

OPTOGEN 2024

9th International Workshop on Technologies
for Optogenetics and Neurophotonics

April 15–17, 2024
Prague, Czech Republic





LIGHT
CONVERSION

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PROGRAM AT A GLANCE



keynote talk



invited talk



industry talk



contributed talk

MONDAY 15. 4. 2024

16:00-17:00	REGISTRATION	
17:00-17:15	WELCOME	
17:15-18:00	Daniel Razansky	Optical and optoacoustic monitoring of ultrasound brain interventions
18:00-18:30	Catherine Hall	Oxygen availability in the brain: When and where could it limit neuronal function?
from 18:30	WELCOME RECEPTION	

TUESDAY 16. 4. 2024

8:00-8:45	REGISTRATION	
8:45-9:30	Hervé Rigneault	Nonlinear flexible endoscopy using specialty optical fibers for 'freely moving' neuron activity imaging
9:30-10:00	Tommaso Patriarchi	EMBO Young Investigator Lecture: A next-generation optical toolkit to study GPCR signaling
10:00-10:30	Sebastian Haesler	Neural circuits underlying curiosity-driven exploration
10:30-10:50	<i>NKT Photonics</i>	
	<i>FEMTONICS</i>	
10:50-11:30	Coffee break	
11:30-12:00	Ferruccio Pisanello	Neurophotonics with tapered optical fibers: from optogenetics to plasmonic neural interfaces
12:00-12:30	Martin Booth	Adaptive optics for deep tissue microscopy
12:30-13:00	<i>Light Conversion</i>	
	<i>DeepEn</i>	
	<i>In-Vision technologies</i>	
13:00-14:00	Lunch break	
14:00-14:45	Martin Lauritzen	Two-photon microscopy imaging at the blood-brain barrier
14:45-15:15	Martin Fuhrmann	From synapses to neuronal networks – in vivo imaging to understand learning and memory
15:15-15:45	Serge Charpak	Two photon fluorescence and phosphorescence microscopy applied to functional imaging in the mouse brain
15:45-16:30	Coffee break	
15:45-18:00	POSTER SESSION	
from 18:00	SOCIAL EVENT	

WEDNESDAY 17. 4. 2024

8:45-9:30	Sanja Bauer Mikulovic	To move or not: Using optogenetics, electrophysiology, and imaging techniques to study goal-directed movement in mice
9:30-10:00	Aleš Stuchlík	Optogenetics and experimental psychosis in rats: Putting it in a context
10:00-10:30	Anna Beroun	Processing of natural and addictive rewards – in vivo activity of central amygdala
10:30-10:50	<i>CLASS 5 PHOTONICS</i>	
	<i>Vector Builder</i>	
10:50-11:30	Coffee break	
11:30-12:00	Peter Saggau	Acousto-optic Approaches to Neurophotonics
12:00-12:30	Ondřej Novák	High-speed optical methods to reveal cell type-specific activity related to seizures in a mouse model of chronic epilepsy
12:30-12:50	<i>Holoeye</i>	
	<i>TOPTICA</i>	
12:50-14:00	Lunch break	
14:00-14:45	Weijian Zong	Miniature two-photon microscopes for studying brain microcircuits in freely moving animals
14:45-15:15	Rob Wykes	Optogenetics and Graphene micro-transistor arrays as tools to investigate spreading depolarizations and seizure susceptibility in awake mice
15:15-15:45	Malte Gather	Organic LEDs for Optical Stimulation – Learning from the Display Industry?
15:45-16:30	Coffee break	
16:30-16:45	Ruth Sims	Scanless two-photon voltage imaging for all-optical neurophysiology
16:45-17:00	Hongbo Jia	Optogenetic tests of a group of urination control cells
17:00-17:15	Stella Aslanoglou	On-fiber printed polymeric neural probes
17:15-17:30	CLOSING REMARKS	

USEFUL INFO

VENUE

OPTOGEN2024 will be held in Martinicky palace in the heart of Prague. Renaissance palace is dating back to the 16th century with original sgraffito decorations on the walls, representing scenes from the Old Testament. The Martinic Palace is right at the corner of the Hradčany Square.

SOCIAL EVENTS

Welcome reception will take place on Monday, April 15, at 18:30 at the conference venue.

The social dinner and concert will take place on Tuesday, April 16 at 18:00 at the Klášterní Pivovar Strahov (Strahovské nádvoří 301).

ORAL PRESENTATIONS

Keynote talks will have a 40min time slot + 5min for discussion.

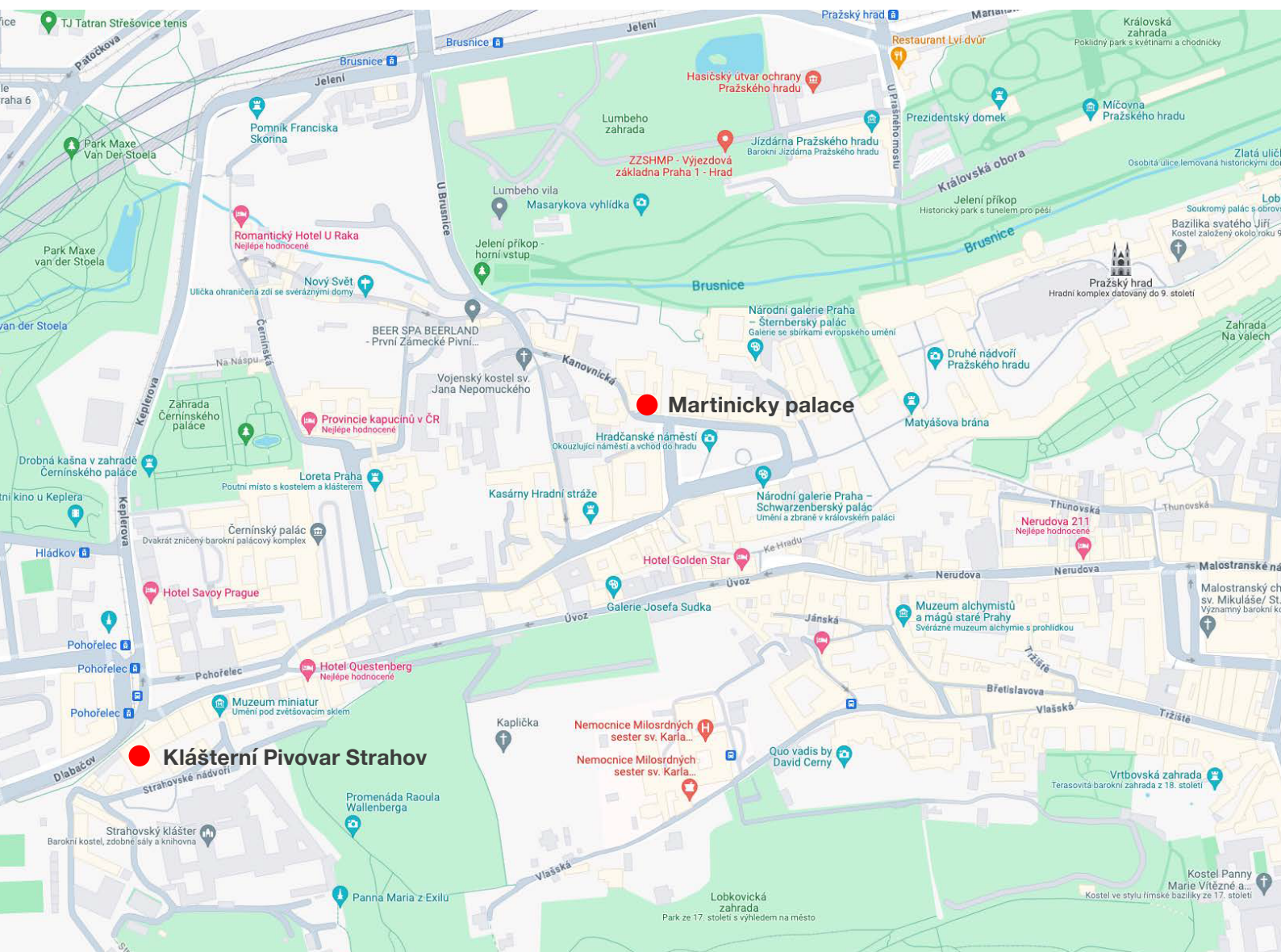
Invited talks will have a 30min time slot, consisting of 25min presentation + 5min for discussion.

Selected contributions will have a 12min time slot + 3min for discussion.

POSTER PRESENTATIONS

Poster should be set by 8:45 on Tuesday (April 16) and will be displayed during both days of the workshop.

The available display area is width: 80 cm, height: 110 cm; please note the orientation is portrait.



INVITED TALKS

Monday 15. 4. 17:15-18:00

Optical and optoacoustic monitoring of ultrasound brain interventions

Razansky D.^{1,2}

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Monitoring brain responses to ultrasonic interventions is becoming an important pillar of a growing number of applications employing acoustic waves to actuate and cure the brain [1]. Progress in studying the brain pathologies and therapeutic ultrasound effects is often hindered by the limited capacity of the commonly used neuroimaging techniques for *in vivo* brain-wide mapping with sufficient resolution and sensitivity. Optical brain interrogation provides a unique means for retrieving functional and molecular information related to brain activity and disease-specific biomarkers [2-5]. However, high resolution brain imaging with optical techniques is commonly limited to superficial cortical layers and often involves highly invasive craniotomy procedures. The hybrid optoacoustic imaging methods have recently enabled deep tissue imaging with optical contrast at high spatial and temporal resolution. The strong optical absorption of hemoglobin and excellent spectroscopic selectivity of the technique allows for the visualization of vascular structures and hemodynamic responses across the whole mouse brain entirely noninvasively [4]. To this end, multi-spectral optoacoustic imaging techniques have been used to image fast neuronal activity via genetically encoded calcium indicators and map the biodistribution of Amyloid beta plaques and tau tangles with targeted optical probes [5], including in deeply embedded hippocampal and thalamic areas inaccessible by conventional intravital microscopy. The marriage between light and sound thus brings together the highly complementary advantages of both modalities toward high precision interrogation, stimulation, and therapy of the brain with strong impact in the fields of ultrasound neuromodulation, gene and drug delivery, or noninvasive treatments of neurological and neurodegenerative disorders.

