SSOM

Swiss Society for Optics and Microscopy

Société Suisse pour l'Optique et la Microscopie

Schweizerische Gesellschaft für Optik und Mikroskopie

Mitteilungsblatt / Bulletin d'information 3+4 / 2013

Heptagon Prize

Winner
Stefan Geissbühler

FEI Switzerland Prize

Winner
Philip Moll

ZEISS/Gloor Prize

Winner
Ioannis N. Papadopoulos

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Table of content

From the President

Dear members,

the human brain has been investigated over the centuries. Non invasive tools are needed to study brain function of living individuals. Such a tool is presented in this issue.

Every second year the SSOM is granting awards to young scientists at the occasion of the General Assembly. This time there were three awards. See the reports of the winners in this issue.

The year 2013 is over. We prepare for the year 2014. All the retrospect of 2013 is done. For the SSOM it was business as usual. Some of the board members changed and I would like to thank the members leaving the board for their work done over their period as board members. In particular our two auditors gave their responsibilities to a new generation.

The SSOM is still in 2014 very well anchored in the European societies such as EOS and EMS. Members of SSOM are either heading or in central positions of these organizations. This is always a chance to have fruitful discussions about the future of scientific community.

Now I wish you a pleasant lecture.

Markus Dürrenberger
President SSOM

Diffusing-wave spectroscopy: Cerebral blood flow in a new light

Human brain function relies critically on blood flow warranting the constant supply of oxygen and glucose. Suppression of local or regional blood flow, such as in ischemic stroke, can lead to irreversible damages of brain tissue, in particular in the cortical microvasculature, and severe impairment of cognitive functions already after a few minutes of reduced oxygen supply. Continuous monitoring of cerebral blood flow is thus very important for rapid diagnosis and therapy planning in neurointensive care units. So far, however, measurements of regional cerebral blood flow in the clinic suffer from the lack of either portability, the use of ionizing radiation, invasiveness, or the use of indirect proxys such as blood flow in large vessels.

Near-infrared diffusing-wave spectroscopy (DWS; also called diffuse correlation spectroscopy, DCS) is an emerging tool for measuring regional cerebral blood flow. DWS allows for completely non-invasive, marker-free measurements through the intact scalp and skull and is particularly sensitive to the microvascular blood flow within the cortex carrying the cognitive functions. DWS complements diffuse optical measurements of blood oxygen saturation with direct blood flow information, yielding the possibility of detecting and mapping oxygen metabolism in the human brain non-invasively and continuously.

As an extension of quasi-elastic light scattering to the regime of multiple scattering, DWS relies on the analysis of temporal fluctuations of the speckle pattern which arises when tissue is irradiated with a laser with a long coherence length. The speckle pattern arises from the interference of multiply scattered photons which travel through the tissue with different phases. The temporal fluctuations of the speckle pattern arise from microscopic motions of tissue components such as erythrocytes or connecting tissue. The central quantity in DWS is the temporal autocorrelation function of the scattered electric field $g^{(1)}(t)$ which is measured with a digital autocorrelator. This quantity can be modelled quantitatively in the diffusive regime of light propagation [1], or is converted to a model-free decay time t_{avg} which is typically around 20-100ms [2]: enhanced regional blood flow following cortical activation leads to a reduction of t_{avg} due to faster speckle fluctuations.

The first experimental demonstrations of DWS brain function measurement were provided independently by the group of A. Yodh at the University of Pennsylvania and ours at Konstanz University [3],[4],[9]. Using motor stimulation protocols and analyzing the data with a 3-layer model for scalp, skull and cortex, scatterer dynamics was found to increase by 38% in the cortex of right-handed subjects during stimulation phases. Compared to conventional NIRS, this functional signal is very large, indicating that it is due to blood flow enhancement rather than to increased blood volume. Stimulation-induced increases of scatterer dynamics in the superficial layers indicated that habituation to motor stimulation leads to enhanced systemic perfusion.

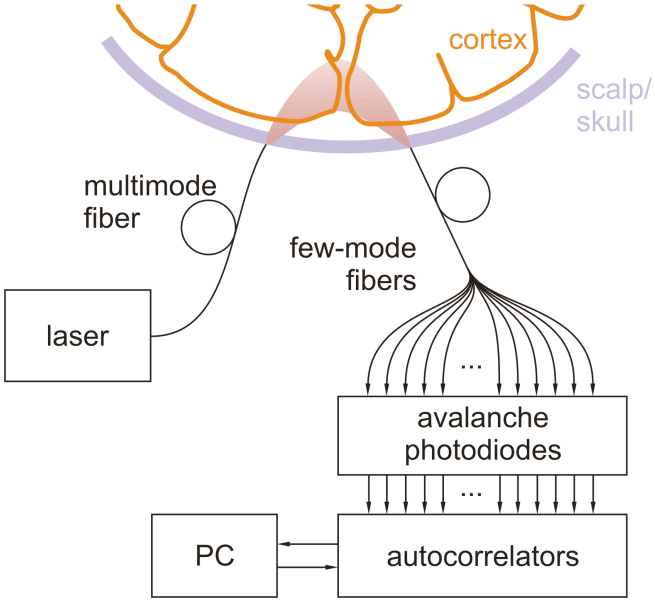

Figure 1: Setup for cerebral blood flow measurement in the human brain. Near-infrared laser light (λ=800nm) is applied at the scalp (light gray) with a multimode fiber. A receiver consisting of a bundle of 32 few-mode fibers picks up multiply scattered light that has travelled through the red shaded cortical volume. Each of the fibers is connected to an avalanche photodiode whose photon count signal is used by the digital multichannel autocorrelator to compute the temporal field autocorrelation function g(1)(τ). Further data analysis, such as averaging over the channels to enhance signal-to-noise ratio, or computation of average decay times or diffusion coefficients, is performed on the PC. Source and receiver fibers are typically separated by 2-3cm, which allows detected photons to reach the activated cortical area at depths of 1-2cm beneath the scalp. Typical photon path lengths at source-receiver distance of 2-3cm are of the order of 40cm.

While the motor cortex is relatively easily accessed by the diffuse photon cloud, the detection of visual activation is considerably more demanding, as the visual cortex is located considerably deeper beneath the scalp than the motor cortex. On the other hand, repeated visual stimulation can be performed without significantly increasing systemic perfusion and blood flow within scalp and skull.
Using our multispeckle setup (see Fig.1) with 2 sensors allows to discriminate functional DWS signals of about 3-4% from the activated visual cortex from a background of systemic perfusion. Indeed, maps of the DWS signal [1] corroborate the localization of visual activation observed in PET studies (see Fig. 2).

The origin of the blood-flow related DWS signal has, since the beginning, been a matter of debate. Time-resolved data from the human forehead show a strong pulsatile variation of the DWS signal which is out of phase with the NIRS signal (see Fig. 3).

