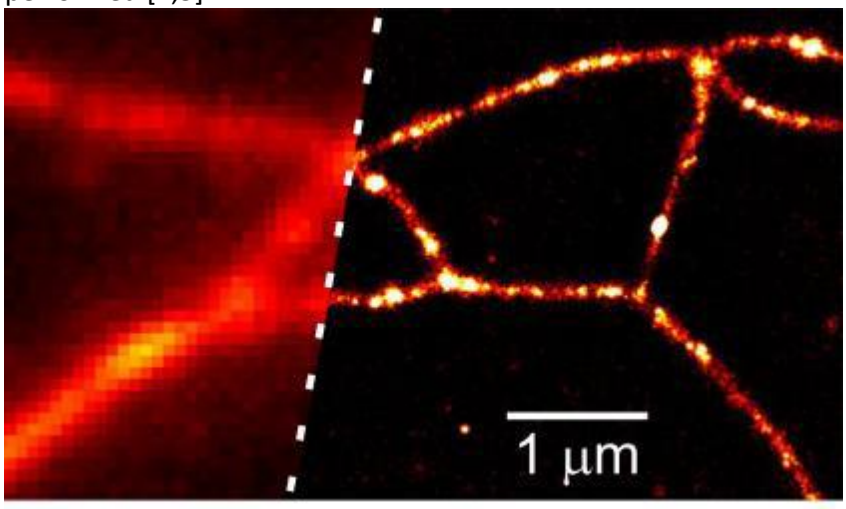
Super-resolution fluorescence microscopy allos for impressive *in situ* imaging of biological structures, but its application in materials science has been rather restricted so far.[1,2] One of the main reasons lies in the fact that there is a lack of powerful dyes that provide suitable labeling and photoswitching properties in materials science systems. Diarylethenes, which can be interconverted between a discrete open- and a closed-ring form can be used as key elements of various light-driven molecular switches.[3] We synthesized different diarylethenes derivatives with high fluorescence quantum yields in their fluorescent closed form and with suitable photokinetics to be readily used for photoactivated localization microscopy (PALM)(see Figure below), superresolution optical fluctuation imaging (SOFI) and reversible saturable optical linear fluorescence transitions (RESOLFT) in polymer systems. Nanoscopic visualization of the self-assembly block copolymer structures was performed.[4,5]



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Probing Lipid Diffusion in Curved Membranes with Fluorescence Microscopy

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Molecular diffusion in biomembranes is central to a number of signaling and trafficking pathways. Conventional fluorescence microscopy and spectroscopy techniques fails to shed light on these dynamic processes, in particular for sub-diffraction limited structures like synaptic vesicles and nanoscopic membrane domains. Additionally, the influence of membrane curvature on viscosity, diffusion speed, or lipid composition becomes dominant at this scale. In this regard, Small Unilamellar Vesicles (SUVs- diameter 30 to 200 nm) serve as an ideal mimicking system in vitro to investigate diffusion-based dynamic processes. We report DynaMIET-FCS by combining Fluorescence Correlation Spectroscopy (FCS) and Metal-Induced Energy Transfer (MIET) (citation) to probe spherical diffusion-based dynamics of a labelled lipid particle in surface-bound SUVs. The underlying physical basis of MIET is that the intensity of a fluorescent molecule becomes increasingly quenched and excited-state lifetime gets reduced when approaching a metal surface, due to electro-dynamic coupling of the excited state of the dipole emitter to surface plasmons on a thin metal film [1]. The core idea here is to use a fluorophore-labelled lipid head group in SUVs and correlate it's intensity fluctuations over time along the axial direction to extract the dynamic timescales involved and diffusion coefficient from the decay curves. In conclusion, DynaMIET-FCS can be successfully applied to address relevant biological questions governed by diffusion based dynamic processes on vesicular structures.

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MULTI-SPECIES DIFFUSION STUDIES IN MEMBRANES UTILIZING SCANNING FCS AND SUPER-RESOLUTION MICROSCOPY

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KEYWORDS: Scanning FCS, STED-FCS, Pattern Matching, Multi-species

Over the last decade, Fluorescence Correlation Spectroscopy (FCS) has been utilized to investigate the dynamics of complex cellular processes. However, this powerful tool has significant drawbacks when observing slower moving molecules such as fluorescently labeled components diffusing in cell membranes. In order to average over a sufficient number of independent events, the optimal measurement time foran FCS measurement has to be increased for slower moving species. This in turn increases the risk of introducing artifacts (e.g., drift, or sample movement) or photobleaching.

