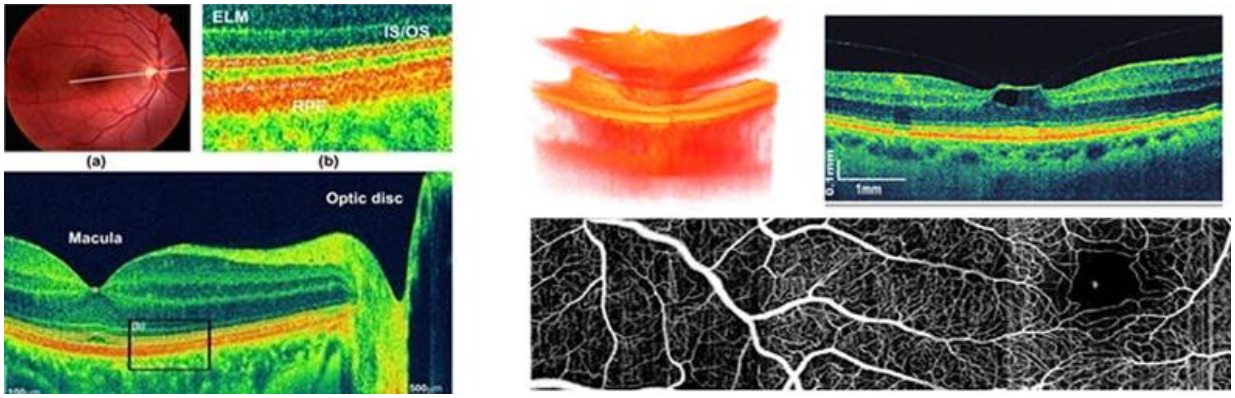

International Conference

New trends in biomedical imaging and data analysis

Max Planck Institute for Dynamics and Self-Organization

July 3-4, 2019, Göttingen, Germany

BE-OPTICAL
Advanced Biomedical Optical Imaging and Data Analysis

Organizers

Jordi Tiana (Universitat Politecnica de Catalunya, Barcelona)
Cristina Masoller (Universitat Politecnica de Catalunya, Barcelona)
Yvonne Mühe (Max Planck Institute for Dynamics and Self-Organization, Göttingen)
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Max Planck Institute for Dynamics and Self-Organization
Universitat Politecnica de Catalunya

Programme

July 3, 2019

Chair: Meritxell Vilaseca

09:15–10:00 **Isabella Guido** (Max Planck Institute for Dynamics and Self-Organization), *Visualisation and analysis of mechanically constrained biopolymers*.
10:00–10:45 **Jesus Malo** (Universitat de València), *Deep networks in the visual brain: from measurements to algorithms*.
10:45–11:05 **Pablo Amil** (Universitat Politecnica de Catalunya), *Outlier mining methods based on network structure analysis*.

11:05–11:30 *Coffee break*

Chair: Cristina Masoller

11:30–12:15 **Robin Henderson** (University of Newcastle), Topological event history analysis.
12:15–12:35 **Donatus Halpaap** (Universitat Politecnica de Catalunya), *Experimental characterization of the speckle pattern at the output of a multimode optical fiber*
12:35–12:55 **Vineesh Kappadan** (Max Planck Institute for Dynamics and Self-Organization), *Study of electro-mechanical restitution in Langendorff-perfused beating rabbit hearts using ratiometric imaging and marker-free motion tracking*.

13:00–14:00 *Lunch*

Chair: Stefan Luther

14:00–14:45 **Yuval Ebenstein** (Tel Aviv University), *Single molecule detection of epigenetic marks.*
14:45–15:05 **Raúl A. Quiñonez Uribe** (Max Planck Institute for Dynamics and Self-Organization), *Arrhythmia termination in murine hearts using millisecond optogenetic stimulation.*
15:05–15:25 **Sayedeh Hussaini** (Max Planck Institute for Dynamics and Self-Organization), *Control of arrhythmogenic cardiac wave activity applying computational optogenetics.*
15:25–15:45 **Jordi Tiana-Alsina** (Universitat Politecnica de Catalunya), *Experimental study of the degree of locking in weakly forced stochastic systems.*

15:45–16:15 *Coffee break*

Chair: Ulrich Parlitz

16:15–17:00 **Claus-Dieter Ohl** (Magdeburg University), *Seeing not touching revealed the physics of nanobubbles.*
17:00–17:45 **Dagmar Krefting** (HTW Berlin), *Reproducible data experiments in biomedical imaging and biosignal analysis*.
17:45–18:30 **Santiago Costantino** (Montreal University), *Capturing live single cells based on visual phenotypes*.