Invited Speaker's short bio. Daniel Razansky is a Full Professor of Biomedical Imaging with double appointments at the Faculty of Medicine, University of Zurich and Department of Information Technologies and Electrical Engineering, ETH Zurich in Switzerland, where he also serves as Director of the joint Preclinical Imaging Center. He earned degrees in Biomedical and Electrical Engineering from the Technion - Israel Institute of Technology and conducted postdoctoral research at the Harvard Medical School. Previously, he was Professor of Molecular Imaging Engineering at the Technical University of Munich and Helmholtz Center Munich in Germany. The Razansky Lab pioneered a number of bio-imaging technologies that were successfully commercialized and put into use in research labs and clinical facilities across the globe, among them the multi-spectral optoacoustic tomography (MSOT) and hybrid optoacoustic ultrasound (OPUS). His research has been recognized by the German Innovation Prize and multiple awards from the ERC, NIH, SNF, DFG and HFSP. He is an elected Fellow of the IEEE, SPIE and Optica Societies.

References

D. Blackmore, D. Razansky, J. Götz, Ultrasound as a versatile tool for short- and long-term improvement and monitoring of brain function, *Neuron* 111, 1174-1190 (2023).

H. Estrada, J. Robin, A. Özbek, Z. Chen, A. Marovsky, Q. Zhou, D. Beck, B. le Roy, M. Arand, S. Shoham, D. Razansky, High resolution fluorescence-guided transcranial ultrasound mapping in the live mouse brain, *Science Adv* 7(50), eabi5464 (2021).

H. Estrada, A. Özbek, J. Robin, S. Shoham, D. Razansky, Spherical array system for high precision transcranial ultrasound stimulation and optoacoustic imaging in rodents, *IEEE Trans Ultras Ferr Freq Cont* 68(1), 107-115 (2021).

Z. Chen, Q. Zhou, X. L. Deán-Ben, I. Gezginer, R. Ni, M. Reiss, S. Shoham, D. Razansky, Multi-modal noninvasive functional neurophotonic imaging of murine brain-wide sensory stimulation, *Adv Sci* 9(24), 2105588 (2022).

R. Ni, Z. Chen, X. L. Deán-Ben, F. F. Voigt, D. Kirschenbaum, G. Shi, A. Villosio, Q. Zhou, A. Crimi, P. Arosio, R. M. Nitsch, K. P. R. Nilsson, A. Aguzzi, F. Helmchen, J. Klohs, and D. Razansky, Multiscale optical and optoacoustic imaging of amyloid- β deposits in mice, *Nature Biomed Eng* 6, 1031-1044 (2022).

Monday 15. 4. 18:00-18:30

Oxygen availability in the brain. When and where could it limit neuronal function?

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Neuronal activity is energetically expensive. To match energy supply with demand, the brain tightly regulates its blood supply, increasing local blood flow in response to increases in neuronal activity. A failure in this regulation may be a key factor in initiating neurodegenerative diseases, as decreases in cerebral blood flow occur before other symptoms and conditions such as hypertension, lung disease or sleep apnoea that lower blood oxygen saturation increase the risk of diseases such as Alzheimer's disease [1–3].

To understand how brain oxygenation could shape brain physiology and pathophysiology, we used haemoglobin spectrometry and 2-photon imaging in awake mice to measure blood oxygenation and neurovascular coupling in brain regions that are differentially sensitive to neurodegeneration (visual cortex: low risk, hippocampus: high risk). Blood oxygenation was lower in the hippocampus than the visual cortex, and neurovascular coupling was weaker [4]. Modelling oxygen diffusion suggested that some neurons in the hippocampus exist at a watershed, whereby a small decrease in oxygenation could lead to tissue becoming hypoxic. To test this, we acutely reduced the amount of oxygen mice inspired, to generate mild hypoxia. This increased blood flow similarly to both regions but the hippocampus then became more hypoxic than the visual cortex. Surprisingly, oxygen consumption rates increased as inspired oxygen was reduced, and hippocampal excitatory neurons showed an increase in basal calcium levels and neuronal activity. This hypoxic increase in activity and oxygen consumption could promote neuronal damage when blood flow and blood oxygen levels are reduced early in neurodegenerative disease.

Invited Speaker's short bio. Catherine Hall is Professor of Neurovascular Physiology at the University of Sussex, United Kingdom. After a PhD at University College London with John Garthwaite on the kinetics of nitric oxide signalling, she did her post-doctoral research with David Attwell, where she quantified the processes consuming most oxygen during neuronal signalling and demonstrated the importance of pericytes for blood flow regulation in health and disease. Since starting her own lab, she has been interested in how the balance between oxygen consumption and supply is altered physiologically and pathophysiologically and how this could drive worsening disease states.

References

- L.J. Launer, G.W. Ross, H. Petrovitch, K. Masaki, D. Foley, L.R. White, et al. *Neurobiol. Aging*. 2000;21(1):49–55.
J.W. Dodd. *Alzheimers Res Ther*. 2015 Mar 21;7(1):32.
A.G. Andrade, O.M. Bubu, A.W. Varga, R.S. Osorio. *J Alzheimers Dis*. 2018;64(s1):S255–70.
K. Shaw, L. Bell, K. Boyd, D.M. Grijseels, D. Clarke, O. Bonnar, et al. *Nature Communications*. 2021 May 27;12(1):3190.

Tuesday 16. 4. 8:45-9:30

Nonlinear flexible endoscopy using specialty optical fibers for ‘freely moving’ neuron activity imaging

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I will present our effort to develop novel flexible endoscopes based on specialty optical fibres that can activate nonlinear contrasts such as 2Photon, 3Photon, harmonic generation (SHG, THG) and coherent Raman (CARS) at the end of miniature imaging probes. I will focus first on a multimodal nonlinear micro-endoscope for real-time, label-free imaging of biological tissues using a specialty hollow core fibre [1][2][3]. The endoscope has a diameter of 2mm and can perform 2Photon and 3 Photon fluorescence, SHG, THG and CARS at 20 frames/s over a field of view of 400 μ m. I will present recent developments that show how this endoscope can be attached to the head of a mouse to follow neuron calcium activity in freely moving experiments. I will then move to the development of a flexible endoscope based on multi-core fibers that represents the ultimate limit in miniaturization [4]. This ‘lensless endoscope’ uses wavefront control on the proximal side to focus and scan a beam at its distal side to perform 2Photon imaging [5]. The endoscope has a diameter of 150 μ m and uses a twisted [6] “tapered multi-core fibre (MCF)” [7], it is designed for integration into ultra-miniaturized endoscopes for minimally invasive two-photon point-scanning imaging.

Invited Speaker’s short bio. Hervé Rigneault is research director at French research agency CNRS. He graduated with an engineer degree in optics in 1991 from Ecole Centrale Marseille and got his PhD from Aix-Marseille University in 1994 in the field of nonlinear optics. He obtained his habilitation from Aix Marseille Univ in 2000. Since then, he is developing optical techniques for life science applications and created the Mosaic group at the Fresnel Institute in 2000 (<https://www.fresnel.fr/spip/spip.php?article1102>). He is the author of more than 240 publications in the field of optics, optical spectroscopy, and molecular imaging. He was awarded with the CNRS Bronze medal in 2000, the IXcore foundation award in 2022 and the CNRS excellence award in 2021 and 2014. He became an Optica Fellow in 2020 and obtained and ERC advanced grant in 2021 in the field coherent Raman imaging for biomedical applications. He is also developing nonlinear endoscopy through specialty fibers that will be the topic of the presentation.

References

- [1]D. Septier, et al., Optics Express 30, 25020-25033 (2022). <https://doi.org/10.1364/OE.462361>
- [2]A. Kudlinski, et al.,” Optics Express 28, 15062-15070 (2020).<https://doi.org/10.1364/OE.389084>
- [3]A. Lombardini, et al., Light: Sci & Appl 7, 10 (2018). <https://doi.org/10.1038/s41377-018-0003-3>
- [4]E. R. Andresen, et al. Optics Letters 38, 609-611 (2013). <https://doi.org/10.1364/OL.38.000609>
- [5]E. Andresen, et al. Opt. Expr. 21, 20713-20721 (2013), <https://doi.org/10.1364/OE.21.020713>
- [6]V. Tsvirkun, et al., Optica 6, 1185-1189 (2019). <https://doi.org/10.1364/OPTICA.6.001185>
- [7]F. El Moussawi, et al., ACS Photonics (2022), <https://doi.org/10.1021/acsp Photonics.2c00661>

A next-generation optical toolkit to study GPCR signaling

Kagiampaki Z.^{1,#}, Rohner V.^{1,#}, Kiss C.^{1,#}, Curreli S.², Dieter A.³, Chernysheva M.¹, Harada M.¹, Duss S. N.⁴, Dernic J.¹, Bhat M. A.¹, Zhou X.¹, Ravotto L.¹, Ziebarth T.⁵, Benke D.^{1,6}, Weber B.^{1,6}, Bohacek J.^{4,6}, Reiner A.⁵, Wiegert J. S.³, Fellin F.², Patriarchi T.^{1,6,*}

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Genetically-encoded sensors are a key emerging technology that is much needed to visualize the extracellular dynamics of neuromodulators [1-3]. In particular these tools allow us to monitor behaviorally-relevant and task-specific catecholamine fluctuations, the kinetics of their release and uptake, as well as the spatial organization of the release events in the brain. Our laboratory is currently focused on the development of highly-sensitive and multicolor genetically-encoded optical probes for catecholamines [4]. In this talk I will introduce our recent progress in this direction. The last part will provide a personal view on exciting new research questions in neurochemistry that are now within reach with these tools at hand, and on what should come next in technological development.