Scanning FCS (sFCS) was developed to counteract these issues. By using fast linear or circular scans, the confocal volume is moved with respect to the sample, thus reducing the residence times of the fluorophores. In this scenario, photobleaching is decreased while increasing the statistical accuracy at the same time. An added advantage of the scanning process is the ability to determine the observation volume without prior calibration.

As we use the confocal time-resolved fluorescence microscope MicroTime 200 STED equipped with a FLIMbee galvo scanner, we also have access to the fluorescence lifetime information. In our case, multi species STED measurements are performed with a single STED laser and labels featuring similar emission wavelengths. The different labels can be discriminated by applying a unique pattern matching analysis method [1].

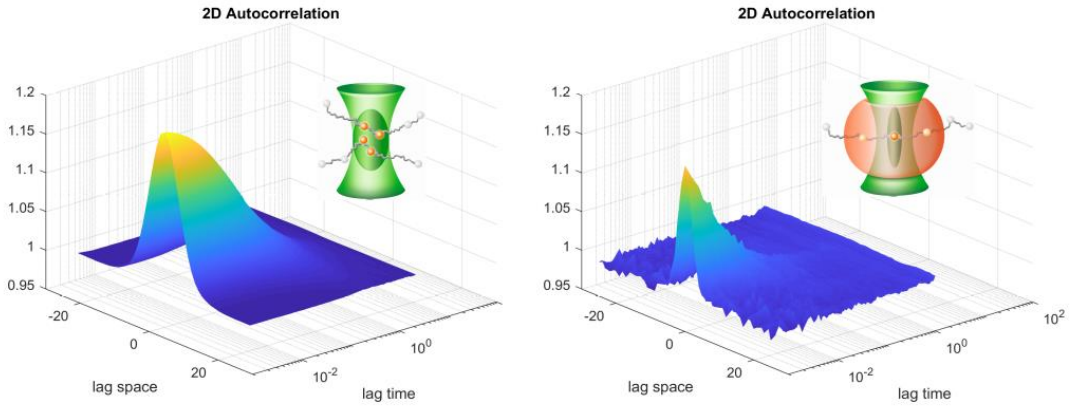

Figure 1. 2D temporal and spatial autocorrelations of Confocal and STED scanning FCS measurements of lipid-dyes in Supported Lipids Bilayers (SLB). In collaboration with S. Isbaner, N. Karedla, and J.Enderlein (Drittes Physikalisches Institut, University of Goettingen).

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The Image Reconstruction of Spinning Disk Confocal-Image Scanning Microscopy

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Spinning Disk Confocal-Image Scanning Microscopy (SDC-ISM) has been demonstrated to be a novel technique to achieve super-resolution image by SDC set up with a pulsing control device and acquisition software. The image reconstruction of ISM image is theoretically easy to implement, which just requires to localize each imaged confocal light spot and then copy and realign them to up-sampled pixel-array (to shrink the light spot) and finally take the sum of all frame reconstructed. The key here would be detecting and localizing all the measured confocal light spots accurately and efficiently.

Therefore, it is necessary to research a detecting and localization algorithm for developing a platform-independent easily-to-use ISM image reconstruction software. Assuming that the measured confocal illumination is Gaussian distributed, so as the measured fluorescence light spot, the image correlation algorithm with a template of an estimated 2D Gaussian function is applied to detect all light spots. Based on 2D Gaussian fitting model, the Gauss-Newton method is used to fit the measured data to a 2D Gaussian function, which give the exact position of each light spot. The experimental results show that except those incomplete light spots around the edge of image all light spots can be detected and the accuracy of localization is good enough to avoid artifact and the efficiency can satisfy the application in volume ISM raw image data (>1GB) processing without introducing multi-thread technology or GPU.

Anterior and posterior segment SS-OCT system integrated into an instrument for autonomous evaluation of the visual function.