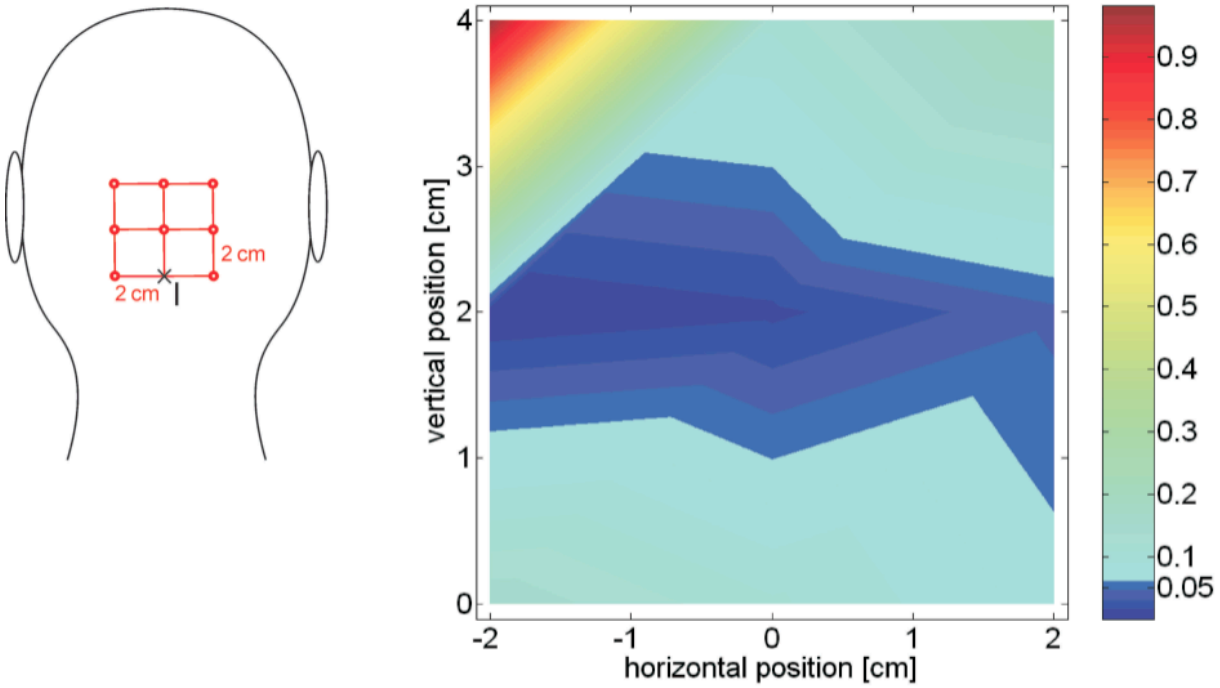

Figure 2: Mapping of brain activation using DWS. Left: positioning of the DWS sensor over the visual cortex. I represents the inion. Right: Map of the p-value for DWS signal changes showing statistical significance in a stripe centered about 2 cm above the inion.

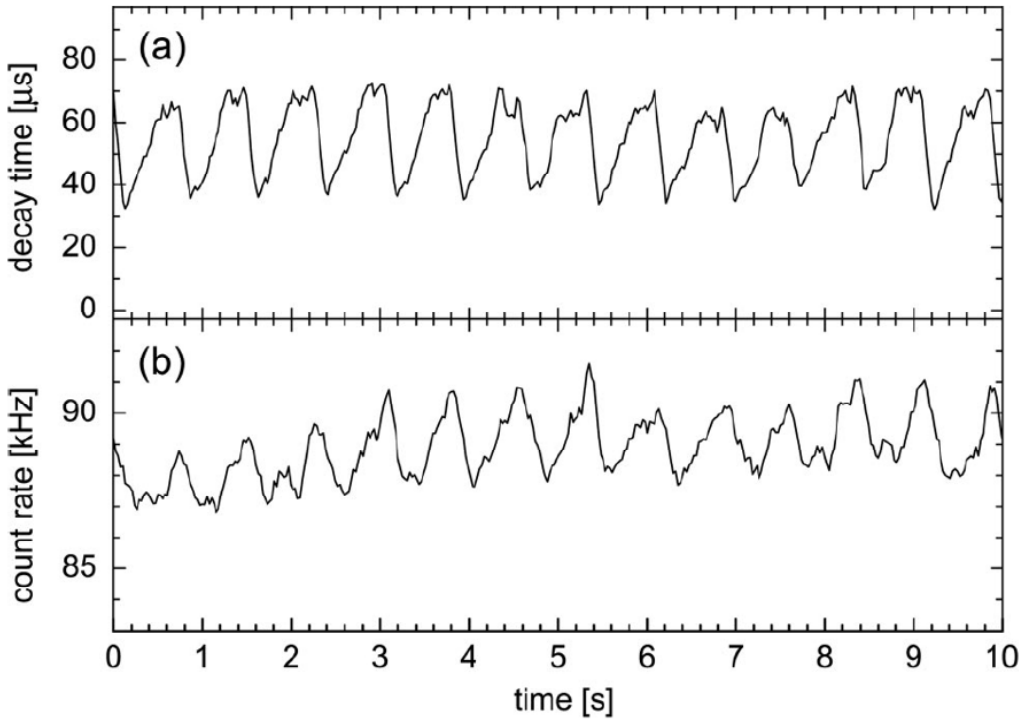

Figure 3: (a) DWS and (b) NIRS signals measured over the human forehead with multispeckle detection [5]. Note the strong variation of the DWS signal over the pulsation cycle and the phase shift between maxima of DWS and NIRS signals.

In-vivo autocorrelation functions from different tissue types are consistently described by diffusion, rather than by shear flow which would be expected for directed erythrocyte motion in blood vessels. Validation of DWS with various techniques sensitive to microvascular blood flow (MRI, microparticles, Doppler) show, in contrast, good correlation of DWS signals with blood flow.

Recently, we have developed an ex-vivo model system to study DWS signals from microvascular blood flow in detail [6] without the tissue heterogeneity complicating the analysis of in-vivo data. The data clearly indicate that DWS does not probe the ballistic, directed motion of erythrocytes in microvessels but rather their diffusive motion brought about by shear-induced collisions with other erythrocytes and vascular walls. Furthermore, our data show that shearing of extravascular tissue only makes a minor contribution to the DWS signal from the brain, while the main part stems from erythrocyte motion. An interesting approach towards using DWS for non-invasively measuring absolute cerebral blood flow has been proposed by Diop et al., using time-of-flight NIRS on indocyanine green boli to calibrate DWS signals [7].

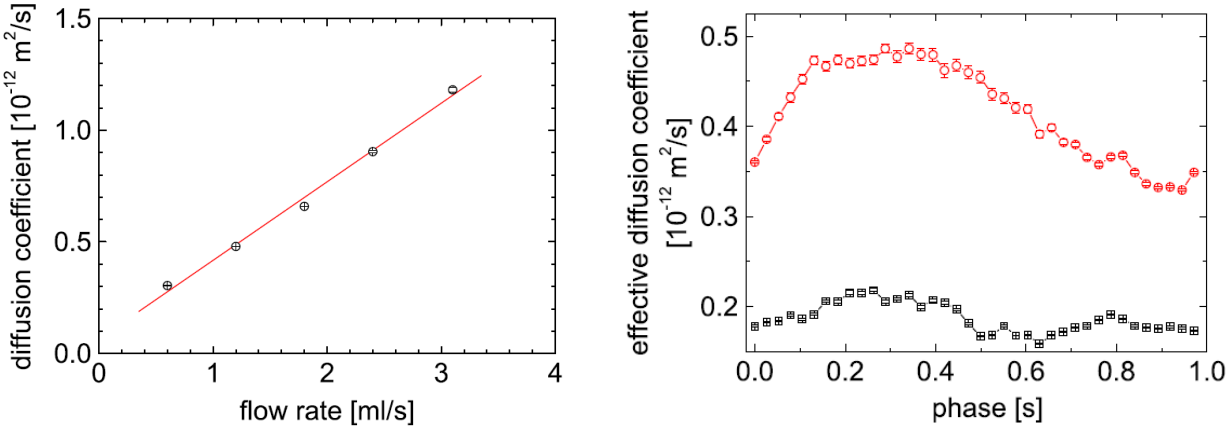

Figure 4: Left: Erythrocyte diffusion coefficient measured with DWS as a function of blood flow rate in an ex-vivo porcine kidney model system. Note the linear scaling of the diffusion coeffiicient with the flow rate. Right: effective diffusion coefficient for perfusion with blood (red) and a non-scattering solution (black) as a function of the phase of the pulsation cycle. Perfusion with non-scattering solution reveals the scattering from extravascular tissue. In the case of blood, the diffusion scales with the flow velocity and shows only a single peak. In contrast, the diffusion coefficient in the case of extravascular tissue scattering exhibits 2 peaks, reflecting the tissue shearing as the origin of the DWS signal [6].

In contrast to cerebral blood flow, the measurement of blood flow by DWS in working muscles is complicated by the dominance of muscle fiber contraction and relaxation. Indeed, time-resolved DWS with a sampling rate of 0.2 kHz shows that contraction of skeletal muscle can be measured quantitatively, possibly allowing for a distinction between fast and slow muscle fibers [8].

