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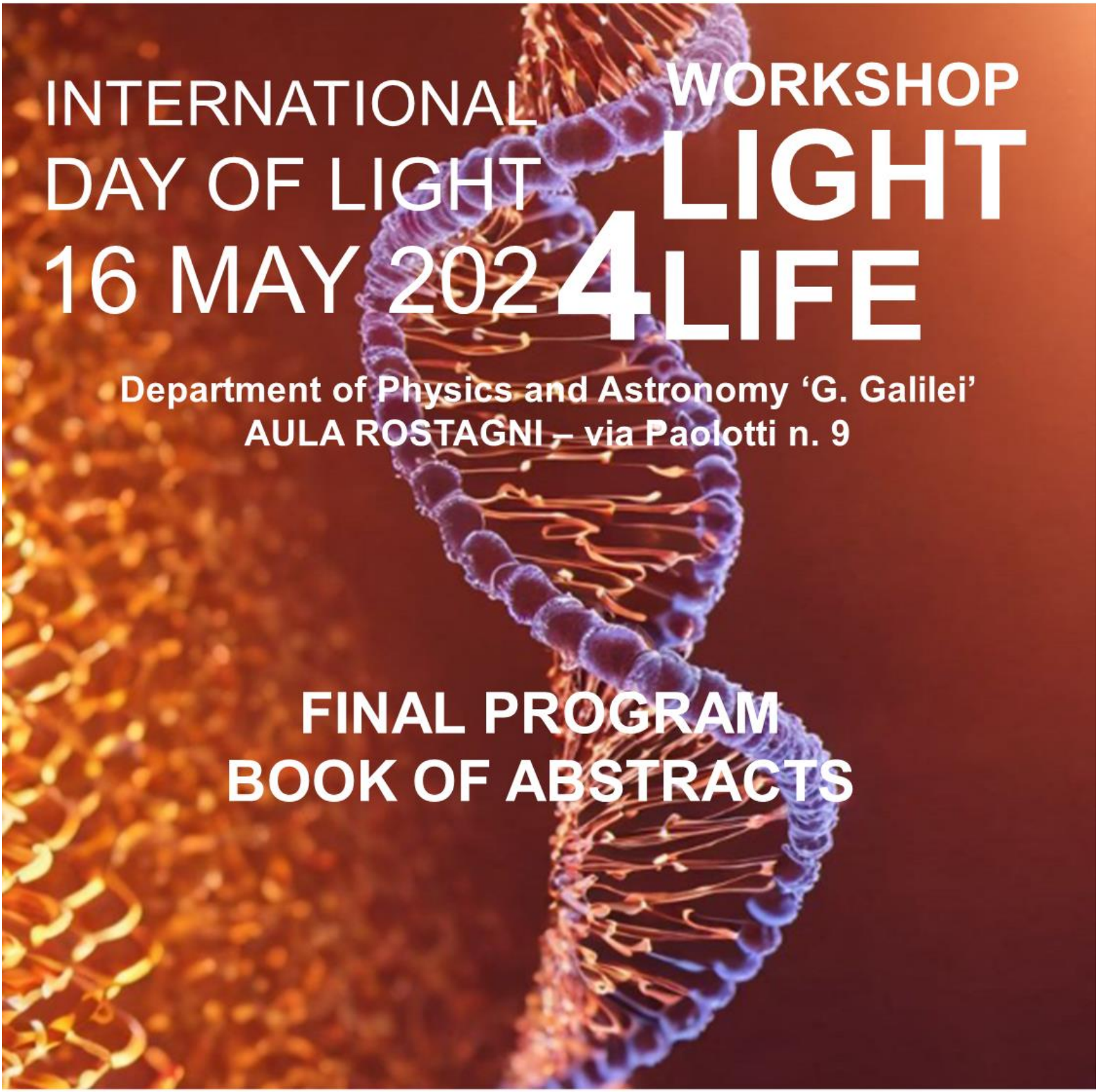

INTERNATIONAL DAY OF LIGHT 16 MAY 2024

WORKSHOP LIGHT 4 LIFE

Department of Physics and Astronomy 'G. Galilei'
AULA ROSTAGNI – via Paolotti n. 9

FINAL PROGRAM
BOOK OF ABSTRACTS

International Day of Light
16 May

Dipartimento di Fisica e Astronomia Galileo Galilei
UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Scienze Biomediche

Dipartimento di Biologia
Università degli Studi di Padova

16 May 1960 – 16 May 2024. THE INTERNATIONAL DAY OF LIGHT

The International Day of Light is held on May 16th every year to promote the public understanding of how light and light-based technologies touch our daily lives and are central to the future development of global society.