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Early detection and objective assessment of ocular dysfunctions and pathologies impact greatly the success of the treatments. An instrument that performs an automatized binocular examination of the patient's visual function while he/she explores a virtual reality environment has been developed by our group in previous works. It reduces the tediousness and subjectivity introduced by both practitioner and patient in standard optometric exams, providing a complete diagnostic report of the patient's visual function that can be used as a base for subsequent clinical decisions. In this work we present a whole-eye imaging module that can be integrated in the abovementioned instrument, improving its ophthalmic capabilities by providing combined morphological and functional clinical information.
The designed optical module consists of a compact swept-source optical coherence tomography (SS-OCT) system that features anterior segment and posterior segment imaging modalities, an eye-tracker and a display system for the generation of real 3D stimuli. The illumination source (Axsun Technologies) is a short-cavity tunable source centered at 1050 nm, 100 nm wide spectrum, 16 mm coherence length and 50 kHz tuning rate. The patient interface consists of a dual path dual-focus configuration which allows for fast switching from anterior to posterior segment modalities. The system achieves an axial resolution of 8 µm in air and an imaging depth range of 16 mm, allowing for whole anterior segment imaging. In this later modality a region of 12x12 mm is scanned with a lateral resolution of about 40 µm while in the retinal modality an area of 6x6 mm is scanned with around 10 µm lateral resolution. Calibration of the system and preliminary results are presented.

Leaflet-dependent diffusion in lipid bilayers using Metal-Induced Energy Transfer Fluorescence Lifetime Correlation Spectroscopy (MIET-FLCS)

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Understanding membrane diffusion provides a deeper insight into transport mechanisms and the functioning of proteins in several cellular processes. In this work, we determine the diffusion of labeled lipids in lipid bilayers in a leaflet-dependent manner. We use the recently established single-molecule Metal Induced Energy Transfer (smMIET)[1] technique together with Line-Scan Fluorescence Lifetime Correlation Spectroscopy (LS-FLCS). In smMIET, the excited-state lifetime of a fluorescent molecule varies monotonically with its distance from a metal surface. This is due to the strong distance-dependent energy transfer from the dye to the surface plasmons of the metal. Here, we use Indium-Tin Oxide (ITO) as a substrate. Due to the steep variation of the fluorescence lifetime with distance from the ITO substrate, we observe a bi-exponential decay of fluorescence from the labeled lipids diffusing in the excitation focus. We estimated the thickness of the bilayer, which is on the order of 5 nm. Using LS-FLCS, we get the temporal- and spatial- autocorrelation functions of the lipids diffusing in the two leaflets. We fit the spatio-temporal autocorrelation functions using a 2D diffusion-sticking model. We found significant sticking of the bottom leaflet to the substrate. The sticking of the top leaflet is indicative of the strong interleaflet coupling in DOPC bilayers. We also observe two distinct sets of population for the top and bottom leaflet of the bilayer by Single Particle Tracking[2]. This method will have huge potential in understanding transport mechanisms in membranes.

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Polymer brushes in motion – measuring flow with nanometre resolution

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Polymer brushes are coatings with surface-tethered polymers and widely used to tailor surface properties including wetting behaviour, friction, and interactions with biomolecules. Brushes change the hydrodynamic properties of a surface in a complex fashion. Experiments on capillaries showed an unexpected large flow reduction by the brush, while recent simulations predict a backflow within the brush layer.[1]
We investigate the interaction of a polymer brush with shear flow and characterise the nanoscale movement of the polymer chains. For this, we fabricate microfluidic channels containing a fluorescently labelled polymer brush on a gold surface. To follow its movement, we utilise Metal-Induced Energy Transfer (MIET):[2] MIET causes a modulation of fluorescence lifetime and brightness in proximity to the gold surface. This enables us to obtain the surface distance (height) of a dye at the chain end by measuring its fluorescence lifetime. By applying flow, we observe a decrease of the average height from 15 nm to 7 nm. Additionally, we correlate the fluorophore's brightness fluctuations over time as in fluorescence correlation spectroscopy (FCS) to quantify the timescale of the vertical motion in the brush. The high spatial and temporal resolution of MIET enables us to quantify the polymer movement on the molecular scale.

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Host-Fungi Interaction Studied with Murine Primary Alveolar Macrophages and Mouse Lung Tissue Sections

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Life-threatening fungal infections due to opportunistic fungal pathogens are realized more and more often nowadays in the clinics. Major risk factors are today's immunosuppressive and invasive medical interventions for patients with severe acquired or congenital immune disorders (e.g. HIV/AIDS, solid organ transplantation and chemotherapy).