In summary, DWS is a novel non-invasive technique holding great promise for non-invasive diagnosis of brain function with unique sensitivity to microvascular blood flow. Combined with near-infrared spectroscopy, DWS allows to measure the rate of oxygen supply to tissue. The fact that the method does not involve extrinsic markers or ionizing

radiation makes it attractive not only for cognitive studies such as presented here, but also for clinical applications, such as stroke diagnosis and treatment, where continuous monitoring of cerebral blood flow at the bedside is imperative for improvement of patient outcome.

by Thomas Gisler

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Acknowledgements
I wish to thank my coworkers at Konstanz University who have, over the years, contributed to development of DWS as a diagnostic technique for biomedical applications, as well as numerous colleagues in the biomedical optics community for interesting discussions. Our research was made possible by continuous funding by Deutsche Forschungsgemeinschaft, Optik-Zentrum Konstanz and Center for Applied Photonics at Konstanz University.

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[6] M. Ninck, M. Untenberger, and T. Gisler, Biomed. Opt. Express 1, 1502–1513 (2010).

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[8] M. Belau, M. Ninck, G. Hering, L. Spinelli, D. Contini, A. Torricelli, and T. Gisler, J. Biomed. Opt. 15, 057007 (2010).

[9] Earlier attempts by Lohwasser and Soelkner [10] used an approach very similar to ours, but did not lead to conclusive results and remained unpublished.

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SSOM General Assembly 2013

Date 15. November 2013

Location Biketec AG, Schwende 1, CH-4950 Huttwil (http://www.biketec.ch)

Program

09.30 Kaffee und Gipfeli
10.00 Werksbesichtigung mit anschliessendem Probefahren auf dem Werksareal
11.45 Mittagessen
13.30 Referat Kurt Schär
14.00 Vereinsversammlung / Preisverleihung im Schulungsraum
16.00 Apéro

Protocol

Beschlussprotokoll der GV 2013

1. Begrüssung und Wahl der Stimmenzähler
   M. Dürrenberger kann 39 Mitglieder begrüssen

2. Protokoll der Mitgliederversammlung 2011
   Protokoll wurde im Bulletin publiziert und wird einstimmig angenommen.

3. Jahresberichte und Rechnung 2011/2012
   Die Jahresberichte wurden im SSOM-Bulletin publiziert und auch in den Jahresberichten der SCNAT und der SATW. Die Vizepräsidenten erläutern die Jahresberichte mit ein paar Worten.
   Gianni Morson präsentiert die Jahresrechnungen 2011 und 2012:
   Der Verlust von 4421.98 Fr. in 2011 ist hauptsächlich auf die Schwankungen des Fonds zurückzuführen. 2012 wurden bei den organisierten und unterstützten Anlässen ein kleiner Verlust eingefahren. Das Kapital beläuft sich per 31.12.2012 auf 154181.14 Fr.
   Mitgliederzahlen:Stand 25. September 2013
   Einzelmitglieder: 319 Personen
   Kollektivmitglieder und Firmen: 57 enthaltend 243 Personen
   Total Mitglieder: 562 Personen

4. Entlastung des Vorstandes
   Der Vorstand und der Kassier werden einstimmig entlastet.

5. Ersatzwahlen Vorstände (Verabschiedungen)

   Sektion Optik:
   - Thomas Südmeyer neu als Beisitzer

   Sektion Mikroskopie:
   - Rolf Erni für Lorenz Holzer

- Dimitri Vanhecke für Barbara Rothen
- Arne Seitz neu als Beisitzer

Sektion Nanotechnology:
- Keine Änderungen

Biomedical Photonics Group:
- Keine Änderungen

6. Ersatzwahlen der Revisoren

Neu stellen sich als Revisoren zur Verfügung:
- Nadine Matthes
- Kurt Pulfer

7. Festlegung des Jahresbeitrags

Folgende Mitgliederbeiträge stehen zur Diskussion:
CHF 30.- für Einzelmitglieder (plus Zuschlag Mik. / Optik)

Zuschläge:	EMS	CHF 7.50	EOS	CHF	22.-
	SCNAT	CHF 4.-	SATW	CHF	17.-
total:		CHF 11.50		CHF	39.-

CHF 150.- für Kollektivmitglieder (max. 10 Personen)

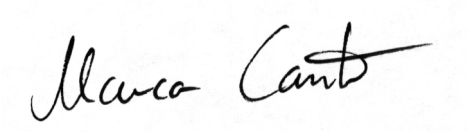
Die Versammlung erteilt M. Dürrenberger mit 18 Ja, 4 Nein, 14 Enthaltungen den Auftrag bis 2014 einen neuen Vorschlage für die Mitgliederbeiträge auszuarbeiten.
Die aktuellen Beiträge werden mit 32 Ja, 2 Nein, 1 Enthaltung beibehalten.

8. Verschiedenes

St. Tschanz macht Werbung für das MICS Symposium in Bern

Lausanne 19.11.2013

Marco Cantoni



SSOM GV in Huttwil, 15. November 2013
bei der
Biketec AG in Huttwil www.flyer.ch








SSOM GV in Huttwil, 15. November 2013

Verabschiedung der alt Revisoren
mit bestem Dank für Ihre Arbeit

Marcel Düggelin
&
Victor Colombo

Research prizes for young scientists

This year the SSOM was able to organize three research prizes for young scientists. The sponsors of the prizes were the companies HEPTAGON, who donated CHF 5000.- for an outstanding work in the field of the development of optics for intelligent micro systems, ZEISS/GLOOR Instruments for an outstanding paper in the field of microscopy, and FEI for an outstanding work in the application of electron- and ion-beams, which CHF 2500.- each.

The jury consisted of Dr. Peter Seitz, Managing Director, Innovation and Entrepreneurship Lab, ETH Zurich and former member of the SSOM board, Dr. rer. nat. Bernhard Braunecker, Leica Research Fellow (retired) , Member of the Swiss Academy of Engineering Sciences, and also former member of the SSOM board,

Prof. Dr. Hans Peter Herzig, Optics & Photonics Technology Lab / EPFL, Past President of the European Optical Society, and Dr. Kurt Pulfer, former president of the SSOM who acted also as the head of the jury. After the extension of the deadline to September 15, ten applications were received. Every contribution was excellent but there were “only” three prizes to be awarded at the General Assembly of the SSOM in Huttwil. Stefan Geissbühler from the EPFL received the HEPTAGON prize from Markus Dürrenberger, who acted as a substitute for Markus Rossi from HEPTAGON. Ioannis N. Papadopoulos from the EPFL received the ZEISS/GLOOR Instruments prize from Hans Flury (Zeiss) and Harry Brandenberger (GLOOR), and Philip Moll from the ETHZ the FEI prize from Simon Wagner (FEI). Actually they were given only a document, the SSOM treasurer will transfer the prize money to them, when he receives the money from the sponsors…

The three laureates presented their contributions at the SSOM General Assembly with a poster and you can read an abstract of their papers in this bulletin.

by Kurt Pulfer

SSOM GV in Huttwil, 15. November 2013

Forschungspreis für den wissenschaftlichen Nachwuchs

Heptagon Preis:
Stefan Geissbühler, EPFL (Theo Lasser), **Structural and Functional Stochastic Super-resolution Microscopy**

FEI Preis:
Philip Moll
ETHZ (Bertram Balogg)
FIB crystal microstructuring and contacting

SSOM GV in Huttwil, 15. November 2013

Forschungspreis für den wissenschaftlichen Nachwuchs

ZEISS/Gloor Instruments AG Preis:
Ioannis N. Papadopoulos
EPFL (Demetri Psaltis)
Miniature endomicroscopes using multimode fibers

Heptagon Prize

Balanced super-resolution optical fluctuation imaging

Background

Fluorescence microscopy is one of the major tools in life science because it allows for the minimally invasive observation of cellular and subcellular processes in living environments, while providing molecular sensitivity and specificity via fluorescent labels that target the proteins of interest. Its spatial resolution is limited by diffraction to about half the wavelength of light in the lateral dimensions and roughly 1.5 wavelengths in the axial dimension[1]. Certainly, many biological processes happen on a much smaller length scale and are thus not accessible with conventional fluorescence microscopy. Within the last two decades, a number of super-resolution microscopy techniques capable of imaging features with diffraction-unlimited spatial resolution have been established. Switching fluorescence signals of single emitters sequentially on and off in either a targeted or stochastic manner enabled the consecutive observation and distinction of objects separated by nanometric distances. This unprecedented resolving power with a far-field optical microscope promises to strengthen our understanding of living processes. However, its successful application is challenging. Super-resolution microscopy sets much higher demands on the sample preparation and labelling quality than researchers are used to. In addition, super-resolution microscopes often require tedious alignment procedures and/or rely on complicated data analysis software. Not surprisingly, commercially available systems are rather expensive and not yet widely spread.