Invited Speaker's short bio. Tommaso Patriarchi is Assistant Professor of Chemical Neuropharmacology at the University of Zurich since 2019. He obtained his PhD in 2015 from the University of Siena, Italy, and worked as a postdoctoral fellow at the University of California Davis. He developed dLight1, the first genetically encoded sensors that enabled high-resolution in vivo imaging of dopamine dynamics in living animals. Research in his lab focuses on developing next-generation optical tools for observing and controlling the action of neuromodulators in the brain. Tommaso is the recipient of several grants and awards, including an ERC Starting Grant 2020, a project grant from the Swiss National Science Foundation, the Young Scientist Lectureship Award by the International Society for Neurochemistry (2023) and is an EMBO Young Investigator since 2024.

References

Simpson EH*, Akam T*, Patriarchi T*, Blanco-Pozo M, Burgeno LM, Mohebi A, Cragg SJ, Walton ME. (2023) Lights, fiber, action! A primer on in vivo fiber photometry. *Neuron*; doi: 10.1016/j.neuron.2023.11.016f

Duffet L, Williams ET, Gresch A, Chen S, Bhat MA, Benke D, Hartrampf N*, Patriarchi T*. (2023) Optical tools for visualizing and controlling human GLP-1 receptor activation with high spatiotemporal resolution. *eLife*; doi: 10.7554/eLife.86628.1

Duffet L., Kosar S., Panniello M., Viberti B., Bracey E., Zych A.D., Radoux-Mergault A., Zhou X., Dernic J., Ravotto L., Tsai Y.-C., Figueiredo M., Tyagarajan S.K., Weber B., Stoeber M., Gogolla N., Schmidt M.H., Adamantidis A.R., Fellin T., Burdakov D., Patriarchi T. (2022) A genetically encoded sensor for in vivo imaging of orexin neuropeptides. *Nat Methods*; doi:10.1038/s41592-021-01390-2

Kagiampaki Z, Rohner V, Kiss C, Curreli S, Dieter A, Wilhelm M, Harada M, Duss SN, Dernic J, Bhat MA, Zhou X, Ravotto L, Ziebarth T, Wasielewski LM, Sönmez L, Benke D, Weber B, Bohacek J, Reiner A, Wiegert JS, Fellin T, Patriarchi T. (2023) Sensitive multicolor indicators for monitoring norepinephrine in vivo. *Nat Methods*; doi: 10.1038/s41592-023-01959-z

Tuesday 16. 4. 10:00-10:30

Neural circuits underlying curiosity-driven exploration

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Curiosity refers to the intrinsic desire of humans and animals to explore the unknown even when there is no apparent reason to do so. The most fundamental form of curiosity may be found among orienting behaviors. Across animal species, novel or surprising stimuli elicit arousal and evoke sensory inspection and exploration. These orienting responses habituate after few exposures, suggesting a very rapid form of non-associative learning. At the level of neural circuits, orienting involves distinct processing steps including the evaluation of sensory stimuli to detect novelty and surprise, the activation of catecholaminergic systems and eventually the initiation of orienting reactions. In my lab, we investigate these processing steps using brain-wide functional ultrasound imaging, large-scale electrophysiology, and cell-type specific manipulations in mice in order to understand how neural circuits transform sensory inputs into curious exploration behaviors.

Short Bio Sebastian Haesler did his PhD at the Max-Planck-Institute for Molecular Genetics in Berlin, Germany, and postdoctoral work at the Center for Brain Science at Harvard University. In 2013, he joined Neuro-Electronics Research Flanders (NERF) as a group leader. Since 2015 he serves as director of NERF. NERF was founded by the leading nano-electronics research center [imec](#), the Flemish life sciences research institute [VIB](#) and [KU Leuven](#), one Europe's oldest research universities. The goal of NERF is to advance our understanding of the brain in health and disease by bringing together research in systems neuroscience and neuroengineering.

Selected Publications

Xiaohua Huang, Horacio Londoño-Ramírez, Marco Ballini, Chris Van Hoof, Jan Genoe, **Sebastian Haesler**, Georges Gielen, Nick Van Helleputte, Carolina Mora Lopez (2022). Actively Multiplexed μ ECoG Brain Implant System with Incremental- $\Delta\Sigma$ ADCs Employing Bulk-DACs. *IEEE Journal of Solid-State Circuits*.

Rik Van Daal, Çağatay Aydın, Frederic Michon, Arno Aarts, Michael Kraft, Fabian Kloosterman, **Sebastian Haesler** (2021). Implantation of Neuropixels probes for chronic recording of neuronal activity in freely behaving mice and rats. *Nature Protocols*.

Joachim Morrens, Çağatay Aydın, Aliza Janse van Rensburg, José Esquivelzeta Rabell, **Sebastian Haesler** (2020). Cue-Evoked Dopamine Promotes Conditioned Responding during Learning. *Neuron*.

Jean Delbeke, **Sebastian Haesler**, Dimiter Prodanov (2020). Failure Modes of Implanted Neural Interfaces. *Neural Interface Engineering: Linking the Physical World and the Nervous System*.

Tuesday 16. 4. 11:30-12:00

Neurophotonics with tapered optical fibers: from optogenetics to plasmonic neural interfaces

Ferruccio Pisanello F.^{1,8,*}, Filippo Pisano F.^{1,6}, Antonio Balena A.¹, Barbara Spagnolo B.^{1,8}, Andriani M. S., Di Zheng¹, Bianco M.¹, Kazmadeh M.¹, Colallard L. J.¹, Piscopo L.¹, Montinaro C.^{1,8}, Al Masmudi M.³, F. Tantussi⁴, De Angelis F.⁴, Perez T. J.⁵, Aslanoglu S.¹, Sabatini B. L.⁷, Valiente M.³, Liset M., De La Prida⁵, De Vittorio M.^{1,2,8}

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The adoption of multimodal optical fibers to access deep brain regions has empowered the neurophotonics community to pioneer new frontiers in optically interfacing with the mammalian brain. These advancements not only enhance optical control and monitoring of neural activity but also integrate multiple capabilities into a single device, including electrophysiological and neurochemical detection. Furthermore, they accelerate the exploration of emerging optical neural interface paradigms, such as surface plasmon resonances, which are still in their early stages of development.

Within this framework, this presentation will delve into neural interfaces that leverage the synergistic combination of nanotechnologies and modal properties of tapered optical fibers (TFs) [1-5]. We will discuss how the wide surface area of the fiber taper enables the use of spontaneous Raman spectroscopy to extract relevant information on the cytoarchitecture of the mouse brain, monitoring molecular alterations linked to circuit dysfunction as well as diagnostic markers of various pathologies. Using an original two-photon lithography approach to pattern the surface of the taper, enabling optical interactions with the brain alongside extracellular electrophysiology and in-situ temperature sensing, allowing to better study the effects of light radiation on neural tissue. This non-planar patterning can achieve resolutions down to a few nanometers through unconventional bottom-up nanofabrication, paving the way for the implementation of Surface Enhanced Raman Spectroscopy (SERS) and thermoplasmonics in neuroscience research.

Invited Speaker's short bio. Ferruccio Pisanello leads the research unit "Multifunctional Neural Interfaces" at the Center for Biomolecular Nanotechnologies of the Italian Institute of Technologies in Lecce. He holds a Master's degree in Telecommunication Engineering from the University of Salento (Lecce) and a Ph.D. in Physics (Quantum Optics) from Pierre et Marie Curie University (Paris). His team is dedicated to pioneering innovative approaches to interface with the central nervous system, leveraging unconventional applications of physical phenomena to develop a new generation of devices capable of extracting multifunctional signals from the brain and influencing its physiology.

References

B. Spagnolo et al, Nature Materials 21, 826 (2022)

D. Zheng et al., Advanced Materials 35, 2200902 (2023)

L. Collard et al, Small 18, 2200975 (2022)

F. Pisano et al, Advanced Optical Materials 10, 2101649 (2022)

F. Pisano et al, Bioarxiv, <https://doi.org/10.1101/2022.06.24.497456> (2022)

Adaptive optics for deep tissue microscopy

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Adaptive optics (AO) has been incorporated into high resolution microscopes for a range of purposes [1]. AO devices, such as deformable mirrors, liquid crystal spatial light modulators or deformable phase plates, introduce phase distortions into optical wavefronts. Most commonly, AO has been widely used to correct the optical aberrations introduced when focusing through inhomogeneous biological tissue. This has enabled effective imaging at depths in tissue where non-AO microscopy is ineffective. A particularly common application is in multiphoton microscopy for structural or functional imaging hundreds of micrometres deep in brain tissue [2]. AO has also enabled microscopy through multi-mode optical fibres, which provide a route to deep tissue imaging through micro-endoscopes [3]. Super-resolution microscopy has revealed details of specimens at resolutions far below the diffraction limit, but these methods are particularly susceptible to the effects of aberrations, with problematic effects occurring even at depths of a few micrometres. AO has again been deployed successfully to overcome these problems [4]. In addition to aberration correction, AO has been used to perform remote focusing, through which the imaged field is focused not through specimen motion, but by modulation of the optical wavefronts. This permits rapid volumetric microscopy without specimen agitation, which has applications in functional neural imaging [5]. AO concepts have also been developed to address additional imaging dimensions. Combinations of AO elements have been shown to benefit imaging through control of spatiotemporal properties of ultrashort laser pulses [6]. Further advances have been made in the introduction of vectorial AO, in which both phase and polarisation errors are corrected [7].