19:30–20:30 Dinner

Chair: Ulrich Parlitz

20:30–20:50 **A. Schlemmer** (Max Planck Institute for Dynamics and Self-Organization), *Efficient research data management with CaosDB.*
20:50–21:20 **G. Datseris** (Max Planck Institute for Dynamics and Self-Organization), *Software to make your scientific life easier.*

July 4, 2019

Chair: Jörg Enderlein

09:15–10:00 **Christian Eggeling** (University of Oxford), *Super-resolution fluorescence spectroscopy of membrane organization.*
10:00–10:45 **Anne Marie Haghiri** (CNRS), *On-chip bioanalysis: identification of trace biomarkers.*
10:45–11:05 **Mariano Gonzalez Pisfil** (PicoQuant), *Scanning FCS and Super-Resolution Microscopy on 2D Lipid membranes*.

11:05–11:35 *Coffee break*

Chair: Alejandro Giacomotti

11:35–11:55 **Adrià Escobet** (University of St. Andrews), *Deep multiphoton imaging with temporal focusing and single-pixel detection (TRAFIX).*
11:55–12:15 **Soheil Mojiri** (Georg-August University), *Out-of-plane bending components in Chlamydomonas flagella observed with multi-plane phase contrast imaging.*
12:15–12:35 **Antu Gortari** (C2N CNRS), *Metasurface-based total internal reflection bioimaging.*
12:35–12:55 **Shun Qin** (University of Göttingen), *Introduction of the maximum likelihood estimation and its application on data analysis.*

13:00–14:00 *Lunch*

Chair: Maciej Wojtkowski

14:00–14:45 **Robert Hubert** (Universität zu Lübeck), *New trends in Megahertz Optical Coherence Tomography (OCT).*

14:45–15:05 **Ana Rodríguez-Aramendía** (Instituto de Microcirugía Ocular), *Anterior segment/retinal swept source optical coherence tomography system (SS-OCT) for comprehensive imaging and biometry of the eye.*

15:05–15:25 **Alfonso Jiménez-Villar** (Nicolaus Copernicus University), *Analysis of the ocular defocus and retinal imaging by scanning laser ophthalmoscope integrated with acousto-optic lens.*

15:30–16:00 *Coffee break*

Chair: Ireneusz Grulkowski

16:00–16:20 **Tommaso Alterini** (Universitat Politecnica de Catalunya), *Spectral analysis of the retina and the choroid in the visible and near infrared: preliminary results of a clinical study*.

16:20–16:40 **Mounika Rapolu** (Polish Academy of Sciences), *In-vivo longitudinal imaging of glioblastoma (GBM) tumor in mouse brain microvasculature using 800nm OCT system*.

16:40–17:00 **Piotr Wegrzyn** (Polish Academy of Sciences), *Two implementations of Spatiotemporal Optical Coherence Manipulation (STOC).*

17:00–17:20 **Maria Masoliver** (Nicolaus Copernicus University), *Dynamics of weakly forced excitable systems.*

17:20–17:30 Closing Remarks

ABSTRACTS

Visualisation and analysis of mechanically constrained biopolymers

Andrej Vilfan, Eberhard Bodenschatz, Ramin Golestanian, Isabella Guido

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Structures of biofilaments and motor proteins are important model systems for the understanding of the out-of-equilibrium behaviour of the cellular cytoskeleton. In Nature, they are involved in key processes in cells such as migration, division, cytoplasmic transport, and cilia beating. Designing in vitro setup of such systems can help their characterisation thanks to the fewer components used and the possibility to introduce controlled perturbations. Here we present experimental and theoretical results on active systems made of microtubules and motor proteins which present interesting collective behaviour due to their constraints. The non-equilibrium nature of the system is due to kinesin motors that in the presence of ATP move along the microtubules. We analyse clamped filaments able to beat like sperm's flagella and active networks that contract and extend forming 3D wrinkling instabilities when confined into a channel. These results clearly show how microscopic interactions between biopolymers and motor proteins give rise to collective dynamics at a different scale in the macroscopic environment.

Deep networks in the visual brain: from measurements to algorithms

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The CERN Data Center processes about 1 PByte of data every day [1]. In case of Google, this amounts to 20 Pbytes a day (estimation in 2008 [2]). A simple calculation shows that, those numbers are *small* compared with the 130 Pbytes/day of visual information faced by your visual brain. How can you do it? It turns out that your natural neurons do a pretty good job crunching data down. In this talk I will show you that neurons at your retina and visual cortex actually compute a sort of Principal Component Analysis [3,4]. That is why, algorithms in image and video coding mimic this kind of computation [5,6]. Interestingly, deep-networks learn these features too [7]. However, don't think you (human) are particularly singular in the universe or touched by some sort of "intelligent designer": reptiles and insects are pretty efficient too [8]. Long ago Charles Darwin figured out the most radical unsupervised learning algorithm: adapt or die [9,10].