My PhD thesis focused on the development and characterization of novel concepts for super-resolution microscopy based on stochastic fluorescence fluctuations in order to extend and/or simplify existing techniques.

Stochastic super-resolution microscopy

Stochastic super-resolution microscopy overcomes the diffraction limit by processing image sequences of stochastically fluctuating fluorophores either with single-molecule localization algorithms (PALM/STORM) or with a statistical analysis based on higher-order cumulants (super-resolution optical fluctuation imaging SOFI[2]). The image sequences can be acquired with a standard wide-field fluorescence microscope that provides single-fluorophore sensitivity.

Theoretically both concepts provide diffraction-unlimited resolution. In practice and under appropriate conditions, single-molecule localization microscopy can yield about an order of magnitude resolution improvement over diffraction-limited imaging, while for SOFI, the useable gain in resolution is often limited to a factor of two to three[3]. But the

requirements on the sample preparation and the imaging system are significantly higher in the former case. For accurate single-molecule localizations, the fluorophores have to be bright and their images have to appear isolated in the individual frames. This requires a rigorous control of the photo-physics of the fluorophores that is adapted to the necessary labelling density for an appropriate sampling of the structure of interest. Most organic fluorophores can be reduced by a thiol – contained in the imaging buffer – to an anionic form that is non-fluorescent and stable for seconds. This reversible process of reducing and oxidizing fluorophores leads to a stochastic blinking that is temporally resolvable by state-of-the-art microscopy cameras. However, most protein networks in cells are intertwined and highly dense structures. In order to fulfil the condition of isolated fluorophore images, the on-time ratio (lifetime of the fluorescent state divided by the blinking period) has to be extremely low and consequently high thiol concentrations as well as high excitation intensities are typically required. Furthermore, additional external background-reduction means, such as total internal reflection (TIR) or light sheet illumination are often necessary.

On the other hand, the cumulants analysis used in SOFI relaxes the requirements significantly. SOFI considers the complete image sequence at once and therefore the signal strength of a single fluorophore in one frame can be much lower than for localization microscopy. Furthermore, the fluorophore images no longer have to appear isolated and consequently, the number of active fluorophores per frame can be higher and the overall acquisition time can be reduced. The thus relaxed brightness and blinking requirements increase the range of suitable fluorophores and make SOFI an attractive alternative to localization microscopy for live-cell super-resolution imaging. In addition, the inherent optical sectioning property of SOFI enables 3D imaging without the need for a special background-reducing illumination.

The main reason for the lower resolution improvement of SOFI as compared to single-molecule localization is its nonlinear response to brightness and blinking differences in the sample. While the resolution of SOFI improves with the cumulant order, the image dynamic range increases exponentially. In the presence of heterogeneous distributions of the molecular brightness and blinking statistics, the sample structures rapidly appear discontinuous, which hampers the interpretation (Fig.1b).

Balanced SOFI

Balanced super-resolution optical fluctuation imaging (bSOFI)[4] extends SOFI by combining several cumulant orders to map fluorescence-related molecular statistics, such as molecular state lifetimes, concentrations and brightness distributions with super-resolution. The estimated molecular statistics are then used to balance the image contrast, i.e., to linearize the brightness and blinking response while maintaining the resolution improvement of the cumulants. We demonstrated an almost five-fold resolution improvement as compared to the diffraction-limited image using a widefield fluorescence microscope with TIR illumination (**Error! Reference source not found.**). In this configuration, the super-resolved brightness and blinking on-ratio maps reflect the exponentially decreasing intensity profile along the optical axis and therefore encode depth information (Fig.1c-d). Likewise, the molecular statistics obtained with balanced SOFI, such as the blinking lifetimes and fluctuation amplitudes, are highly sensitive to static differences and/or dynamic changes in the chemical microenvironment of the fluorophores and can thus report functional information that has not been exploited before, for instance, the local pH or the concentration of reducing and oxidizing agents.

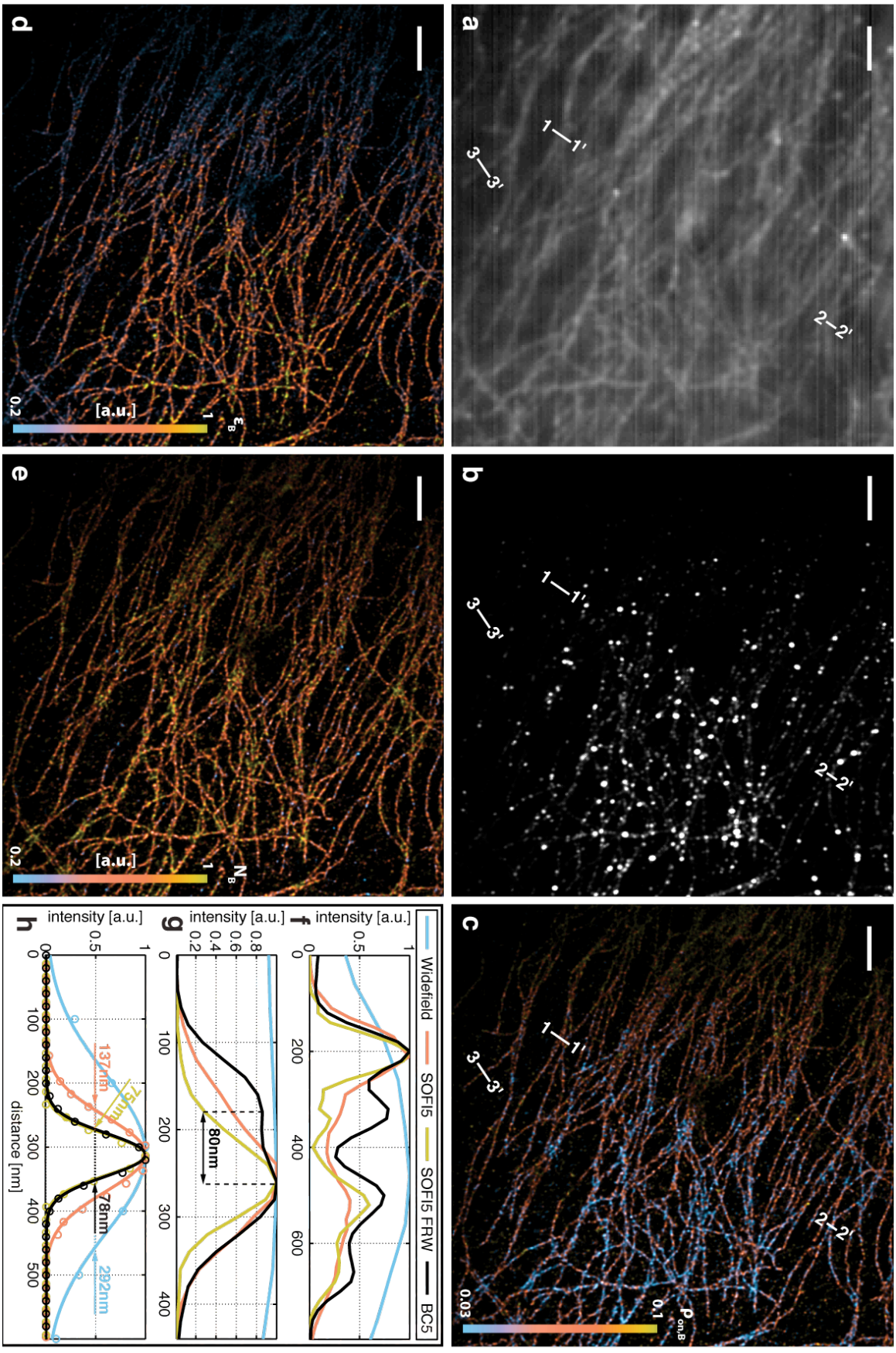


Figure 1: HeLa cells with Alexa647-labelled microtubules. (a) Widefield image. (b) Conventional 5th order SOFI. (c-e) Color-coded molecular on-time ratio, brightness and density overlaid with the 5th order balanced cumulant [BC5]. (f-h) Profiles along the cuts 1-1', 2-2' and 3-3'. Scale bars: 2µm.