Biography. Prof Booth is chair in Optical and Photonic Engineering at the University of Oxford. His research involves the development and application of adaptive optical methods in microscopy, laser-based materials processing and biomedical imaging. In particular, his group have developed numerous implementations of adaptive optics for aberration correction in high and super resolution microscopes. He has held Royal Academy of Engineering and EPSRC Research Fellowships and in 2016 received an Advanced Grant from the European Research Council. In 2014 he was awarded the International Commission for Optics Prize. He was appointed Professor of Engineering Science in 2014 and Chair in Optics and Photonics in 2023. He is a fellow of SPIE, Optica, and the Institute of Physics. He has over 180 publications in peer-reviewed journals, over twenty-five patents, and has co-founded two spin-off companies, Aurox Ltd and Opsydia Ltd.

References

- [1]KM Hampson, et al., Nature Reviews Methods Primers 1 (68), 1-26, (2021) <https://doi.org/10.1038/s43586-021-00066-7>
- [2]Q Hu, et al., Light Sci Appl 12, 270 (2023) <https://doi.org/10.1038/s41377-023-01297-x>
- [3]R Turcotte, et al., Optics Lett., 44 (10), 2386-2389, (2019). <https://doi.org/10.1364/OL.44.002386>
- [4]MGM Velasco, et al., Optica 8 (4), 442-450, (2021) <https://doi.org/10.1364/OPTICA.416841>
- [5]M Žurauskas, et al., Biomed. Opt. Exp. 8 (10), 4369-4379 (2017) <https://doi.org/10.1364/BOE.8.004369>
- [6]B Sun, et al., Light: Science & Appl. 7, e17117, (2018). <https://doi.org/10.1038/lsa.2017.117>
- [7]C. He, et al., eLight 3, 23 (2023). <https://doi.org/10.1186/s43593-023-00056-0>

Two-photon microscopy imaging at the blood-brain barrier

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Invited talks - 250 words abstract. The need for new treatments for brain diseases is growing with the increasing lifespan of western populations. However, drug transport across the blood-brain barrier (BBB) is a great challenge because of the low permeability of the barrier. This presentation will outline key mechanisms for transporting cargo across the BBB in vivo as applied to normal brains¹, brains with low levels of activity at the sphingosine-1-receptor² and in the 5XFAD model of Alzheimer's disease³. Our recent work has outlined new methodologies to quantify paracellular transport in brain arterioles and capillaries, adsorption mediated transcytosis (AMT), receptor mediated transcytosis (RMT) and the density of glycocalyx in cortical microvessels in vivo⁴. This enables us to provide a detailed analysis of mechanisms and pharmacokinetics of drugs and drug vehicles in health and disease. Our work has revealed a segregation of transport functions along the microcirculation from the arteriolar to the venous end of the vascular tree. Furthermore, we have developed new tools to examine trafficking of large molecules and drug carriers in brain extracellular space, which enables us to assess drug bioavailability in brain tissue and important aspects of pharmacodynamics. Our projects combine research on drug transport systems with research on BBB and the neurovascular unit. The prospect of this work is to make a breakthrough in mechanistic insight to the functional organization of the BBB, and to push boundaries in the universal efforts to influence drug delivery across the BBB for the benefit of patients.

Invited Speaker's short bio. Professor Martin Lauritzen has been a pioneering clinician-scientist in the field of cerebrovascular biology for over four decades. He has worked on our understanding of neurovascular functions, including the blood-brain barrier (BBB) and the role of cortical spreading depolarization/depression (CSD) in migraine and acute brain injury. His first major contribution was his early work on migraine, and CSD as the mechanism of this neurological disorder, and later he focused on the role of CSDs in acute neurologic injury. A second major focus of Martin's research program has been the mechanistic understanding of neurovascular coupling, i.e., the regulation of cerebral blood flow (CBF) by neural activity and the generation of functional Magnetic Resonance Imaging (fMRI) signals in health, aging, and disease. Martin has developed advanced new imaging and image analysis tools for multimodal measurements of brain activity that underlie the cellular and molecular origin of neurovascular coupling. A third and recent focus is the exploration of the BBB by multiphoton microscopy and the quantification of permeability characteristics along para- and transcellular pathways. Martin's lab operates at the interface between neuroscience and clinical neurology, bridging the worlds of biology and technology, advancing measurement methods, and applying novel methods for addressing central neuroscience questions relevant for neurovascular physiology, regulation of CBF and metabolism, and the interpretation of findings in brain aging and disease states.

References

- (1) Kutuzov, N.; Flyvbjerg, H.; Lauritzen, M. Contributions of the glycocalyx, endothelium, and extravascular compartment to the blood-brain barrier. *Proc Natl Acad Sci U S A* 2018, *115* (40), E9429-E9438. DOI: 10.1073/pnas.1802155115.
- (2) Mathiesen Janiurek, M.; Soylu-Kucharz, R.; Christoffersen, C.; Kucharz, K.; Lauritzen, M. Apolipoprotein M-bound sphingosine-1-phosphate regulates blood-brain barrier paracellular permeability and transcytosis. *Elife* 2019, *8*. DOI: 10.7554/eLife.49405.
- (3) Zhukov, O.; He, C.; Soylu-Kucharz, R.; Cai, C.; Lauritzen, A. D.; Aldana, B. I.; Bjorkqvist, M.; Lauritzen, M.; Kucharz, K. Preserved blood-brain barrier and neurovascular coupling in female 5xFAD model of Alzheimer's disease. *Front Aging Neurosci* 2023, *15*, 1089005. DOI: 10.3389/fnagi.2023.1089005 From NLM PubMed-not-MEDLINE.
- (4) Kucharz, K.; Kristensen, K.; Johnsen, K. B.; Lund, M. A.; Lonstrup, M.; Moos, T.; Andresen, T. L.; Lauritzen, M. J. Post-capillary venules are the key locus for transcytosis-mediated brain delivery of therapeutic nanoparticles. *Nat Commun* 2021, *12* (1), 4121. DOI: 10.1038/s41467-021-24323-1.

Tuesday 16. 4. 14:45-15:15

From synapses to neuronal networks – *in vivo* imaging to understand learning and memory

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Memory loss is a characteristic of dementia like Alzheimer's disease (AD). AD affects the brain progressively on different levels, at synapses, individual neurons and neuronal networks. We combine intravital imaging with optogenetic and chemogenetic neuronal manipulations to address the cellular and molecular mechanisms underlying this devastating disease. We discovered that contextual memories in the hippocampus that are represented by engram cells, are still present in mice with Alzheimer's disease like pathology. The reason, why the memory cannot be recalled properly anymore, are superimposed cells that encode a novelty experience. Thus, interference of different memory traces prevents the recall of the memory [1]. The mouse does not recall the learned context, but rather thinks it is experiencing a novel context. This is reflected on the neuronal network level in the hippocampus.

Impaired inhibitory neuron function has been hypothesized to underly hyperactivity and epileptiform activity in mice with an AD-like pathology. We have shown that somatostatin positive interneurons play an important role in gating information from cortical areas and contribute to memory impairment under AD-like conditions [2]. In addition, parvalbumin positive (PV+) interneurons regulate oscillations in the hippocampus. We tested, whether optogenetic stimulation of PV+ interneurons in a closed loop configuration was sufficient to improve memory in AD mice. Indeed, optogenetic enhancement of theta oscillations improved spatial and recognition memory. These results might be used for future therapeutic interventions aimed at restoring neuronal network function in AD.

Short bio

Education/Degrees:

- 1997 – 2002 Diplom Biologe t.o., University of Stuttgart
- 2002 – 2006 Doctoral thesis, Prof. Herms, Center of Neuropathology & Prion Research, Ludwig-Maximilians University of Munich
- 2007 Dr. rer. nat. (summa cum laude), Prof. Hock, Technical University of Munich
- 2017 Habilitation, Mechanisms of Neurodegeneration, University of Bonn

Academic Career:

- 2007 – 2010 Postdoctoral fellow, Center of Neuropathology & Prion Research, Ludwig-Maximilians University of Munich
- 2010 – 2018 Junior Research Group Leader, German Center for Neurodegenerative Diseases (DZNE), Bonn
- 2018 Research Group Leader, German Center for Neurodegenerative Diseases (DZNE), Bonn
- Since 2020 Professor for Neuroimmunology and Imaging, Bonn University and DZNE

Awards and Honors:

- 2016 DZNE Prize
- 2019 ERC CoG 2019, European Research Council Consolidator Grant

References

- Poll S, Mittag M, Musacchio F, Justus LC, Giovannetti EA, ...**Fuhrmann M.** (2020) Memory trace interference impairs recall in a mouse model of Alzheimer's disease. **Nat Neurosci.** 23(8):952-958.
- Schmid LC, Mittag M, Poll S, Steffen J, Wagner J, Geis HR, Schwarz I, Schmidt B, Schwarz MK, Remy S, **Fuhrmann M.** (2016) Dysfunction of Somatostatin-Positive Interneurons Associated with Memory Deficits in an Alzheimer's Disease Model. **Neuron** 92(1):114-125.
- Ambrad Giovannetti E., Poll S, Justus D, Kaneko H, Fuhrmann F, Steffen J, Remy S, Fuhrmann M. (2018) Restoring memory by optogenetic synchronization of hippocampal oscillations in an Alzheimer's disease mouse model. **bioRxiv.** 363820, doi:10.1101/363820

Tuesday 16. 4. 15:15-15:45

Two photon fluorescence and phosphorescence microscopy applied to functional imaging in the mouse brain.