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https://www.youtube.com/watch?v=cv9hje42i_E

Outlier mining methods based on network structure analysis

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Anomaly or outlier detection in high-dimensional datasets is a fundamental challenging problem across disciplines that has also practical implications, as removing outliers from the training set improves the performance of machine learning algorithms. While many outlier mining algorithms have been proposed in the literature, they tend to be valid or efficient for specific types of datasets (time series, images, videos, etc.). Here we propose two methods that can be applied to generic datasets, as long as there is a meaningful measure of distance between pairs of elements of the dataset. Both methods start by defining a graph, where the nodes are the elements of the dataset, and the links have associated weights that are the distances between the nodes. Then, the first method assigns an outlier score based on the percolation (i.e., the fragmentation) of the graph. The second method uses the popular IsoMap nonlinear dimensionality reduction algorithm, and assigns an outlier score by comparing the geodesic distances with the distances in the reduced space. We test these algorithms on real and synthetic datasets and show that they either outperform, or perform on par with other popular anomaly detection methods. A main advantage of the percolation method is that is parameter free and therefore, it does not require any training; on the other hand, the IsoMap method has two integer number parameters, and when they are appropriately selected, the method outperforms all the other methods tested.

Topological Event History Analysis

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Topological data analysis, or TDA, has grown in popularity in recent years. Methods have been developed by researchers in algebraic topology, computational geometry, computer science and other fields, and applications have been diverse. We summarise some of the ideas of TDA, in particular persistent homology, and apply the methods to compare patterns of neutral hydrogen in the interstellar medium.

We also show similarities in principle between aspects of TDA and some features that are familiar in statistical event history analysis. This suggests that use of event history techniques can bring added value to topological data analyses. We show this in two ways.
The first is the use of Nelson-Aalen estimators in the analysis of persistent homology of random fields. The standard Nelson-Aalen machinery can be applied with little adaptation to the analysis of lifetimes of topological features in a field and so bring added value to analyses. We illustrate with an analysis of brain imaging data.
The second case relates to metric trees embedded in metric spaces. TDA has been applied successfully in the analysis of, for example, branching neural networks and brain artery trees. We show that Cox proportional hazards or other survival models can bring valid inferential procedures in the presence of censored branches. This method is illustrated in an analysis of trees made up by blood vessels in the human eye.

Experimental characterization of the speckle pattern at the output of a multimode optical fiber

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Speckle patterns produced by coherent waves interfering with each other are undesirable in many applications (for example, in laser projection systems) but on the other hand, they contain useful information that can be exploited for applications (for example, speckle imaging modalities in microscopy use speckle information to reconstruct the object that generates the speckle). It is therefore important to understand how speckle can be enhanced or reduced by tailoring the coherence of laser light. Using a conventional semiconductor laser and a multimode optical fiber we study experimentally how the speckle pattern depends on the laser pump current and on the image acquisition settings. By varying the pump current from below to above the lasing threshold, and simultaneously tuning the image acquisition time to compensate for the change in brightness, we find conditions that allow to record images with similar average intensity, but with speckle contrast (the standard deviation of the intensity over the average intensity) as low as 0.16, or as high as 0.99.

Study of electro-mechanical restitution in Langendorff-perfused beating rabbit hearts using ratiometric imaging and marker-free motion tracking

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Optical mapping is used for visualizing membrane voltage and Ca2+ concentration on the surface of isolated, Langendorff-perfused intact hearts. Despite recent progress in imaging of beating hearts, accurate measurements of quantities such as the duration of action potentials are still challenging due to residual motion artifacts present in the fluorescence signal. Motion artifacts can be significantly reduced by using the electromechanical uncoupler Blebbistatin, which may, however, affect electrophysiological properties. We show that marker free motion tracking combined with ratiometric fluorescence imaging can be used to measure more reliably the APD from Langendorff-perfused, beating rabbit hearts. We use this technique to study electro-mechanical restitution properties of the heart and also to investigate the effects of Blebbistatin onto the electrophysiology.