Simultaneous 3D and multi-color SOFI

In SOFI, spatio-temporal cross-cumulants between neighbouring pixels are routinely applied to increase the sampling within the plane so that the resolution is not limited by the size of a pixel. Accordingly, when multiple depth planes are acquired simultaneously, we can perform cross-cumulation in all three dimensions and supplement the acquired planes with additional intermediate planes. Consequently, the acquisition time is reduced and the bleaching-limited photon budget from marker fluorophores is better exploited. Using an eight-plane detection scheme (Fig.2a), we imaged the outer membranes of mitochondria in C2C12 cells without mechanical scanning and demonstrated an almost three-fold 3D resolution improvement using a third-order analysis[5] (Fig.1b-j). Similarly to cross-cumulation in space, cross-cumulation in the spectrum can be performed if multiple colour channels are acquired in parallel (for example by exchanging a 50:50 beam splitter in Fig.2a by a dichroic mirror). The resulting increase in spectral sampling can be used to unmix multiple fluorophore colors with overlapping spectra, not limited to the number of physical colour channels. The concept of super-resolution is therefore extended to the spectral dimension and simultaneous multi-color super-resolution imaging is simplified setup-wise.

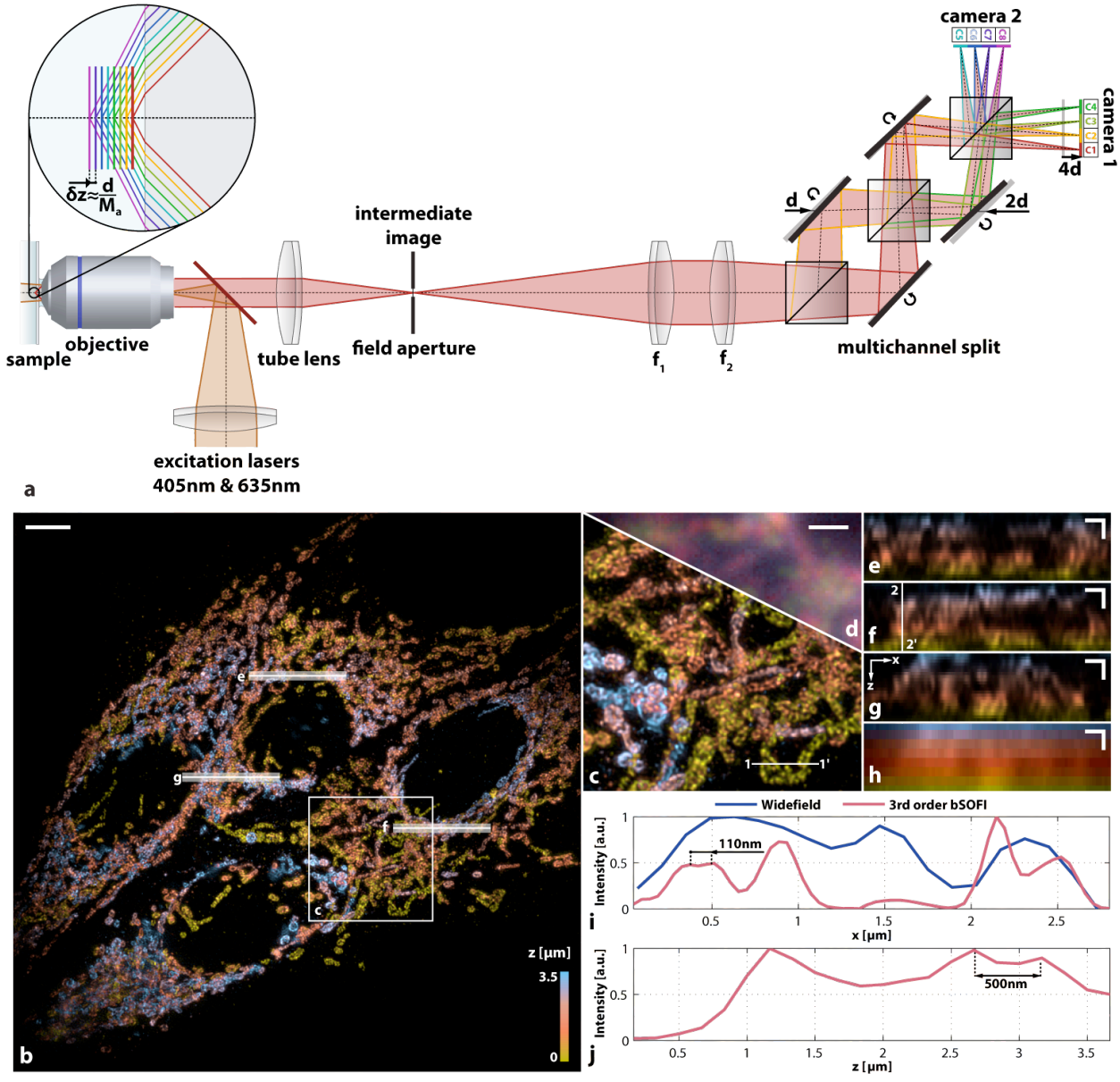


Figure 2: Multiplane SOFI. (a) Multiplane setup. Eight simultaneously acquired focal planes are obtained by splitting the fluorescence into several channels using three 50:50 beam splitter cubes and by introducing path length differences d and 2d with the mirrors, as well as 4d with camera 1. The resulting separation between the focal planes in the object space is then approximately d/Ma, with Ma being the axial magnication of the system. The images of the focal planes are projected side-by-side on the cameras. A field stop in the intermediate image prevents overlaps between the image frames. (b) Maximum intensity projection of the 3rd-order bSOFI image of Alexa647-stained mitochondria in C2C12 cells. The eight planes cover a total volume of 65x65x3.5μm^3. (c) Zoomed region highlighted in (b). (d) Diffraction-limited view of the same area as (c). (e-g) x-z sections highlighted in (b) averaged over 25 pixels along y. (h) Same x-z section as (g) from diffraction-limited stack. (i) x-profiles from the cut 1-1' highlighted in (c). (j) z-profile from the cut 2-2' highlighted in (f). Scale bars: (b) 5μm (c,d) 2μm (e-h) 1μm in x and z.

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FEI Switzerland Prize

The role of anisotropy in iron-pnictide high-temperature superconductors addressed by Focused Ion Beam sample fabrication

The capability to measure electronic properties in microscopic samples and to overcome the experimental issues arising from small sample size, small masses and small signals is of key importance in the research of novel materials, challenging scientists to push measurement techniques to their limit. The aim of our work is to explore the

potential of a Focused Ion Beam (FIB) as a tool to perform resistivity measurements on microscopic samples by depositing micron-sized electric contacts, as well as to microstructure the material.

These FIB-based methods were applied to iron-pnictide crystals, a recently discovered class of high temperature superconductors. Typical for many novel compounds in condensed matter physics, single crystals of these materials are only available as small crystallites, about the size of the diameter of a human hair. The main scientific goal is to uncover the superconducting transport properties of SmFeAs(O,F), which shows the highest T_c of 55K in the pnictides. These transport properties are of theoretical interest for the still ongoing debate about nature of unconventional superconductivity, as well as of direct practical consequence as technologically relevant parameters such as critical fields and currents are probed.

Three key material properties determine a superconductors application potential: (1) Tolerate high magnetic fields, i.e. have a large H_{c2}, (2) Sustain large amount of currents, i.e. have a large j_c, and (3) not to be prohibitively anisotropic. During this project, all of these key properties have been reliably measured in microscopic crystals employing FIB based structuring and contacting. As it turned out, all of these criteria are met, indicating that the single crystal behavior of SmFeAs(O,F) is not a 'showstopper' on the path towards the application of SmFeAs(O,F) in wires.