This anniversary was started by UNESCO (United National Educational, Scientific and Cultural Organization), with the inaugural celebration taking place in 2018. This annual day followed the larger event, the International Year of Light, which took place throughout 2015 and was sponsored by UNESCO.

This specific day was chosen as it is the anniversary of May 16, 1960, the day when Theodore Maiman, an American physicist and engineer, successfully fired the first laser. Since then, lasers have improved dramatically and become a pervasive and essential light-based technology in many sectors of research, industry, and society.

To celebrate this event, we organized the one-day workshop LIGHT 4 LIFE to showcase the state-of-the-art of Padua University research in the fields of optical devices, platforms, and techniques for advanced microscopy and light-based technologies for life sciences.

The workshop is organized by the FNIP program, a scientific initiative that brings together young scientists from the departments of Physics and Astronomy (DFA), Biology (DiBio), and Biomedical Science (DSB) of the University of Padova and the Neuroscience Institute of CNR.

The event has the patronage of the Italian Physics Society (SIF), the Italian Society of Optics and Photonics (SIOF), and the University of Padova.

The event is organized and promoted by the FNIP scientific program. For more information visit the official webpage: https://fnip.biomed.unipd.it/

ORGANIZING COMMITTEE
Gianluca Ruffato (DFA)
Filippo Pisano (DFA)
Andrea Vogliardi (DFA)
Nicoletta Plotegher (DiBio)
Claudia Cecchetto (DSB)
Matteo Bruzzone (DSB)
Jacopo Agrimi (DSB)
Letizia Mariotti (CNR)
Marco Brondi (CNR)

SECRETARIAT
Paola Zenere (DFA)
Silvana Schiavo (DFA)

Practical information:

Coffee breaks will be served at 11.00 and 16.00 in the conference room (Aula Rostagni, DFA). Lunch will be served from 13.10 to 14.00 in the garden of the Department of Physics and Astronomy (weather permitting, otherwise in the conference room).

PROGRAM OF LIGHT 4 LIFE WORKSHOP

08:00

Registration
1/1-1 - Aula "A. Rostagni", Dipartimento di Fisica e Astronomia - Edificio Marzolo 08:30 - 09:00

09:00

Welcome to L4L - IDL 2024
09:00 - 09:10

Opening talk: Photon-Resolved Microscopy: a New Microscopy Paradigm for Life-Science Research
Dr Giuseppe Vicidomini
09:10 - 10:00

10:00

Light wavefront engineering to modulate neuronal activity at cell resolution *Prof. Marco Dal Maschio*
10:00 - 10:20

Dual-functional metalenses for the polarization-controlled generation of structured beams *Dr Andrea Vogliardi*
10:20 - 10:40

Adaptive optics in biological imaging: enhancing resolution and fidelity *Dr Stefano Bonora*
10:40 - 11:00

11:00

Coffee break
11:00 - 11:30

Going beyond fluorescence: bringing label-free optical spectroscopy in deep brain regions using a single thin optical fi...
Dr Filippo Pisano

Single-molecule force spectroscopy with optical tweezers *Dr Annamaria Zaltron*
11:50 - 12:10

12:00

Droplet microfluidic platform for extracellular vesicle isolation and handling *Dr Davide Ferraro*
12:10 - 12:30

The use of light to guide neuronal connection in vitro and in vivo *Prof. Cecilia Laterza*
12:30 - 12:50

Detecting membrane contacts and associated Ca2+ signals by reversible chemogenetic reporters *Dr Riccardo Filadi*
12:50 - 13:10