Single molecule detection of epigenetic marks

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Arrhythmia Termination in Murine Hearts using Millisecond Optogenetic Stimulation

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Cardiac arrhythmia is one of the major health threats in developed countries. Current treatments include electrical shocks, which deliver a high amount of energy to the heart and the surrounding tissues, making it painful and possibly harmful, and drugs, which lack specificity and could lead to side- effects. In optogenetics, cardiac cells can be stimulated using light, offering a tool to study the electrical activity of the heart with unprecedented spatial and temporal resolution and with no harm. In our study, we use global epicardial illumination as a mean to control and terminate cardiac arrhythmias. ChR2 transgenic mice hearts are Langendorff- perfused and arrhythmia is induced before illuminating the whole heart surface with 3 LEDs surrounding the perfusion bath. Applying millisecond stimuli of 1, 2, 5 and 10 ms e investigate the mechanisms of global illumination cardioversion. As seen in other studies, longer pulses terminate arrhythmia at a higher rate. Moreover, optical mapping revealed two possible mechanisms behind optogenetic cardioversion; an early annihilation of the arrhythmic waves and a later perturbation, being the first one the most dominant. This study offers a deeper understanding of the potential and effects of optogenetics in arrhythmia termination and control.

Optogenetics Control of Cardiac Arrhythmias

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In a diseased heart there is abnormality in the formation and propagation of excitation wave which results in cardiac arrhythmias. The conventional high-energetic electrical treatment methods which reset the function of the conduction system are under suspects and entail severe side effects. One new promising method known as optogenetics consists of selectively embedding photo-responsive proteins in the cardiomyocyte membranes which can be non-invasively and remotely stimulated by the corresponding excitation wavelength. Nevertheless, less is known about the potential interactions of these photo-sensitive ion channels and cardiac activity. In order to deepen the knowledge of mechanisms underlying arrhythmia evocation as well as potential low-energetic defibrillation approaches (e.g. multi-site pacing), numerical investigations applying optogenetics could be beneficial. Furthermore, computational optotgenetic modeling offers the possibility to evaluate and optimize specific experimental questions, like the dependency of different light parameters. In this study, we have modified the Bondarenko model [Am J Physiol Heart Circ Physiol 287 (2004)] of ventricular mouse heart for optogenetics by adding the mathematical model of a light-activated protein named Channelrhodopsin-2 (ChR2). To investigate the kinetic behavior of ChR2 we a pplied different intensity of photo-stimulation, under prolonged illumination at different holding voltages, to the cells. Using our light-activated model, we studied the applicability of optogenetics in controlling arrhythmia. After inducing a free spiral wave in the continuous medium of the single cell model, we illuminated the domain at different number of pacing sites, with varying pulse length and light intensity. Our studies show the light-triggered action potential of ventricular myocyte has a similar morphology to the electrically-triggered action potential. The response of the ChR2 to the blue light shows an inward currents followed by depolarizing the cell. Increment of photo-stimulation induced action potential with prolonged depolarization. Increase of photo-current in response to higher light intensities shows the current amplitude depends on light intensity. In agreement with the experiments, our simulations indicate that terminating arrhythmia at lower pulse lengths needs higher light intensity and increasing pulse duration leads to decreasing required photo-stimulation intensity. However, we applied sub-threshold illumination to interact with the spiral tip. Our preliminary results regarding spiral tip interface indicated the drifting of spiral wave to the boundary along the interface. In which, this displacement increases upon the higher light intensity.

Experimental study of the degree of locking in weakly forced stochastic systems.

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Controlling an stochastic nonlinear system with a small amplitude signal is a fundamental problem with many practical applications. Quantifying locking is challenging, and current methods, such as spectral or correlation analysis, do not provide a precise measure of the degree of locking. Here we study locking in an experimental system, consisting of a semiconductor laser with optical feedback operated in the regime where it randomly emits abrupt spikes. To quantify the locking of the optical spikes to small electric perturbations, we use two measures, the success rate (SR) and the false positive rate (FPR). The SR counts the spikes that are emitted shortly after each perturbation, while the FPR counts the additional extra spikes. We show that the receiver operating characteristic (ROC) curve (SR versus FPR plot) uncovers parameter regions where the electric perturbations fully control the laser spikes, such that the laser emits, shortly after each perturbation, one and only one spike [1]. To demonstrate the general applicability of the ROC analysis we also study a stochastic bistable system under square-wave forcing and show that the ROC curve allows identifying the parameters that produce best locking.

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Seeing not touching revealed the physics of nanobubbles

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Surface attached nanobubbles have not only been a challenge to understand their diffusional stability but even more they challenged the experimentalist to prove their existence reliably. Only a multimodal optical-atomic force microscopy approach provided data to measure size and content of these soft objects. I will try to convince you that hydrophobic attraction and pinning of the bubbles is sufficient for the understanding of all reliable experiments.