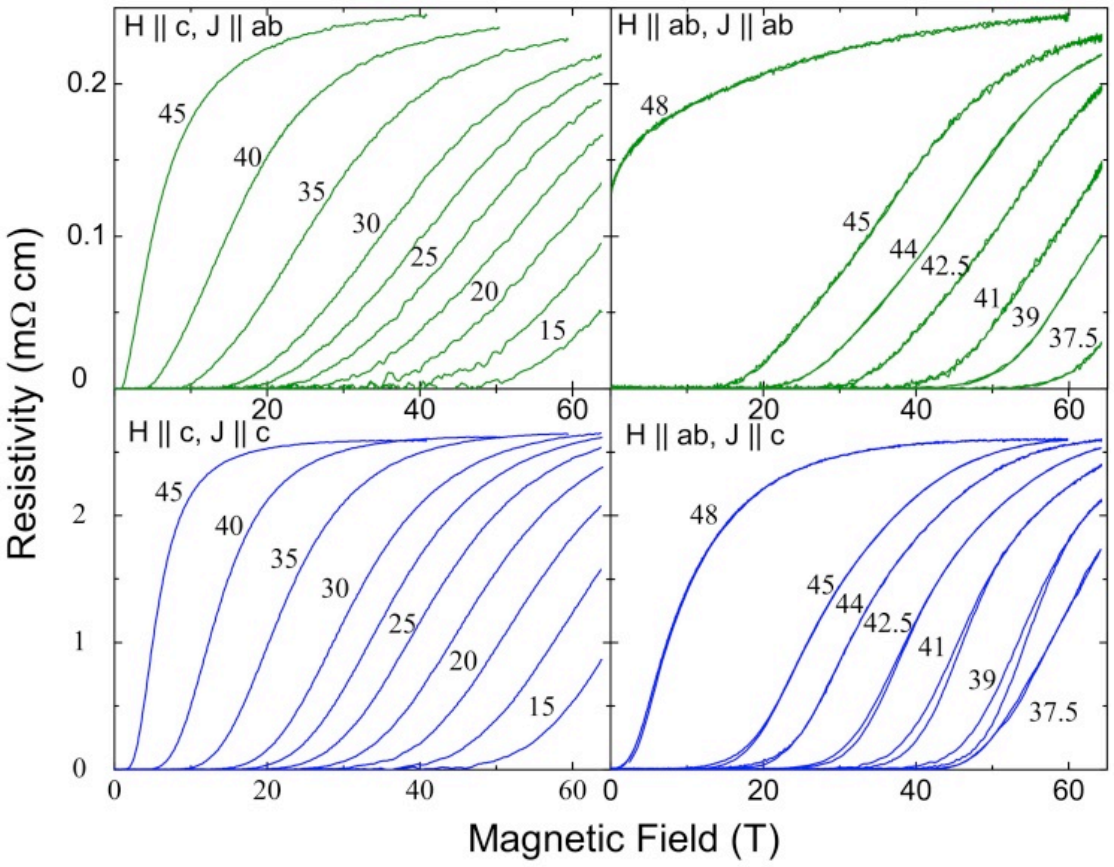

Figure 1: Resistivity of a FIB shaped SmFeAs(O,F) crystal upon destruction of superconductivity by strong magnetic field for various fixed temperatures below Tc. At zero field, the crystal is in a zero-resistance state. The onset of resistivity at a temperature dependent value of the field marks the upper field boundary for superconducting applications.

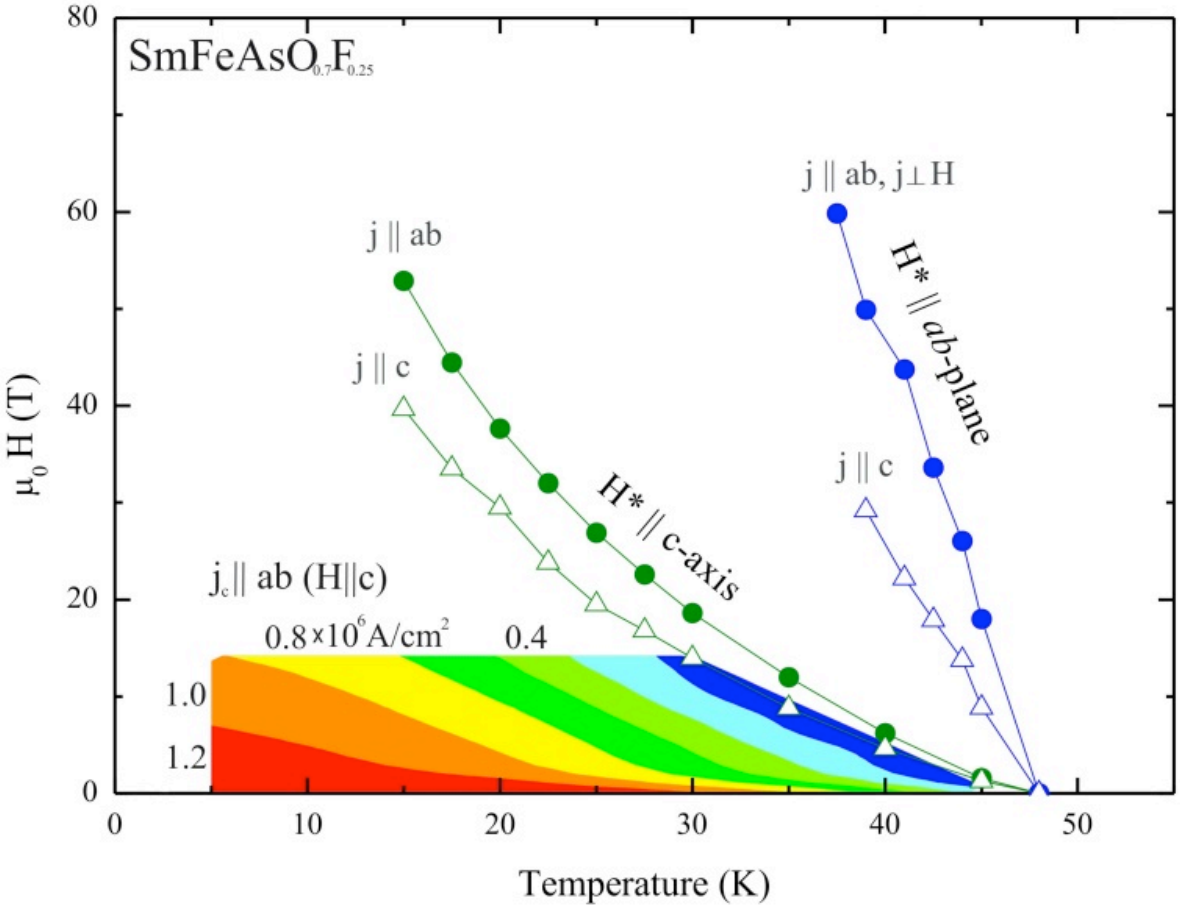

Figure 2: Map of the explored field-temperature region. The upper lines indicate the field-temperature points of the loss of zero-resistance for the different field- and current-directions. The critical current density, the maximal current the material can carry in its zero-resistance state, is given in the color-plot. The critical currents in these single cystals are remarkably high, on the order of 106 A/cm^2.