Aspergillus fumigatus, a filamentous opportunistic fungus is one of the most important causes of invasive mycoses in Europe. For asexual reproduction, the fungus forms thousands of airborne conidia. Due to their hydrophobic exterior and small size (~ 2-3 µm in diameter) conidia easily reach the lung alveoli and can cause an infection. Although, several antifungal drugs have become available to combat *Aspergillus fumigatus* infections, the mortality of this devastating disease remains as high as 90% in immunocompromised patients [1]. Another opportunistic pathogen is *Lichtheimia corymbifera*. Although *Lichtheimia* species represent a common cause of mucormycosis in Europe virulence and pathogenesis of this genus has not been investigated in detail yet [2].

In healthy individuals, the immune system rapidly eliminates conidia and they do not develop a life-threatening infection. The fast recognition of pathogenic fungi is one important way to eliminate penetrating fungi. Recognition of fungal specific patterns is mediated by pattern recognition receptors (PRR). One important PRR is CLEC7A, also known as Dectin-1 and is present on macrophages and neutrophils, both immune cells that fight against fungal infections [3].

Under immuno-suppressive regimens fungal clearance is notably affected. Receptors might be impaired to some extent and thus fungal recognition. To get a deeper insight into receptor organisation and distribution under immuno-suppressive regimens *d*STORM microscopy will be used to visualize Dectin-1 on isolated primary macrophages and on specific immune cells inside mouse lung tissue sections.

Additionally isolated primary alveolar macrophages are co-cultured with *Lichtheimia corymbifera* conidia and stained for actin filaments. Actin re-organisation is indispensable for a fast engulfment of conidia and thus fungal clearance.

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Defibrillating the heart with light

Raúl Quiñonez Uribe, Stefan Luther, and Claudia Richter

Cardiac arrhythmias are defined by an abnormal heart rhythm. In developing countries, sudden cardiac deaths due to ventricular arrhythmias account for ~15% of all deaths. Available treatments such as drug therapy and electrical shock therapy are known for showing side effects and tissue damage. Moreover, they lack specificity as well as spatial and temporal precision. In cardiac optogenetics, a light sensitive ion channel, Channelrhodopsin-2 (ChR2), is used to trigger action potentials in cardiomyocytes solely making use of the cell inner machinery. This enables low energy, non-damaging optical control of cardiac tissue with a spatio-temporal resolution never achieved before. Different studies using ChR2-transgenic mouse hearts have shown the capability of optogenetic cardioversion. However, all of them have used local illumination. Therefore, in order to investigate alternative optogenetic defibrillation methods, we addressed the response of the arrhythmic heart to a panoramic stimulus by building a whole-heart epicardial illumination setup based on 3 LEDs surrounding the heart. Sustained ventricular arrhythmias were induced in Langendorff perfused ChR2-transgenic mouse hearts before being optically stimulated on its whole surface. Electrical response was recorded using a MAP electrode and changes on the surface voltage were tracked via optical mapping. Light intensity and stimulation length were varied to interrogate their role on the effectiveness of the cardioversion. 1 second single pulse at 0.56 mW/mm2 terminated arrhythmias with an efficiency of >95%, and by increasing the light intensity to 1.1 mW/mm2 an efficiency of >90% was also achieved with a 10 ms single pulse. Combining panoramic photostimulation with optical mapping techniques revealed the influence of light intensity as well as pulse length changes on excitation patterns via unpinning or annihilation of characteristic wave dynamics. Considering this method efficiently defibrillated the heart using pulse lengths in three different orders of magnitude, it proves to be a successful and versatile cardioversion method. The possible tradeoff between intensity and pulse length can be of great utility in the understanding of optogenetic cardioversion mechanistics as well as in the design of novel illumination technologies with specific energy requirements.

Active Optical Components in Scanning Laser Ophthalmoscope to Visualize Ocular Structures.