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In the few years since its demonstration in 1990, the use of two-photon laser scanning microscopy to measure neuronal activity and blood flow with high spatio-temporal resolution in the brain of anaesthetised and awake rodents has expanded rapidly. By collecting fluorescence and phosphorescence photons emitted by exogenous and endogenous sensors, it is now possible to decipher the dynamics of different intracellular, extracellular and intravascular compartments during simple sensory stimulation or complex tasks. I will review some of our findings that allow interpretation of the extent to which mesoscopic imaging, such as fUS and BOLD fMRI, reflects cellular activity [1-7]. I will then describe an unpublished work in which we are investigating the role of pH and CO₂ in triggering neurovascular coupling, the ensemble of signalling pathways triggered by neuronal activation and is responsible for the vascular signal underlying the BOLD fMRI signal.

Short bio. Since its origin, my laboratory has associated neuroscientists and physicists to develop new tools to study brain activity at microscopic and mesoscopic levels in vivo. Over the last decade, my team has pursued several optical developments while investigating various aspects of neurovascular coupling, functional hyperemia and oxygen consumption in the mouse olfactory bulb and neocortex. More recently, we have started to bridge cellular and mesoscopic (fUS) imaging by combining two-photon microscopy, fUS and BOLD fMRI in the same animal.

MD.Ph.D.; Postdocs in Zurich (B. Gaehwiler, Brain Research Institute) and New York (R. Llinas, NYU). Head of lab first at ESPCI and then at ParisDescartes University and Sorbonne University.

References

- Aydin, AK, Verdier, C, Chaigneau, E, & Charpak, S (2022). The oxygen initial dip in the brain of anesthetized and awake mice. *Proceedings of the National Academy of Sciences of the United States of America*, 119(14), e2200205119.
- Aydin AK, Haselden WD, Goulam Houssen Y, Pouzat C, Rungta RL, Demené C, Tanter M, Drew PJ, Charpak S*, Boido D*. (2020) Transfer functions linking neural calcium to single voxel functional ultrasound signal, *Nat Commun*. 11:2954. doi: 10.1038/s41467-020-16774-9
- Boido D, Rungta RL, Osmanski BF, Roche M, Tsurugizawa T, Le BD, Ciobanu L*, Charpak S* (2019) Mesoscopic and microscopic imaging of sensory responses in the same animal. *Nat Commun* 10:1110. doi: 10.1038/s41467-019-09082-4
- Rungta RL, Chaigneau E, Osmanski BF, Charpak S (2018) Vascular Compartmentalization of Functional Hyperemia from the Synapse to the Pia. *Neuron* 99:362-375.
- Rungta RL, Osmanski BF, Boido D, Tanter M, Charpak S (2017) Light controls cerebral blood flow in naive animals. *Nat Commun* 8:14191.
- Ducros M, Goulam HY, Bradley J, de S, V, Charpak S (2013) Encoded multisite two-photon microscopy. *Proc Natl Acad Sci U S A* 110:13138-13143.
- Parpaleix A, Houssen YG, Charpak S (2013) Imaging local neuronal activity by monitoring PO(2) transients in capillaries. *Nat Med* 19:241-246.

Wednesday 17. 4. 8:45-9:30

To move or not: Using optogenetics, electrophysiology, and imaging techniques to study goal-directed movement in mice

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Invited talks abstract: Animals move with different motivations, driven by the pursuit of rewards, avoidance of punishment, or social interaction with their conspecifics. However, not all stimuli elicit the same action responses, indicating the significance of the internal state of the animal. Employing a combination of optogenetics, electrophysiology, and imaging techniques, we delve into the neural activity within the medial septum-hippocampus system to understand how mice's goal-directed movements are predicted.

Invited Speaker's short bio. Sanja Bauer Mikulovic studied biomedical engineering at the Technical University of Vienna and received her doctorate in neuroscience from the University of Uppsala in Sweden. Subsequently, she conducted research in Sweden in the Department of Neuroscience as a postdoc on the role of specific types of interneurons in the hippocampus and their importance for oscillations underlying cognitive and emotional behaviour. In 2018 she received an international postdoc grant, which enabled her to conduct her research in parallel at the Karolinska Institute in Stockholm and at the German Center for Neurodegenerative Diseases (DZNE) in Bonn. Since January 2021 she is leading her own group Cognition and Emotion.

Wednesday 17. 4. 9:30-10:00

Optogenetics and experimental psychosis in rats: Putting it in a context

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Recent advances in schizophrenia research have increasingly focused on its neural underpinnings. Central to this area of study is decision-making and cognitive flexibility, which are significantly impaired in schizophrenia and are considered critical endophenotypes of the disorder. A notable development in understanding these cognitive impairments is the link to altered functions of NMDA receptors, leading to increased cortical activity. This has been modeled in animal studies using NMDAR antagonists to induce schizophrenia-like cognitive inflexibility. Research has identified a decrease in GABAergic inhibitory activity, contributing to an EI imbalance and desynchronizing prefrontal gamma and hippocampal theta rhythms. This study aimed to establish an acute MK-801 administration model in rats to mimic schizophrenia-like cognitive inflexibility and to investigate the potential therapeutic role of optogenetic modulation. We employed the attentional set-shifting task to assess cognitive flexibility in rats, wherein they learned to switch or reverse relevant rules. We applied in vivo optogenetic stimulations of parvalbumin-positive interneurons at specific light pulses in the prefrontal cortex and ventral hippocampus during this task. Our experiments showed that specific optogenetic stimulation of parvalbumin-positive interneurons (PVI) in the prefrontal cortex and ventral hippocampus effectively rescued cognitive flexibility in rats treated with MK-801. These results significantly contribute to the understanding of the role of PVI in the cognitive impairments associated with schizophrenia. They open new avenues for research into the mechanisms underlying these deficits and potential therapeutic interventions for this severe psychiatric disorder. The talk will also put the problem of dysfunction of PVI into a broader context.

Ales Benjamin Stuchlik. I am currently engaged as a Senior Research Scientist and serve as the Head of the Laboratory of Neurophysiology of Memory at the Institute of Physiology of the Czech Academy of Sciences. Additionally, I hold a position as a Research Scientist at the National Institute of Mental Health in Klecany, Czech Republic. My research primarily investigates the neural underpinnings of spatial cognition, encompassing the involvement of various brain structures and the neurochemical dynamics in spatial navigation. Furthermore, my work extends to examining cognitive impairments and the deterioration of neural functions in various brain disorders, utilizing animal models to elucidate these phenomena.

Wednesday 17. 4. 10:00-10:30

Processing of natural and addictive rewards – in vivo activity of central amygdala

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Natural rewards and drugs of abuse tap into the same brain circuitries and create strong, appetitive memories. It is unclear, however, why only addictive substances create such irreversible memories that lead to compulsion. In our research, using light-sheet imaging we took a holistic view of the entire, cleared mouse brain and identified regions and pathways activated in response to sugar and cocaine. Among several network hubs that were particularly involved in the processing of both rewards, we focused on the central amygdala – a brain structure that assigns emotional value to incoming stimuli. Looking closer on the populational level, focusing on the dopamine-sensitive neurons we looked at the activity of D1 and D2-type dopamine receptors-expressing neurons while a mouse received either sweet water or cocaine injection. Two-photon *in vivo* imaging revealed that the overall activity of D1-type neurons increases following reward exposure while the activity of D2-type neurons is decreased. Thus, we visualized how the intra-amygdalar network is modulated by rewards exposures.

Anna Beroun's short bio. Dr. Anna Beroun obtained her PhD from Göttingen University; in her research, she focused on cocaine-induced long-term synaptic plasticity. Combining optogenetics and electrophysiology techniques, she discovered that cocaine self-administration in rats leads to increased glutamatergic drive between the prefrontal cortex and the nucleus accumbens. After obtaining a PhD in 2013, she began a postdoctoral fellowship at the Laboratory of Neurobiology, Nencki Institute of Experimental Biology in Warsaw, Poland. Working with mouse long-term alcohol exposure models that mimic human aspects of addiction, she discovered that neurons in the central amygdala create silent synapses in response to alcohol exposure. Such silent synapse accumulation correlates with increased motivation to obtain alcohol. Moreover, in the dentate gyrus of the hippocampus, levels of silent synapses increase during alcohol relapse. This increase is larger in mice with higher addiction scores. Three years ago Anna became a group leader at Braincity, a newly established research center at the Nencki Institute of Experimental Neurobiology. Her group's research focuses on the synaptic plasticity of appetitive learning and addiction. More specifically their goal is to identify the basis of silent synapse formation and to follow their fate in various models of learning. Harnessing this transient appearance of silent synapses might give tools to alter the plasticity that leads to addiction.

Wednesday 17. 4. 11:30-12:00

Acousto-optic Approaches to Neurophotonics

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Experimental protocols based on advanced optical techniques have become state-of-the-art in Neuroscience. Key requirements of the employed techniques are high spatio-temporal resolution, sufficient penetration depth overcoming light scattering, and absence of photodamage to living brain tissue. To comply with these fundamental needs, various approaches to Neurophotonics have been taken and many advanced systems have been developed and are successfully utilized.