Reproducible data experiments in biomedical imaging and biosignal analysis

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Reproducibility of scientific findings has gained increasing interest in the last years [1]. It is now evident that traditional methods to describe the process of data analysis are not sufficient to ensure that reported and published scientific findings can be reproduced [2]. Besides inethical methods like selective data and misuse of statistical methods; even for well-intended researcher it is often not easy to describe the experiment in a reproducible manner and allow others to replicate it [3]. There are different levels of reproducibility. Methods reproducibility is given, when a certain method gives the same results on the same input data. In computational experiments same means exactly the same, while wet-lab methods should give reasonably similar results. Result reproducibility is given, when another method gives similar results on similar input data [4]. In the talk, the

challenges in modern medical image and biosignal processing methods - including artificial intelligence methods such as deep learning - are presented and approaches to increase reproducibility are discussed.

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C**apturing live single cells based on visual phenotypes**

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The ability to isolate rare live cells within a heterogeneous population based solely on visual criteria remains technically challenging, due largely to limitations imposed by existing sorting technologies. Here we present optical methods that permits labeling and capturing cells of interest. We will introduce cell labelling via photobleaching (CLaP), a method that enables instant, specific tagging of individual cells based on a wide array of criteria such as shape, behaviour or positional information. CLaP uses laser illumination to crosslink biotin onto the plasma membrane, coupled with streptavidin conjugates to label individual cells for genomic, cell-tracking, flow cytometry or ultra-microscopy applications. We show that the incorporated mark is stable, non-toxic, retained for several days, and transferred by cell division but not to adjacent cells in culture. Furthermore, attaching streptavidin-coated magnetic beads to their membranes using the lasers of a confocal microscope and a simple magnet allows highly-specific isolation of the labeled cells, which then remain viable and proliferate normally.

Super-resolution fluorescence spectroscopy of membrane organization

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Molecular interactions are key in cellular signaling. They are usually ruled by the organization and mobility of the involved molecules. We present different fluorescence spectroscopic tools that are able to determine such organization mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. We depict how novel details of molecular dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS) or spectral detection. We highlight how STED-FCS and spectral STED microscopy can reveal novel aspects of for example membrane bioactivity such as of the existence and function of potential lipid rafts.

On-chip bioanalysis: identification of trace biomarkers

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Over the last fifteen years, a new field has emerged: microfluidics, which involves handling very small volumes of biological fluids for medical applications. In the field of biomedical analysis, current macroscopic methods based on chromatography techniques coupled to mass spectrometry remain long and tedious, which may prove detrimental for certain pathologies where a fast and early diagnosis is often desired. On-chip analytical methods are therefore very promising, since analysis can be carried out in less than 30 minutes with a microliter of biological liquid. In the first part of my talk, I will present two recent examples of bioanalytical chips for the detection of trace biomolecules: 1/ nanofluidic devices allowing enrichment by a factor of 1000 in a few minutes in a selective way [1] and 2/ graphene-based biosensors for direct DNA electrochemical detection at the sub-femtomolar level [2-3].
More recently, microfluidics technologies have been also used to develop “organs-on-chips” with living cells that are cultured within 3D devices. Concerning this emerging field, I will conclude my talk by presenting a novel microfluidic device for blood oxygenation, which exhibits a large surface area of gas exchange between blood microchannels and air/oxygen microchannels. Designed at 4 inches wafer scale [4], this device exhibits the largest surface area compared to previously reported devices. I will show that its oxygen transfer rate is strongly related to the thickness of the thin membrane inserted between both blood capillaries and air microchambers.

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Scanning FCS and Super-Resolution Microscopy on 2D Lipid membranes

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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 labelled components diffusing in cell membranes. In order to average over a sufficient number of independent events, the optimal measurement time for an FCS measurement has to be increased for slower moving species. This in turn increases the risk of introducing artefacts (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, STED measurements combined with Graphene Induced Energy Transfer (gMIET) are performed on supported lipid bilayer (SLB). With STED + gMIET, it is possible to study the diffusion properties of the upper and bottom layers of an SLB, achieving sFCS with 3D super-resolution.