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Scanning laser ophthalmoscope (SLO) is an example of ophthalmic imaging technique commonly used in the clinical practice to visualize the eye's fundus. SLO operates at the same principle as the confocal scanning laser microscopy. The light spot is scanned across the sample, and the reflected / back-scattered light is detected by a photodetector. Thus, an image with high resolution and a very good contrast can be obtained in real time. Different improvements have been recently accomplished in SLO in order to get a better performance. These include adaptive optics; different aperture stops or the use of fluorescence.
Tunable lenses are active optical elements which allow to modify the focal length corresponding to an applied signal, e.g. the voltage. Currently, the application of tunable optics in optical coherence tomography (OCT) have shown a significant impact to observe new ocular structures [1]. Therefore, tunable lenses might become a considerable element to take in mind for future optical systems.
In this work, we report a SLO prototype integrated to a high-speed tuneable lens to image different layers of the retina. The SLO prototype was designed with Zemax software in order to determine the best configuration to image the peripheral retina. Thus, Double-Gauss lenses with long diameter were selected to get a visual field of 40 degrees. A monochromatic light source with a wavelength of 830 was chosen with an optical power of 1.0mW. This setup was integrated with a customized acousto-optic lens which operated at a frequency of 250 kHz [2]. Consequently, a rapid depth scanning enables SLO to image different focal planes of the retina and to reproduce the fundus eye in 3D.
Different experimental settings were performed to test the setup. Firstly, a simple eye model was developed with a positive lens and a mirror in order to simulate the cornea and retina. Thus, the point spread function (PSF) of the reflected signal is determined depending on the focusing scenario provided by the acousto-optic lens. Secondly, different conditions of refractive error were simulated, replacing the positive lens by a set of trial lenses. Consequently, the SLO is calibrated according to the tunable focus and the optical power of the eye model. Finally, some images of the fundus eye were acquired in vivo from subjects with different age. Different retinal layers and vitreous images were identified with this setup.

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Activity-induced ionotropic glutamate receptor dynamics at super-resolution in vivo

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The activity-dependent rearrangement of ionotropic glutamate receptors mediates manifold forms of synaptic plasticity. However, fundamental principles governing receptor dynamics remain incompletely understood. Here, we investigate how the spatial and temporal activity patterns control the subunit-specific mobility of synaptic glutamate receptors (GluR) at the neuromuscular junction (NMJ) of *Drosophila melanogaster* larvae. To do so, we use structured illumination microscopy (SIM), as well as *direct* stochastic optical reconstruction microscopy (*d*STORM) and photoactivated localization microscopy (PALM) and combine these super-resolution methods with electrophysiology. We are especially interested in the subunit arrangement of GluRs, the quantification and positioning of GluRs as opposed to Brp clusters across the synaptic cleft and local protein translation of BRP and/or Glutamate receptor subunits.

Lattice Light-Sheet Microscopy for Whole-Cell Single Molecule Imaging

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Super-resolution microscopy such as direct stochastic optical reconstruction microscopy (*d*STORM) has been proven to be a valuable tool for many biological questions. Samples labeled with fluorescent dyes can be imaged with a lateral resolution of typically 15 nm, far beyond the diffraction barrier of ~200 nm. However, when expanding this technique to whole cells, new problems arise.
In conventional 3D localization microscopy, the sample is illuminated with collimated laser light from below (epi illumination). Nevertheless, only molecules within a thin slice in the sample (~1 µm) can be localized in 3D at once due to the shallow depth of field of the required high-NA objectives. This leads to premature photobleaching of not-yet-localized fluorophores. Using Lattice Light-Sheet (LLS) illumination, only a thin plane in the sample, usually 1.1 µm thick, is illuminated. The plane coincides with the detection focal plane such that all illuminated molecules can be localized. Another effect of using LLS for dSTORM is the dramatically improved signal-to-noise ratio due to the elimination of background fluorescence.
Nevertheless, super-resolving a whole-cell volume requires the careful selection of experiment parameters as well as sophisticated data processing and evaluation. Here, I demonstrate how key parameters of the LLS microscope can be optimized for *d*STORM imaging and how a typical localization microscopy workflow needs to be adjusted for best results.

Next generation Expansion Microscopy: combining physical specimen expansion with super-resolution microscopy.

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The recently developed technique Expansion Microscopy (ExM) [1] physically expands the specimen enabling the visualization and investigation of proteins and other biological molecules with nanoscale precision. Therefore, the specimen is embedded in a dense swellable polymer in which a modified fluorescent tag is targeted to the biomolecule of interest. Additionally, the label is anchored into the polymer mesh. In water, the polymer expands isotropically ~4.5-fold, enabling ~70 nm lateral resolution using diffraction-limited microscopes. Combining this innovative imaging technique with super-resolution microscopy methods such as stimulated emission depletion (STED), structured illumination microscopy (SIM) or *direct* stochastic optical reconstruction microscopy (*d*STORM) paves the way towards fluorescence imaging with so far unmatched spatial resolution. We developed a new method, called UltraExM, and demonstrate its potential using different cellular structures and molecular assemblies. In particular, UltraExM enabled us to unveil the centriolar chirality, an ultrastructural signature of centrioles, which was so far only visualizable by electron microscopy.