13:00

Lunch
13:10 - 14:00

14:00	**Towards personalized medicine: investigating Parkinson's disease by patient-derived midbrain organoids** *Prof. Mario Bortolozzi*	
	Astrocyte-mediated phagocytosis: identification of novel players using a genetic screening	*Prof. Laura Civiero* 14:20 - 14:40
	Highlighting mitochondria-ER contact sites	*Prof. Marta Giacomello* 14:40 - 15:00
15:00	**A powerful model swims in the light blue: using the zebrafish to study neurodegeneration**	*Dr Francesca Terrin* 15:00 - 15:20
	FLASH PRESENTATIONS	15:20 - 16:00
16:00	**Coffee break**	16:00 - 16:30
	Roundtable: where are we going next?	16:30 - 17:00
17:00	**Closing remarks**	17:00 - 17:15

FLASH PRESENTATION CONTRIBUTIONS [3 min, 2 slides]:

FP1: "Trifurcated Splitting of Water Droplets on Engineered Lithium Niobate Surfaces", Dr. Sebastian Cremaschini

FP2: "Biophysical characterization of a novel gene mutation associated with the X-linked Charcot-Marie-Tooth disease using light-based techniques", Dr. Erva Bayraktar

FP3: "Dynamic chemogenetic reporters to investigate organelle coupling", Dr. Michela Rossini

FP4: "3D reconstruction of neural networks in human brain organoids", Dr. Diego Lopez-Pigozzi

FP5: "Fabrication of metalenses: fundamentals and key aspects", Dr. Daniele Bonaldo

FP6: "Tuning the properties of glasses through light-generated defects", Dr. Lara Piemontese

FP7: "Manipulation of water droplets by optical patterns imprinted on engineered LiNbO3 surfaces", Dr. Alessio Meggiolaro

FP8: "Temporal Dynamics of Calcium Signaling: Clustering Adrenal Cells Producing Aldosterone through Ca2+ FURA-2 with the use of functional statistical analysis", Dr. Juan Fernandez de Velasco

DETAILED PROGRAM AND ABSTRACTS

9.00. WELCOME TO L4L WORKSHOP

Chair: Dr. Gianluca Ruffato (DFA)

9.10 – 11.00. OPENING TALK: Photon-Resolved Microscopy: a New Microscopy Paradigm for Life-Science Research

Speaker: Dr. Giuseppe Vicidomini (Molecular Microscopy and Spectroscopy, IIT, Genova)

Fluorescence optical microscopy has long been a valuable and minimally invasive tool for visualizing biological structures and functions at the cellular level and beyond. However, understanding many fundamental biological processes crucial to human health and disease remains beyond the capabilities of conventional optical microscopy. Super-resolved microscopy, which shifts our perspective from considering fluorophores as mere passive markers to active participants in image formation, has significantly expanded the capabilities of optical microscopy, marking a new era for life sciences. Inspired by the transformative potential of shifting perspectives, we introduce the innovative concept of photon-resolved microscopy. By examining fluorescent light in terms of its most elemental components—the photons—we unveil even greater potential for fluorescence microscopy. Our exploration begins by illustrating how this fresh approach can reinvigorate one of the most widely used and traditional microscopy architectures: the confocal laser-scanning microscope. Subsequently, we reveal the synergies between photon-resolved microscopy and more advanced techniques, including super-resolved microscopy. This change in perspective holds the promise of not only enhancing the capabilities of fluorescence microscopy but also unlocking new horizons in studying intricate biological processes.

SESSION 1: ADVANCED LIGHT CONTROL

Chair: Dr. Filippo Pisano (DFA, UNIPD)

11.00: Light wavefront engineering to modulate neuronal activity at cell resolution

Speaker: Prof. Marco Dal Maschio (DSB, UNIPD)

Light wavefront engineering represents a valuable tool to control the electric field intensity distribution at the sample volume by modulating the phase and/or the amplitude of the light wavefront in a conjugated space. For imaging purposes, this approach has been traditionally adopted on one side to compensate for optical aberrations due to the sample or the medium so as to improve either the resolution or the signal-to-noise ratio; on the other side, to achieve particular illumination spatial profiles so as to increase the background rejection. Along with novel applications for imaging, in the last ten years wavefront engineering has become a fundamental tool in neuroscience research when light is used not to record but also to control the neuronal circuit activity, taking advantage from the development of light sensitive ion channels rendering light sensitive the activity of the cells in the brain. Following a brief background, I will present the methods and the application for applying these methods in neuroscience research.