Deep multiphoton imaging with temporal focusing and single-pixel detection (TRAFIX)

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Optical imaging has seen exceptional advances over the last decade. Super-resolution and fast volumetric imaging are now key tools for biological and medical sciences. Whilst these advances have been startling, a remaining challenge in all optical microscopy is to penetrate deeper into tissue. To address this limitation, aberration correction has been applied to multiple imaging techniques improving image quality at depth. The effects of scattering on a laser beam can now also be characterised and compensated for, achieving focusing through turbid media. However, correction usually requires a “guide star” embedded in the sample or it needs to be applied in a point-by-point basis, making it challenging and time-consuming.
Alternatively, here we present TRAFIX as an approach to extend the imaging depth of fluorescence microscopy without correction or characterisation of the scattering medium. Our technique combines patterned temporal focusing illumination with single-pixel detection to obtain wide-field images through turbid media. The ability of temporal focusing beams to propagate in scattering media with minimal distortion is used to project

light patterns 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 intensity is measured in a single-pixel detection scheme. We demonstrate the potential of TRAFIX by imaging fluorescent beads, human embryonic kidney cells and fluorescent micropatterns through various scattering samples including rat brain tissue and unfixed human colon tissue at maximum imaging depths up to 7 scattering mean free path lengths. We show that TRAFIX compares favourably with standard point-scanning two-photon microscopy yielding a fivefold higher signal-to-background ratio than the latter, while minimising photobleaching of the sample. Finally, we discuss the outlook for the technique in terms of imaging speed, three-photon excitation and novel image reconstruction algorithms with compressive sensing.

Out-of-plane bending components in Chlamydomonas flagella observed with multi-plane phase contrast imaging

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Cilia and flagella are whip-like cellular appendages found in microorganisms and animals. These structures are mainly responsible for locomotion and causing fluid flows across cell surface. The central core of cilia is a cytoskeletal structure called axoeneme, which exhibits rapid motility in three spatial dimensions by hydrolyzing adenosine triphosphate (ATP). In vivo investigation of axoneme dynamics provide a deep insight into motion mechanism and cilia-driven flow dynamics. Addressing this goal however, requires an imaging technique, which is fast, label free and provides high spatial resolution at the same time. Despite the needs, existing methods does not offer a simple solution fulfilling these experimental criteria, due to either a technically demanding nature or complexity in image analysis.
Here, we propose the combination of the conventional phase contrast apparatus in illumination and a customized beam splitter in the detection path, which enables the simultaneous acquisition of eight planes through the specimen depth.
Most importantly, the method does not require any complex post-processing analysis. Using this approach, we could image the fast motion of reactivated axonemes, within a 3D volume of $\sim 40 \times 40 \times 4\,\mu m^3$ with an imaging speed of 200 volume images per second.
The tracking of the axoneme is performed using a gradient vector flow snake, which then is used to calculate the global length and curvature numerically. Our preliminary results demonstrate oscillatory behavior of the curvature and out of plane bending of the axoneme.

Metasurface-based total internal reflection bioimaging

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In recent years there has been a significant effort to push electromagnetic metasurfaces with the ability to abruptly change light properties into visible wavelengths. These advancements have opened a new range of possibilities to reshape light using ultra-thin optical devices and there is one field that is starting to gather attention: bioimaging. One technique particularly well suited for the study of molecules near a cell membrane is Total Internal Reflection Fluorescence (TIRF) microscopy, which relies on an evanescence field created by light being totally internally reflected within a glass substrate due to its high incidence angle. As of today, TIRF is generally implemented using bulky high-NA, small field of view oil objectives. We introduce the realization of metasurface-based TIRF microscopy substrates consisting of periodic arrays of asymmetric structures fabricated in titanium dioxide on glass substrates. These structures were numerically optimized couple 50-90%
of the incoming normally incident light into the first diffraction order, which outputs at an angle that suffices total internal reflection in water and eliminates the requirement for oil objectives or prisms to achieve TIRF. Our optical analysis show that we have an intensity up to 5 times higher on the first order than on the other orders, meaning that most of the light is being redirected asymmetrically and propagates throughout the length of the glass substrate. Being able to utilize lower-magnification air objectives and having a large evanescence field area provide unique TIRF conditions not accessible by traditional methods.

Introduction of the Maximum Likelihood Estimation and Its Application on Data Analysis

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Maximum Likelihood Estimation is a very powerful technique for data analysis. Herein, we study the concept of Maximum Likelihood Estimation and introduce some efficient algorithms to solve the involved minimization problem in the application of Gaussian fitting problem and image deconvolution. We show that for Poisson noise model, Maximum Likelihood Estimation method is more appropriate than the Least Squares method.