One such neurophotonics technique is based on dynamic diffractive optical elements which can be acoustically steered with ultrasound in the radio frequency domain. These acousto-optic devices (AODs) are highly versatile elements and allow us to implement various types of advanced laser-based microscopes useful for Neurophotonics.

In my talk, I will first introduce the fundamentals of acousto-optic devices [1] and then review several AOD-based systems with demonstrated achievements or potential use in Neurophotonics, including *Three-dimensional Random-access Scanning* [2], *Encoded Multiplane Light-sheet Microscopy* [3], and *High-speed Scan-less Camera-free Imaging* [4].

Short Bio Peter Saggau is an Emeritus Professor of Neuroscience at the Baylor College of Medicine in Houston, TX, USA., and an External Collaborator at the Italian Institute of Technology in Genoa, Italy. He was a Senior Director and Head of Research Engineering at the Allen Institute for Brain Science in Seattle, WA, USA. He is an Elected Fellow of the Institute of Physics, London, GB. Dr. Saggau holds degrees from the School of Engineering at the Technical University Munich and the Medical School of the Ludwig-Maximilians University in Munich, Germany.

Dr. Saggau's research interest is focused on Advanced Imaging Techniques to overcome the challenges inherent to optical imaging structure and function of living brain tissue. His research efforts resulted in numerous publications and several patents.

Dr. Saggau's research has been funded by several agencies, including the Whitaker Foundation (Arlington, USA), the Human Frontier Science Program (Strasbourg, France), the National Institutes of Health (Bethesda, USA), and the National Science Foundation (Arlington, USA). He is on the editorial board of *Microscopy Research and Technique* (Wiley). Dr. Saggau has trained many pre- and postdoctoral students as well as numerous undergraduate students and research interns.

References

M. Duocastella, S. Surdo, A. Zunino, A. Diaspro, and P. Saggau. Acousto-optic systems for advanced microscopy. *J. Physics Photonics*, 3, 012004, 2021.

G.D. Reddy, K. Kelleher, R. Fink, and P. Saggau. Three-dimensional random access multiphoton microscopy for functional imaging of neuronal activity. *Nature Neuroscience*, 11, 713-720, 2008. [3] A. Zunino, F. Garzella, A. Trianni, P. Saggau, P. Bianchini, A. Diaspro, and M. Duocastella.

Multiplane encoded light-sheet microscopy for enhanced 3D imaging. *ACS Photonics*, 2021.

[4] A. Marchese, P. Ricci, P. Saggau, and M. Duocastella. Fast and adjustable frequency-encoded microscopy using acousto-optic deflectors. *Nanophotonics* 13, 2024.

High-speed optical methods to reveal cell type-specific activity related to seizures in a mouse model of chronic epilepsy

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Focal cortical dysplasia (FCD) type II is a prevalent malformation of cortical development characterized by cortical dislamination and atypical cellular morphologies (notably dysmorphic neurons; DNs), frequently associated with drug-refractory epilepsy [1]. Molecular genetic studies of human cases have identified somatic gain-of-function mutations in the PI3K/AKT/mTOR signaling pathway as the main cause of FCD type II [2]. Mechanisms underlying the endogenous epileptogenicity and ictogenicity of FCD type II remain poorly understood. To elucidate the cellular and network substrates of FCD epileptogenicity across multiple temporal and spatial scales, we implemented state-of-the-art optophysiological techniques of intravital calcium and voltage [3] imaging combined with EEG recording, cell-specific optogenetic stimulation [4], and morphological analysis. Voltage imaging revealed distinctive firing patterns in DNs compared to healthy pyramidal cells (PCs), with DNs exhibiting a larger fraction of bursting neurons and higher intraburst firing frequency. Interestingly, DNs exhibited lower overall spontaneous activity compared to PCs. For the first time, we documented a potential causal role of DNs in ictogenesis using two-photon calcium imaging during spontaneous epileptic seizures in awake animals. Optogenetic stimulation of DNs expressing channelrhodopsin-2 induced seizures and interictal epileptiform activity in FCD type II animals, whereas similar stimulation in healthy PCs failed to evoke ictal activity. Further results will be discussed together with newly identified therapeutic targets.

Invited Speaker's short bio. Ondrej Novak completed his Master's degree in physics at the Faculty of Mathematics and Physics, Charles University in Prague (CU). Driven by a fascination for biological systems, his focus shifted towards advanced optical methods for investigating mammalian brain structure and function at cellular and network levels. During his Ph.D. studies under Prof. Syka at the Institute of Experimental Medicine, Czech Academy of Sciences, he pioneered the use of intravital two-photon imaging in the auditory cortex of awake animals. During his internship at Janelia Research Campus in Karel Svoboda's lab in 2017, he contributed to the development and testing of new genetically-encoded indicators of neuronal activity (VoltronST [3], iGABASnFR, iAchSnFR) and innovative imaging techniques (two-photon kHz framerate microscopy [5]). In 2018, Ondrej Novak became a junior group leader of the Laboratory of Neural Circuit Optophysiology set up at the Second Faculty of Medicine, Charles University. His team specializes in state-of-the-art optophysiological methods to explore pathophysiological mechanisms underlying drug-refractory epilepsy and brain tumors, with a focus on identifying novel therapeutic approaches for treating brain disorders. The lab collaborates closely with other research groups at the Department of Physiology, Second Faculty of Medicine, Charles University, and plays a pivotal role in the Epilepsy Research Centre Prague (EpiReC).

References

- M. Proietti Onori et al., RHEB/mTOR hyperactivity causes cortical malformations and epileptic seizures through increased axonal connectivity. *Plos Biology* 19, 34 (2021).
- J. S. Lim et al., Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. *Nature Medicine* 21, 395-+ (2015).
- A. S. Abdelfattah et al., Bright and photostable chemigenetic indicators for extended in vivo voltage imaging. *Science* 365, 699-+ (2019).
- J. Chvojka et al., Mouse model of focal cortical dysplasia type II generates a wide spectrum of high-frequency activities. *Neurobiology of Disease* 190, 10 (2024).
- A. Kazemipour et al., Kilohertz frame-rate two-photon tomography. *Nature Methods* 16, 778-+ (2019).

Wednesday 17. 4. 14:00-14:45

Miniature two-photon microscopes for studying brain microcircuits in freely moving animals

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Understanding complex cognitive functions starts with elucidating how information is encoded and transmitted within individual brain microcircuits. To achieve this goal, we need recording techniques capable of capturing the activity of large populations of neurons with a temporal precision close to the timescale of spikes and a spatial resolution high enough to resolve their spatial organization. Moreover, these techniques should be compatible with well-established and well-validated behavioral paradigms. Traditional extracellular recording techniques have drawbacks regarding their ability to identify genetically defined cell types and are of limited use for studies of subcellular dynamics. Two-photon (2P) functional imaging stands out by offering subcellular spatial resolution and near-spike temporal resolution, so it has emerged as one of the workhorses to study neural populations' coding and computational properties. However, its application had been limited by the bulky nature of conventional 2P imaging systems, restricting studies to head-fixed animals. Over the last two decades, considerable progress has been made in developing portable microscopes specifically tailored for freely-moving-animal functional imaging. This talk introduces our recent work in developing new generations of 2P microscopes with resolution, field of view, speed, and z-scanning capability similar to that of 2P benchtop microscopes. I will highlight key applications from my group and our collaborators, showcasing how this technology contributes to studying the neuronal computation rulesets in cortical microcircuits. Additionally, I'll discuss the current limits and perspective for future developments.

Invited Speaker's short bio. Weijian Zong is currently the group leader of the neurophotonics lab at the Kavli Institute for Systems Neuroscience, Norwegian University of Science and Technology in Trondheim, Norway. He had his post.doc training in the lab of Prof. Edvard Moser and Prof. May-Britt Moser. He holds a BSc in Electrical Engineering from Peking University and a Ph.D. in biophotonics from the Academy of Military Medical Sciences, supervised by professors Heping Cheng and Ming Fan. Dr. Zong's research is focused on developing cutting-edge imaging techniques to understand brain function and behavior. His research has contributed significantly to the development of miniature two-photon microscopy for brain imaging in freely behaving mice.

He holds several invention patents in optical and imaging and has received numerous fellowships and awards for his work, including the Tycho Jæger's Prize in Electro-Optics in 2022, the Marie Skłodowska-Curie Individual Fellowship in 2019, China's top 10 scientific achievements and Shitsan Pai (贝时璋) Award for Young Biophysicists Award in 2017.

Optogenetics and Graphene micro-transistor arrays as tools to investigate spreading depolarizations and seizure susceptibility in awake mice.

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Spreading depolarization (SD) is a slowly propagating wave of neuronal and glial depolarization often associated with suppression of neuronal activity termed cortical spreading depression; (CSD). We have recently developed an *in vivo* preclinical platform that uses optogenetic approaches to induce on-demand SD; 5-10s sustained activation of Channelrhodopsin (hChR2-H134R) expressed in excitatory neurons. Opto-SD can be electrographically mapped and pharmacologically characterized using arrays of graphene micro-transistors in awake head-fixed mice [1]. Further refinements to this approach allow both optogenetic induction and electrographic detection to be conducted non-invasively. Using pupillometry as a proxy for arousal state we demonstrate that SD results in an initial pupil dilation (arousal) during the depolarization phase, followed by pupil constriction (loss of arousal), time-locked to the duration of CSD. Sustained activation of Halorhodopsin (eNpHR3.0) expressed in excitatory neurons can paradoxically induce SD. Sometimes SDs occurred after the end of illumination when a surge in extracellular potassium was observed resultant from activation of the potassium-chloride cotransporter KCC2. However, on other occasions SDs arose during the period of eNpHR3.0 activation when most neurons in the network were hyperpolarized and extracellular potassium low. This suggests a cell volume-mediated SD induction mechanism [2]. To investigate both seizure and SD susceptibility in awake chronically epileptic mice, we applied linear graphene micro-transistor probes to target the hippocampus and overlying cortex [3]. Brief optogenetic stimulations that only result in time-locked responses in the local field potential in control mice were able to reliably induce seizures and seizure-associated SD in epileptic mice.