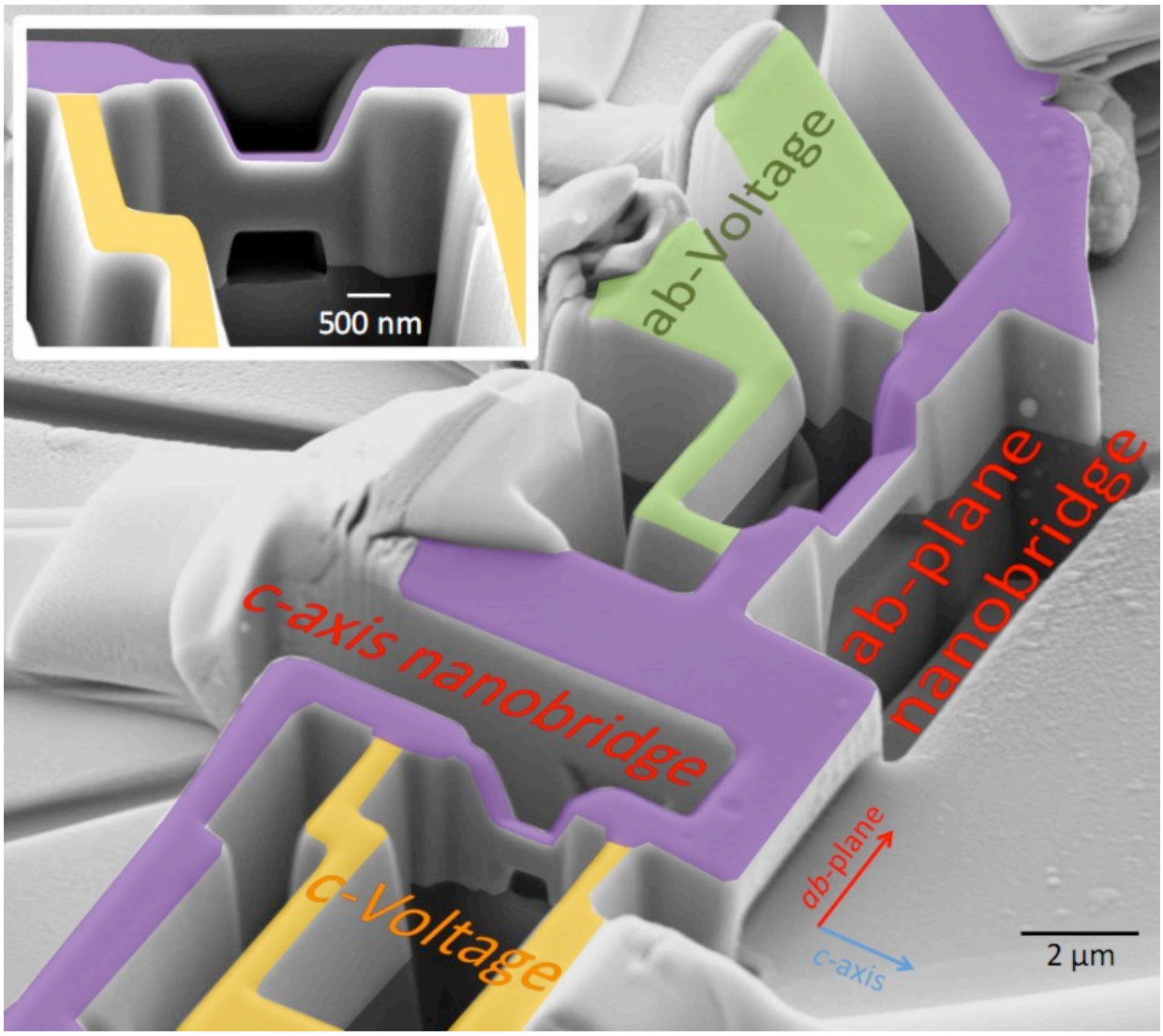

Figure 3: Typical example of a FIB microstructured and contacted crystal sample. In a first step, the irregularly shaped microcrystal was carved into a 80x10x2 μm^3 slab. This slab was then the starting material of the depicted structure. In the next step, the slab was electrically contacted at multiple points by FIB platinum deposition. The small thickness of the slab allowed to grow platinum electrodes directly ontop of the crystal. In a final step, it was then again structured into the shape shown.

This FIB scheme enables us to select the crystallographic orientation for the transport measurements. In this particular sample, the two symmetry directions of the tetragonal system SmFeAs(O,F) were selected: current flowing along the FeAs-layers (ab-plane) and perpendicular to them (c-axis). Voltage and current contacts for four-terminal measurements were directly carved out of the metallic crystal. The crystal part between the voltage contacts was in a last step further tailored into a bow-tie shape, thus creating free-standing crystal bridges with a cross-section of about 500x500 nm^2. This last step is essential to ensure that the smallest conductor cross-section, or equivalently the highest current density, is achieved between the voltage contacts and not in another part of the current path. This structure is thus well suited to study critical current densities along different directions in this crystal.

by Philip J.W. Moll

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Zeiss / Gloor Instruments Prize

Miniature endomicroscopes using multimode fibers

Endomicroscopy enhances the capabilities of endoscopy by delivering images of very high resolution from deep inside the body. Conventional clinically available endoscopes are based on optical fiber bundles where each of the single mode fibers acts as a single pixel of the final image [1]. Those devices can be used as versatile flexible endoscopes, however the achieved resolution is larger than 5μm.

Recently, multimode fibers [2-5] have been explored as a means to enable the generation of miniature endomicroscopes, delivering high-resolution images deep inside tissue. Indeed, contrary to fiber bundles were the fiber cores are spaced apart from each other to avoid any mode mixing, multimode fibers can support a very large number of modes in the fiber core, therefore greatly increasing the density of the available degrees of freedom per surface area. This means that for a fiber bundle with a certain number of single mode cores/pixels, a multimode fiber can offer the same number of modes in only a fraction of the bundle size. Doing a simple calculation of the modes supported by a multimode fiber and comparing it against the pixel count of state of the art fiber bundles, we can see that multimode fibers offer a huge potential in terms of miniaturizing endoscopic devices.

However the increased number of degrees of freedom and the resulting miniaturization capability do not come without a cost. The modes inside the multimode fiber are free to exchange energy among each other in a process known as modal mixing, therefore any information coupled inside the fiber will reach the output completely scrambled. In order to overcome this limitation we deploy techniques of wavefront shaping of the light that propagates through the multimode fiber. Wavefront shaping techniques, and more particularly Digital Phase Conjugation allows to deterministically transmit information through the fiber finally leading to the capability of focusing and scanning submicron sized foci across the whole fiber facet [6].

Combing the small size of multimode fibers, their capability of transmitting very large amounts of information along with the versatility of digital techniques in excitation and acquisition, we have demonstrated a novel lensless, high-resolution miniature endomicroscope that can revolutionize medical optical imaging and photomedicine. The proposed miniature imaging modality is capable of delivering a highly detailed image of a single neuron along with images of the far smaller dendrites [5]. The quality of the fluorescent images enables medical diagnosis based on the cellular phenotype of the investigated sample. The submicron resolution achieved, along with the miniaturization of the endomicroscopic device enabled by the wavefront shaping of light in multimode

fibers, opens up the possibility of in-vivo biopsy based on cellular imaging directly at the position of the sample of interest, even very deep inside the organism.

A specific example of the usefulness of such a miniature endomicroscope based on multimode fibers is the diagnosis of hearing loss based on the imaging of cellular structures of the inner ear. Based on work done in our lab [7], hearing lose can be directly linked to a severe change in the structure of the so called hair cells in the cochlea cavity of the inner ear. Accessing this part of the ear requires a very thin endoscopic probe and at the same time, imaging the cellular structure implies a very high achievable resolution. State of the art endoscopes cannot facilitate both these requirements. However, the endomicroscope shown in Fig.1 can provide high-resolution images of the hair cell structure of the cochlea while the size of the device is still very small. The ability of using only optical and digital techniques to focus and scan the light at the distal tip of the endomicroscope, without the presence of any active component would allow the easy adoption of such a technology from physicians.

Moreover, apart from fluorescent imaging, the proposed technique has been exploited to provide Optical Resolution Photoacoustic Microscopy (OR-PAM). Using the multimode fiber to transmit nanosecond laser pulses while focusing and scanning the light using the techniques described in [6], we are able, by exploiting the photoacoustic effect, to perform multimodal imaging using the optical absorption properties of the sample as a contrast mechanism. During the process, the needle endomicroscope is used as a miniature source bringing the optical excitation directly against the sample of interest, which can be buried deep inside scattering biological tissue. An ultrasound transducer placed at the surface collects the generated acoustic signal. In conventional photoacoustic microscopy the resolution of the acquired image depends on the achievable size of the optical excitation focus. As the technique is used to form images deeper inside tissue, the optical excitation field cannot render a tight focus spot because of the tissue scattering properties. Therefore, optical resolution photoacoustic microscopy is limited to the ballistic regime of light propagation in scattering media (~1mm in biological tissue). In our implemented technique, the miniature endomicroscope can be used as a minimally invasive method of generating micron-sized foci directly on the sample of interest far deeper than the ballistic range, while a transducer placed at the surface of the tissue picks up the generated sound.