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Hiking:

Parc natural de S'Albufera de Mallorca: Spectacular marsh, the everglades of Mallorca. The S'Albufera Natural Park is possibly the most extensive and bird-rich wetland to be found on any mediterranean island. Its 1,646.5 ha have enjoyed protection since 1988 and now have a visitor's reception area, a permanent exhibition and a good number of hides, observation platforms and marked itineraries.

Formentor: Spectacular views from high cliff. Cap de Formentor is a spectacular place, located on the northernmost point of the Balearic Island Mallorca in Spain. Its highest point, Fumart, is 384m above sea level. It has many associated bays, including Cala Fiquera, Cala Murta and Cala Pi de la Posada.The 13.5 km road which runs from Port de Pollença to Cap de Formentor was built by the Italian engineer Antonio Parietti. His masterpiece on Mallorca, however, was the snake to Sa Calobra. Instead of being overwhelmed by what stood in his way on the cliffs, Parretti observed the Tramuntana winds and understood: where the slope was too steep, he made a curve. When he had to remove part of the cliffs, he placed the waste in other places where it was needed. The result was the two roads, which are nestled together in the mountains like abandoned silk ribbons.

Biking:

The hotel has a *huerzler.com* bike station one can rent road bikes and helmets. Please bring your own cycling shoes and check if they fit to the pedal system. They don't rent out Mountain bikes. Go on their webpage to reserve your bike and equipment if you like.

http://www.huerzeler.com/en/cycling-stations/detailview-radsportstationen/?hID=11

Mallorca is the winter training arena of all professional cyclist, for the enthusiast it's a must cycling on their roads. If one fears the Spanish traffic, be advised to cycle in groups.

Wine:

Close to the hotel is one of the most famous vinery's of the world. The vines of canvidalet are served on pricy tables from Tokio to Los Angeles. One can visit the vineyard daily from 8am to 4pm, details on their webpage.

http://www.canvidalet.com

A second hint is to visit the village of Binissalem, it's the rising star on the vine market, comparable to Napa valley in the early eighties. If you are interested to go there have a look here.

http://www.wine-searcher.com/regions-binissalem-mallorca

Culture and history:

Alcudia: Visit the old roman village and historic center of Alcudia, there will be a market every Tuesday and Sunday morning.

Pollencia: One can combine a visit at the market, held every Sunday morning with a walk in the historic village.

Catamaran tour:

The port of Alcudia is the starting point to some of the greatest catamaran tours on the island, please check their webpage if you are interested in this water experience.

http://www.click-mallorca.com/ausfluge-sehenswurdigkeiten/puerto-alcudia/pollensa-katamaran-ausflug/#.V9fJdY9OK00

All-inclusive rules

1. On arrival you will be given a card with your picture and that will be your all-inclusive-card.

2. Please show your all-inclusive-card at Bars and restaurants of Resort.

3. Your card is personal and not transferable to other guests. If you want to invite someone you need to pay full price.

4. Your all-inclusive-card will be valid from arrival until 12:00 midday on your departure day. And you can use your all-inclusive-card from 07:30h till 24:00h.

5. Please finish your drink before ordering another.

6. Conditions: all members of the same reservation and room must have the same board basis.

What is included?

- Breakfast Buffet: from 07:30h to 10:00h in Restaurants *Ancora* and *Denario*.
- Afterhours Buffet: continental breakfast from 10:30h to 11:30h at the Grill Aquarius.
- Lunch: 12:00h to 16:00h in our a la carte Grill Aquarius or buffet from 12:30h till 14:00h at Restaurant *Denario*.
- Dinner: from 18:30h to 22:00h at buffet restaurant *Denario* and *Ancora*.
- Thematic dinner: 2 times a week at buffet restaurant *Denario* and *Ancora* (Eastern, Italian, French, Spanish, Mallorcan, etc.)
- Snacks: hot and cold snacks from 10:00h till 12:00h and 16:00h to 18:00h in Restaurant Grill Aquarius.
- Drinks: you can enjoy a good selection of drinks all day from 10:00h to 24:00h.
- In our Resort all soft drinks and alcoholic beverages are served to the table.
- Bar Luna and Triton time table is from 10:00h to 24:00h.
- Mini-bar in rooms with soft drinks and beer are filled up every day.
- Free access to sauna, turkish bath, jacuzzi and gym.