Invited Speaker's short bio. WykesNeuroLab is comprised of two research laboratories based in the United Kingdom. One within the Department of Clinical and Experimental Epilepsy at the UCL Queen Square Institute of Neurology; the other in the Division of Neuroscience (affiliated with the Centre for Nanotechnology in Medicine), at the University of Manchester. We develop and apply innovative neurosciences technologies to detect and investigate mechanisms underlying pathological brain activity. A recent focus is application of implantable Graphene-based transistor arrays to map spreading depolarisations in preclinical models of epilepsy, stroke and glioblastoma. <https://www.wykesneurolab.com/>

References

Masvidal-Codina E,, Guimerà-Brunet A, Wykes RC (2021). Characterization of optogenetically-induced cortical spreading depression in awake mice using graphene micro-transistor arrays. Journal of Neural Engineering PMID: 33690187.

Parrish RR,, Wykes RC, Voipio J, Trevelyan AJ (2023). Indirect effects of Halorhodopsin activation: potassium redistribution, non-specific inhibition and spreading depression. Journal of Neuroscience. PMID: 36639898.

Bonaccini Calia A,, Wykes RC*, Guimerà-Brunet A*, & Garrido JA*. (2022). Full bandwidth electrophysiology of seizures and epileptiform activity enabled by flexible graphene micro-transistor depth neural probes. Nature Nanotechnology. DOI: 10.1038/ s41565-021-01041-9. * Corresponding authors.

Organic LEDs for Optical Stimulation – Learning from the Display Industry?

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Today the technology of choice for modern smartphones and TV alike, organic light-emitting diodes (OLEDs) are now also gaining traction for a range of biomedical applications [1]. Here their main advantage over conventional LEDs and other light sources is that they can be readily integrated on almost any substrate, from the TFT backplane of a display to a silicon neuroprobe and a flexible and ultrathin plastic film. Over the past years, we systematically optimized OLED technology for use in optogenetics, in particular in terms of brightness[2,3], spectrum[4,5], and stability and passivation [6]. Very recently this allowed us to produce shank-shaped micro-OLED arrays with 1024 individually addressable pixels, each ca. 20 µm in size that allow to control neuronal activity with unprecedented local specificity[7, 8]. In our latest work, we also integrated OLEDs directly on acoustic antennae, thus obtaining what we believe is the world's most compact wirelessly powered light source [9].

Short bio. Malte C. Gather is Humboldt Professor and founding director at the Centre for Nano- and Biophotonics at University of Cologne and holds a co-appointment at University of St Andrews. His research interests are at the interface of biophotonics and organic semiconductors, with particular focus on bio-implantable LEDs and lasers, mechanobiology, and strong light-matter coupling. He studied physics and material sciences at RWTH Aachen University and Imperial College London and received his PhD from University of Cologne in 2008. He previously worked at University of Iceland, Harvard University, TU Dresden and University of St Andrews.

References

- C. Murawski, M.C. Gather, “Emerging Biomedical Applications of Organic Light-Emitting Diodes” *Adv. Opt. Mater.* 9, 2100269 (2021)
- Y. Deng, C. Murawski, C.-M. Keum, K. Yoshida, I.D.W. Samuel, M.C. Gather, “Development and optimization of very high luminance p-i-n junction based fluorescent organic light-emitting diodes” *Adv. Opt. Mater.* 8, 1901721 (2020)
- S. Hillebrandt, C. Keum, Y. Deng, J. Chavas, C. Galle, T. Hardin, F. Galluppi, M.C. Gather, “High brightness, highly directional organic light-emitting diodes as light sources for light-amplifying prosthetics in the optogenetic management of vision loss” *Adv. Opt. Mater.* 11, 2200877 (2023)
- A. Mischok, S. Hillebrandt, S. Kwon, M.C. Gather, “Highly efficient polaritonic light emitting diodes with angle-independent narrowband emission” *Nature Photonics* 17, 393–400 (2023)
- C. Murawski, A. Mischok, J.H.H. Booth, J.D. Kumar, E. Archer, L. Tropic, C.-M. Keum, Y. Deng, K. Yoshida, I.D.W. Samuel, M. Schubert, S.R. Pulver, M.C. Gather, “Narrowband Organic Light-Emitting Diodes for Fluorescence Microscopy and Calcium Imaging” *Advanced Materials* 31, 1903599 (2019)
- C. Keum, C. Murawski, E. Archer, A. Mischok, Y. Deng, S. Hillebrandt, M.C. Gather, “A substrateless, flexible, and water-resistant organic light-emitting diode” *Nat. Comm.* 11, 6250 (2020)
- A.J. Taal, I. Uguz, S. Hillebrandt, C.-K. Moon, V. Andino-Pavlovsky, J. Choi, C. Keum, K. Deisseroth, M.C. Gather, K.L. Shepard, “Single-neuron-resolution optogenetic stimulation in the deep brain with a complementary metal-oxide-semiconductor probe containing 1024 monolithically integrated organic LED pixels” *Nat. Electron.* DOI: 10.1038/s41928-023-01013-y (2023)
- S. Hillebrandt, C.-K. Moon, A.J. Taal, H. Overhauser, K.L. Shepard, M.C. Gather, “High-density Integration of Ultrabright OLEDs on a Miniaturized Needle-shaped CMOS backplane” *Adv. Mater.* DOI: 10.1002/adma.202300578 (2024)
- J.F. Butscher, S. Hillebrandt, A. Mischok, A. Popczyk, J.H.H. Booth, M.C. Gather, “Wireless Magneto-electrically Powered Organic Light-Emitting Diodes” *Science Advances* (in press)

Scanless two-photon voltage imaging for all-optical neurophysiology

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Parallel light-sculpting methods have been used to perform scanless two-photon photostimulation of multiple neurons simultaneously during all-optical neurophysiology experiments [1–3]. In this work, we demonstrate that scanless two-photon excitation also enables high-resolution, high-contrast, voltage imaging by efficiently exciting fluorescence in a large fraction of the cellular soma during high duty-cycle recordings [4]. We present a thorough characterisation of scanless two-photon voltage imaging using existing parallel approaches and lasers with different repetition rates. We demonstrate voltage recordings of high frequency spike trains and sub-threshold depolarizations in intact brain tissue from neurons expressing the soma-targeted genetically encoded voltage indicator JEDI-2P-kv [5]. Using a low repetition-rate laser, we perform recordings from multiple neurons simultaneously in-vivo. We further demonstrate that scanless light-sculpting illumination methods enable two-photon voltage imaging of rhodopsin-based voltage indicators. These indicators are typically much brighter than those based on voltage sensing domains, but have not previously been demonstrated to function under two-photon excitation [6,7]. Finally, by co-expressing JEDI-2P-kv and the channelrhodopsin ChroME-ST in neurons of hippocampal organotypic slices, we perform single-beam, simultaneous, two-photon voltage imaging and photostimulation. This enables in-situ validation of the precise number and timing of light evoked action potentials and will pave the way for rapid and scalable identification of functional brain connections in intact neural circuits.

References

- [1] M. Dal Maschio, J. C. Donovan, T. O. Helmbrecht, and H. Baier, *Neuron* **94**, 774 (2017).
- [2] I.-W. Chen, E. Ronzitti, B. R. Lee, T. L. Daigle, D. Dalkara, H. Zeng, V. Emiliani, and E. Papagiakoumou, *J. Neurosci.* **39**, 3484 (2019).
- [3] A. R. Mardinly, I. A. Oldenburg, N. C. Pégard, S. Sridharan, E. H. Lyall, K. Chesnov, S. G. Brohawn, L. Waller, and H. Adesnik, *Nat. Neurosci.* **21**, 881 (2018).
- [4] R. R. Sims, I. Bendifallah, C. Grimm, A. Mohamed-Lafirdeen, X. Lu, F. St-Pierre, E. Papagiakoumou, and V. Emiliani, *Res. Sq.* **Under rev**, (2023).
- [5] Z. Liu et al., *Cell* **185**, 3408 (2022).
- [6] H. C. Davis, F. Phil Brooks III, J. D. Wong-campos, and A. E. Cohen, *bioRxiv* 89 (2023).
- [7] T. D. Weber, M. V. Moya, K. Kılıç, J. Mertz, and M. N. Economo, *Nat. Neurosci.* **26**, 1642 (2023).

Optogenetic tests of a group of urination control cells

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Optogenetics toolboxes provide causal evidence for understanding the neural circuit basis of specific brain functions. However, neural circuits are interconnected and the interpretation of manipulating one or few nodes in a circuit could become fundamentally different if more complete measurements are present. Here, our recent neurophysiological data is one such case. We studied the role of ESR1⁺ cells in the Pontine Micturition Center (PMC) for reflective urination control using optogenetically activating or suppressing these specifically tagged cells under cystometry conditions (method of assessing urination function, constantly infusing saline to the bladder). The PMC, alias as the Barrington's nucleus, has been well known as a key node in the brainstem for driving two muscle groups to complete a urination process [1], i.e., bladder contraction and sphincter relaxation. A previous study [2] demonstrated that PMC^{ESR1+} cells primarily drive sphincter muscle relaxation to void, as male mice tend to voluntarily do so in the presence of female odor. That study marked a new territory as other earlier studies had identified another type of cells in the PMC, the CRH⁺ cells, which primarily accounted for contracting the bladder [3].