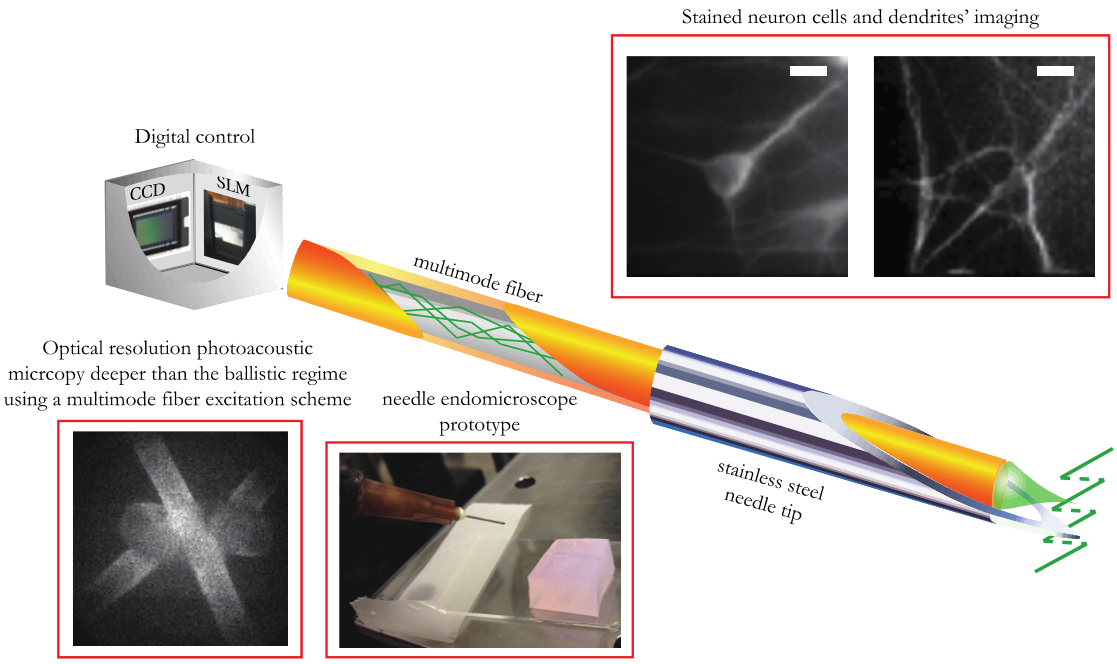

Figure 1: Multimode fibers supporting a huge number of modes along with an all-digital control of excitation and acquisition can pave the wave towards miniature endomicroscopes that can be exploited for different medical diagnosis application. High resolution imaging of cellular structures enables, cellular diagnosis directly in vivo. Also, the same miniature probe can be used as a source for biological photoexcitation (optogenetics) and phototherapy (laser ablation).

Imaging through a multimode fiber endomicroscope is sensitive to bending and the needle tip apart from acting as the tissue penetration mechanism is the supporting structure of the fiber. To enhance the capabilities of the endomicroscope and to enable the fabrication of semi flexible endomicroscopes using multimode fibers, we have explored techniques that allow us to compensate the effects of bending. Using a coherent beacon source placed at the distal tip of the multimode fiber we demonstrated that by intensity correlation, it is possible to identify the fiber conformation and maintain a focus spot while the fiber is bent over a certain range. Once the fiber configuration is determined, previously calibrated phase patterns could be stored for each fiber conformation and can be used to scan the distal spot and perform imaging [8].

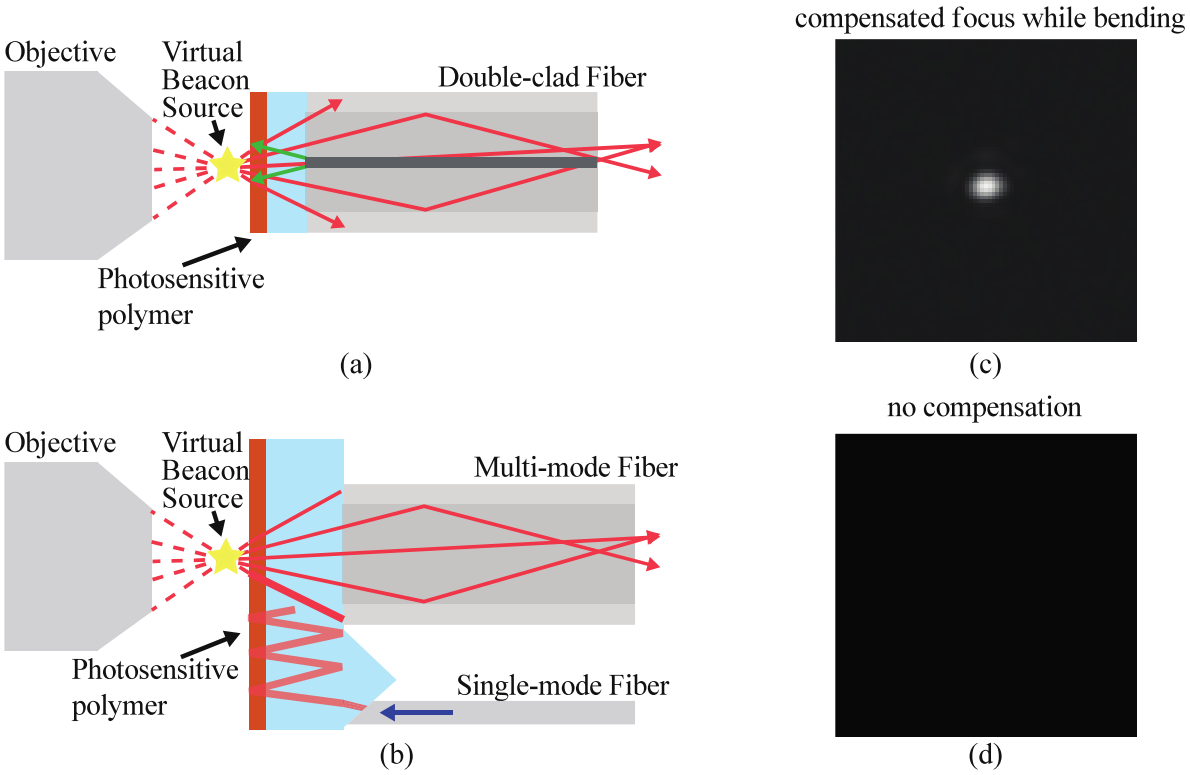

Figure 2: (a) and (b). The virtual beacon point source is experimentally implemented3 by recording at the fiber tip, a permanent hologram of a spherical wave whose virtual focus point is in front of the fiber facet. (c) and (d) Focusing through the multimode fiber with and without bending compensation using the virtual beacon source.

In conclusion, we propose and have demonstrated a new paradigm of miniature endomicroscopes using multimode fibers as the optical element. Exploiting wavefront shaping techniques and optical field manipulation in the digital domain, we have gained access to a very powerful toolbox that can bring optical microscopy and photoexcitation directly inside the body through the exploitation of high information capacity miniature optical elements like multimode fibers. This type of new endomicroscopes can pave new ways towards in-vivo cellular diagnosis, phototherapy (laser ablation) and photoexcitation (optogenetics).

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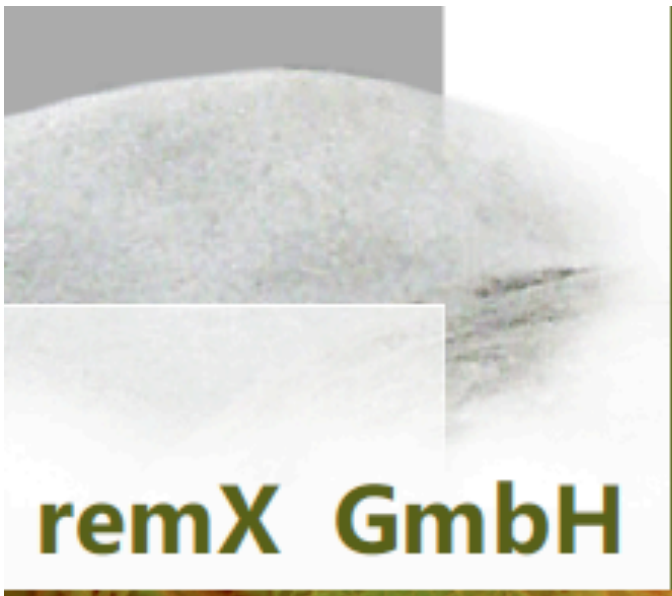

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