New trends in Megahertz Optical Coherence Tomography (OCT)

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Optical coherence tomography (OCT) is a technique enabling three-dimensional tomographic imaging of biological tissue in vivo with microscopic resolution. The idea is similar to ultrasound imaging, however OCT uses infrared light instead of sound for an indirect time of flight measurement. More than 25 years after its invention, OCT has now generated a multi-billion dollar market with ten thousands of devices deployed worldwide. But also, OCT still is one of the hottest topics in fundamental optics research, covering mature systems for large scale medical in-patient studies but also new fundamental optics and photonics approaches for future entirely new generations of imaging systems.

One of the main drivers of these new directions was and is the ever increasing speed. The development of Fourier Domain Mode Locking (FDML) mechanism in lasers was key to the implementation of the first Megahertz-OCT (MHz-OCT) engines which can acquire, process and display more than one million full depth scans or 4 billion voxel elements per second. This high imaging speed makes it now also possible to use the full holographic phase information of the back-scattered light field – even in vivo.
Numerical refocusing of the image plane, numerical adaptive optics for aberration correction, nanometre scale dynamic functional imaging of physiological response and Brownian motion based label-free contrast enhancement mechanisms are now possible in vivo. MHz imaging speed also enabled the transition from OCT imaging for offline diagnosis to real-time 4-dimensional volumetric imaging at video update rate with low latency for integration into augmented reality surgical microscopes.
The talk will discuss the technology of high speed OCT and selected applications.

Anterior segment/retinal swept source optical coherence tomography system (SS-OCT) for comprehensive imaging and biometry of the eye

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A dual-path dual-focus SS-OCT system that enables consecutive acquisition of retinal images and whole anterior segment images has been developed. A flip mirror allows to rapidly switch from one interface to the other. In the posterior segment imaging modality, the diffraction-limited optical design yields a lateral resolution of 10 µm and a field of view (FOV) of 21º. The anterior segment modality, designed to exhibit a longer depth of focus, shows a lateral resolution of 43 µm and 13 x 13 mm FOV. An extended coherence length swept source permits to achieve an imaging depth range of 14 mm, enabling the visualization of the whole anterior segment, from the cornea to the back of the crystalline lens. The measured axial resolution of the system is 8um.
A group of healthy volunteers have been imaged with the developed system. It allows for comprehensive imaging of the whole anterior segment and the retina of the subjects. We show that complete biometric information of clinical relevance can be assessed from the OCT data sets

Analysis of the Ocular Defocus and Retinal Imaging by Scanning Laser Ophthalmoscope Integrated with Acousto-Optic Lens

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The eye is an optical system whose retinal image is limited by different aberrations. The main aberration of the eye is defocus which might be responsible of retinal detachments.

One of the techniques which allows to assess the clinical conditions of retina is confocal Scanning Laser Ophthalmoscope (cSLO). It is a retinal imaging modality based on confocal microscopy and it can image high-contrast en-face fundus images. Different techniques have been integrated in order to improve the performance in cSLO. Some examples are adaptive optics cSLO or ultra-wide field SLO. By other hand, lenses with variable focal length (tunable lenses) have many applications in different fields of optics. In microscopy, the use of an acousto-optic tunable lens (AOL) into an Optical Coherence Microscopy (OCM) has allowed to image biological samples increasing the depth of focus.

In this work, we developed a prototype cSLO system integrated with an AOL to image different eye compartments and to analyze the central defocus of the eye. The cSLO was designed by a engineering software in order to achieve a configuration of 20 degrees of Field of View for a focal telescope of M=1x. A monochromatic He-Ne laser source with a wavelength of 632.5nm was used to image the retina. A customized AOL with a focus tuning frequency at 275 KHz was integrated into the system to get a rapid depth scanning. In-vivo retinal images from myopic and hyperopic patients were acquired. acquired by the system.

Spectral analysis of the retina and the choroid in the visible and near infrared: preliminary results of a clinical study

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Many systemic and ocular pathologies can affect structures of the eye fundus, impairing the visual performance and, in the last stages, causing visual loss. The study of the ocular fundus helps in the investigation and early diagnosis of diseases related to the retina and the choroid, as several structures and substances can be highlighted and monitored. Some of the most frequent ocular diseases affecting the retina and the choroid are glaucoma, Age-Related Macular Degeneration (AMD), diabetic retinopathy, and tumors. These diseases generally involve changes in the location and thickness of retinal structures and of the concentration of anomalous substances such as drusen. There are various optical and imaging systems used in the daily clinical practice to diagnose these diseases and evaluate the effectiveness of treatments, which have become very popular because of their non-invasive basis. Some of the most used devices are color fundus photography and optical coherence tomography (OCT). Recently, multispectral imaging technology has come in to view, offering enhanced visualization of anomalies of the ocular fundus and increasing the amount of extractable spectral information, thus avoiding problems such as metamerism caused by the poor spectral sampling of the color (RGB) fundus cameras. In this regard, we developed a new multispectral fundus camera that allows acquiring 15 spectral images from 400 nm to 1300 nm, since it includes CMOS and InGaAs sensors. To validate this system, a preliminary clinical study was conducted on healthy and diseased eyes in order to notice