However, our present study shows that the PMC^{ESR1+} cells not only drive sphincter relaxation but also drive bladder contraction, both at the same time. Our experiments involve a pathway-specific 'loss-of-function' on top of a 'gain-of-function' test, i.e., we combined specific nerve transection with optogenetic stimulation of PMC^{ESR1+} cells that drives reflective urination events. These manipulations with simultaneous measurements of bladder pressure and sphincter muscle myogram reveal a dual-control role of PMC^{ESR1+} cells that is fundamentally different from one-way relays. Particularly, PMC^{ESR1+} cells do not primarily account for the sphincter relaxation, because there existed non-voiding contraction events that the bladder contracted, the sphincter did not relax, and yet PMC^{ESR1+} cells were activated (but to a much lower level than that in voiding events).

Our finding resonates with a simple physical picture, that rapidly emptying a filled fluid container requires both internal pressure elevation and valve opening, i.e., dumping the body's hazardous liquid waste once in a while and as fast as possible, for which PMC^{ESR1+} cells could be a candidate of such dual-control performer. Reflective urination is a conserved, life-maintaining function across almost all vertebrate species (except birds) including humans, in contrast, social peeing with urine is not what humans do – humans evolved to use many other ways of marking territories in societies, those involving much more 'munitions' than the limited volume of urine and many more diverse neural circuits, yet to be studied by using mouse models and optogenetics.

References

1. Masahiro Kawatani, William C. de Groat, K.I., Katsuya Uchida, Kenji Sakimura, Akihiro Yamanaka, Takayuki Yamashita, and Masahito Kawatani, *Downstream projection of Barrington's nucleus to the spinal cord in mice*. 126, 2021.
2. Keller, J.A., et al., Voluntary urination control by brainstem neurons that relax the urethral sphincter. *Nature Neuroscience*, 2018. **21**(9): p. 1229-1238.
3. Hou, X.H., et al., Central Control Circuit for Context-Dependent Micturition. *Cell*, 2016. **167**(1): p. 73-86 e12.

On-fiber printed polymeric neural probes

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Tapered fibers (TFs) have recently emerged as implantable photonic probes for optogenetic control of neural activity in deep brain regions [1]. Compared to their flat-cleaved counterparts, TFs are less invasive for the brain tissue and offer controlled light emission along the taper by exploiting the modal properties of the gradually narrowing waveguide [2]. Silica TFs can be patterned to control the modal content or host electric elements for simultaneous performance of optogenetic stimulation and electrophysiological recordings [3], however, establishing efficient interfacing between them and the brain tissue is challenging due to the mechanical mismatch in place. The unstretchable and stiff silica fiber implants often induce inflammation and consequently neuronal death and tissue encapsulation in the implant surroundings, which is highly detrimental for *in vivo* chronic applications [4]. It is becoming evident that replacing silica with a material that matches most closely with the mechanical properties and micro-scale movements of the brain could allow for a more seamless integration of the neural probes into the hosting tissue [5]. As a result, over the last years, there has been an increased interest in developing implantable optical waveguides from soft and elastic polymeric materials [6]. In this work, we demonstrate a method for the single-step fabrication of soft and flexible polymeric air-clad tapers using two-photon polymerization (2PP) and investigate their prospective use in optogenetics and fiber photometry. We take advantage of 2PP since it has been exploited as a versatile additive manufacturing technique that allows the fabrication of 3D structures of arbitrary shape with sub-micrometer resolution and tuneable mechanical properties based on the employed printing parameters [7]. In addition, 2PP has been successfully employed for the fabrication of optical fiber integrated photonic elements [8, 9]. To fabricate soft polymeric tapers, we used the commercially available IP-PDMS photoresist, which is a photocurable type of polydimethylsiloxane (PDMS). 2PP was performed using Nanoscribe's Photonic Professional system equipped with a femtosecond laser ($\lambda=780$ nm). 3D structures were printed in a layer-by-layer sequence using the Dip-in Liquid Lithography (DiLL) mode on top of polished multimode fiber facets connectorized with ceramic ferrules. Following the exposure, not cross-linked material was removed and solid tapers were encapsulated with a thin conformal film of Parylene-C. Polymeric tapers were then characterized in terms of light delivery and collection performances *ex vivo*. Moreover, the mechanical tissue damage induced by the polymeric tapers was assessed *in vitro*, by measuring the insertion force required for successful implantation into phantom brains. Our preliminary results suggest that IP-PDMS tapers hold great promise as a more biocompatible and compliant alternative to glass TFs.

References

- Pisano F. et al., Nature Methods, 16, 1185-1192 (2019)
- Pisanello F., Microelectronic Engineering, 215, 110979 (2019)
- Spagnolo B. et al., Nature Materials, 21, 826-835 (2022)
- Rivnay J. et al., Science Advances, 3 (6), e1601649 (2017)
- Forró C. et al., Advanced Healthcare Materials, e2301055 (2023)
- Guo J. et al., Sensors, 19 (17) (2019)
- Lemma E. D. et al., IEEE Transactions on Nanotechnology, 16(1), 23-31 (2017)
- Kim J.A. et al., Advanced Optical Materials, 8(9), e1901934 (2020)
- Yu J. et al., Optics Express, 28(25), e38127-38139 (2020)

LIST OF POSTERS

ID Number	Presenter	Poster title
1	Youri Bolsius	Bicistronic constructs for all-optical interrogation of neuronal circuits using two-photon optogenetics
2	Šárka Danačíková	Characterization of metabolic changes during in vitro differentiation of human induced pluripotent stem cells towards neurons for personalized diagnosis of epileptogenic variants
3	Karolína Hrůzová	The effect of manipulation of the parvalbumin-positive interneurons in the medial prefrontal cortex on anxious behavior.
4	Matthias König	Calcium imaging of optogenetically activated neurons through a semitransparent light source
5	Endre Marosi	Combining optogenetics and large scale electrophysiology recordings for network characterization underlying medial septum driven locomotion induction
6	Lukas Michal	Ischemic stroke lesion size and development does not correlate with hemorrhagic transformation occurrence
7	Jakub Otáhal	The effect of SFN and acetazolamide on the blood flow and electrophysiological parameters of adult rats
8	Enrico Patrono	Medial prefrontal cortex but not ventral hippocampus optogenetic stimulation of parvalbumin-positive interneurons is able to rescue navigational flexibility in an MK-801 mouse model of schizophrenia.
9	Yeva Prysiashniuk	Contrast-free evaluation of blood-brain barrier permeability in pediatric brain tumors: first case studies using multi-TE Arterial Spin Labeling MRI
10	Hoda Shamsnajafabadi	Validating human-derived organoids as a suitable model for testing optogenetic therapies
11	Jan Svoboda	Clarifying the alternation of mitochondrial energy metabolism in mice model of focal cortical dysplasia
12	Maria Samuela Andriani	Multiplexing optical signals in fiberoptodes: from the visible to the near infrared spectral range
13	Marco Bianco	Additive micro-fabrication on tapered optical fibers: integration of optical, electrical and thermal readout channels
14	Andreas Bucherer	Flexible neural probe with fluidic functionality realized using resist lamination and laser patterning
15	Liam Collard	Data driven approaches to Raman imaging through a multimode optical fiber
16	André Gomes	STED microscopy at the end of a holographic multimode fibre endoscope
17	Sabina Hillebrandt	A needle-shaped CMOS-based bioimplant with 1024 individually switchable high-brightness OLEDs
18	David Kala	Focal epilepsy is associated with thalamic ultrastructural abnormalities
19	Mohammadrahim Kazemzadeh	Holographical and Physics informed Deep Learning Approaches for fiber characterization and Image Transmission through Multimode Optical Fiber
20	Bára Krbková	Towards imaging via tapered multi-mode optical fibres
21	Robert Kuschmierz	High resolution lensless fiber endoscopy for optogenetics and neurophotronics
22	Antonio Lorca Cámara	A multicolor two-photon light-patterning microscope exploiting the spatio-temporal properties of a fiber bundle

23	Niall McAlinden	A wireless head stage for the mode selective coupling of light into tapered fibres using μ Lasers
24	Tomáš Pikálek	Non-linear microscopy through a multimode fibre endoscope
25	Ferruccio Pisanello	Bringing label-free vibrational spectroscopy in the deep-brain
26	Linda Piscopo	Spatio-temporal photonics at the tip of a plasmonic multimode optical fiber using supercontinuum light
27	Linda Piscopo	Advancing Neural Implants: SERS-Active Tapered Fibers via Low-Temperature Plasmonic Deposition
28	Jan Sanda	Automatic detection of focal cortical dysplasia lesions in pediatric patients
29	Andrea Sattin	Aberration corrected GRIN lenses for extended field-of-view deep brain imaging in freely moving animals using miniaturized two-photon microscopes
30	Felix Schmieder	Optogenetics with Human Stem-Cell-Derived Cardiomyocytes and Neuronal Networks
31	Barbara Spagnolo	Exploring the influence of Parylene-C thickness on optical properties of optoelectronic devices
32	Miroslav Stibůrek	Simultaneous recording of intracellular calcium and extracellular electrical signals through a hair-thin multimode fibre
33	Shy Shoham	Ultrasound modulation assisted multiphoton imaging

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