differences depending on the spectral band used, especially in those with disorders. Patients underwent standard ophthalmologic and optometric evaluations, including ocular fundus evaluation with conventional color retinography and OCT. Multispectral images were shown to provide additional and more specific information, especially from deeper layers of the retina and the choroid. The spectral information of longer wavelengths provided superior contrast of structures that in color fundus photography might remain hidden, especially the choroidal vasculature and lesions caused by drusen induced retinal pigment epithelium (RPE) degeneration, in which there was less concentration of melanin. In addition, thanks to the enhanced penetration depth of infrared wavelengths, drusen could be better visualized through some spectral bands as well as choroidal tumors, which were not visible in OCT images due to the poor image quality caused by scattering of preceding media.

In-vivo longitudinal imaging of glioblastoma (GBM) tumor in mouse brain microvasculature using 800nm OCT system

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We present the in-vivo imaging for a group of three mice (N=3) used for the experiments using 800 nm OCT system for Glioblastoma (GBM) tumor injected into the mouse brain to quantify and understand the growth mechanism and progression. The imaging of the tumor is challenging because the scattering properties are changed during angiogenesis and makes it difficult for imaging. The contrast agents could help to overcome this difficulty and we made our initial efforts to study the contrast enhanced signal and apply it for GBM tumor growth. The results obtained shows that GBM tumor model is successfully implanted and is imaged for period of 15 days. The experiments also show the potential for longitudinal studies with our current OCT system. We except to see the tumor vasculature more pronounced with introduction of contrast agents and it would be useful to quantify the biological growth process of tumor, pattern in the tumor shape, boundaries and size of the growth. This could lead to interesting findings to understand the angiogenesis in tumor. The experiments with intralipid with 1300 nm and 800 nm would be a reference for the contrast enhancement for our forthcoming studies on GBM tumour with contrast agents. But we still need to focus to optimize the repeatability of the system to achieve more high quality angiography. Efforts have been made to achieve the high quality angiography to analyze the growth and progression for longitudinal studies using fractal analysis for small tumors and also by stabilizing the OCT system for repeatable measurements. This experiments would shed light in a good direction to understand the basic and fundamental mechanisms and biological process involved of growth and progression of small tumors to quantify the angiogenesis process.

Two Implementations of Spatiotemporal Optical Coherence Manipulation (STOC)

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Full-field swept-source optical coherence tomography (FF-SS-OCT) achieves high-resolution and high-speed images of the sample by parallel interferometric detection. However, the image quality is very susceptible to the cross-talk-generated noise. This issue results from the so-called signal degrading speckles and can be reduced, if the spatially incoherent light source (thermal lamp or LED) is employed but reduces the imaging speed. These results lead to a hypothesis that the cross-talk-generated noise and low-order geometrical aberrations can be overcome by damping the spatial coherence of the incident light beam. Here, we present a novel method, in which the cross-talk-generated noise is suppressed with the spatiotemporal optical coherence (STOC) manipulation. In STOC, the phase of incident light is modulated in time with a set of phase patterns displayed sequentially on the spatial light modulator (SLM). The modulation is synchronized with light acquisition to effectively control the spatial coherence of the detected light. We investigated two different experimental realization of spatiotemporal optical coherence manipulation for suppressing coherence cross-talk in in FF-SS-OCT. The results highlight the differences between them concluding that increased complexity of the experimental STOC implementation improves suppression of the spatial coherence.

Our results examining the STOC performance facilitate optimal design of the imaging systems based on the desired level of decorrelation and maximum number of phase masks which could be used in the experiment.

Dynamics of weakly forced excitable systems

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I will discuss the dynamics of weakly forced excitable cells modeled with the stochastic FitzHugh-Nagumo model, that has been widely used to study the dynamics of cardiac cells or neurons. We study the response to weak, subthreshold sinusoidal excitation. Using a well-known symbolic method of time series analysis known as ordinal analysis we find that the sequence of spikes has preferred and infrequent symbolic patterns whose probabilities depend on the period and on the amplitude of the forcing. Future work is aimed at testing the model predictions in empirical time traces recorded in in-vitro experiments.