11.20: Dual-functional metalenses for the polarization-controlled generation of structured beams

Speaker: Dr. Andrea Vogliardi (DFA, UNIPD)

The ability to generate different structured beams in a compact optical path by controlling the input polarization has been a challenge of the last few years in the optics and photonics field. In this regard, we propose designing, fabricating, and characterising new dielectric dual-functional metaoptics that generate 3D orbital angular momentum beams or vector beams along custom-define trajectories with on-demand different behaviours acting on the input light's polarization. Our meta-optics are designed as an array of periodic subwavelength metastructures (the so-called meta-atoms) composed of silicon nanofins on a silicon substrate. Each nanorod acts like a half-wave plate that exploits both the geometrical and dynamical phases in a different way depending on its position on the entire optic. The optical elements have been fabricated in the form of phase-only metasurfaces (meta-atoms) with high-resolution electron-beam lithography and characterized with a custom-made optical bench. The main result of this work is the design of tiny high-resolution optics generating longitudinally-variant vector beams and spheres of light that are able to impart new peculiarities to the light. In particular, the proposed metaoptics could open new applications of structured light for holography, super-resolution imaging, optical trapping and particle tweezing.

11.40: Adaptive optics in biological imaging: enhancing resolution and fidelity

Speaker: Dr. Stefano Bonora (CNR, Institute of Photonics and Nanotechnology, Padova)

The use of adaptive optics (AO) in biological imaging has emerged as an important technique for overcoming the inherent optical aberrations present in biological specimens. We will provide an overview of the principles, applications, and recent advancements in AO technology within the realm of biological imaging. We will discuss the implementation of AO in various imaging modalities, including confocal microscopy, two-photon microscopy, and optical coherence tomography, highlighting its capability to enhance image quality and enable high-resolution imaging. Furthermore, we explore the integration of AO with adaptive lenses in imaging techniques such as light-sheet microscopy and super-resolution microscopy.

---------- 11.00 – 11.30: COFFEE BREAK ----------

SESSION 2: ADVANCED PLATFORMS AND OPTICAL TOOLS

Chair: Dr. Claudia Cecchetto (DSB, UNIPD)

11.30: Going beyond fluorescence: bringing label-free optical spectroscopy in deep brain regions using a single thin optical fiber

Speaker: Dr. Filippo Pisano (DFA, UNIPD)

Optical approaches for in vivo neural monitoring using genetically-encoded fluorescent molecular reporters offer a precious window on brain functions, and on the mechanisms of development, ageing or disease progression. Nonetheless, the existing methods are still shortsighted with respect

to the complex biomolecular alterations that accompany these physiological and pathological dynamics. As a result, our grasp of the multifaceted components of brain activity is still partial. To surpass these limitations, this talk will discuss the opportunities offered by the broad physical phenomenologies underlying light-brain interactions to capture a more comprehensive picture of neural mechanisms using label-free optical spectroscopy in deep brain regions. In particular, I will present a vibrational fiber photometry method, based on spontaneous Raman scattering, that allows monitoring the bio-molecular content of arbitrarily deep brain volumes of the mouse brain – in vivo – to gather information on molecular alterations caused by traumatic brain injury and to detect diagnostic markers of brain cancer using a single thin optical fiber. This approach, which can be employed alongside conventional photometry techniques, has the potential to empower emerging research on brain-immune and brain-cancer bidirectional dynamics.

11.50: **Single-molecule force spectroscopy with optical tweezers**

Speaker: Dr. Annamaria Zaltron (DFA, UNIPD)

Optical Tweezers exploit light to manipulate objects at the micro- and nanoscale, demonstrating to be a powerful tool for investigating the biological world. Force spectroscopy measurements with optical tweezers allow the application of controlled mechanical stimuli and displacements on individual molecules of DNA, RNA and proteins, while monitoring the time evolution of the system as it undergoes biochemical reactions. In this way, it is possible to derive information on the elastic and kinetic properties of the molecule, characterizing its molecular pathways and its free energy landscape. In this talk, the working principles of optical trapping and its application to single-molecule experiments will be presented; the study we are carrying out with optical tweezers on the Thymidylate Synthase consensus RNA will be also discussed.

12.10: **Droplet microfluidic platform for extracellular vesicle isolation and handling**

Speaker: Dr. Davide Ferraro (DFA, UNIPD)

Extracellular vesicles (EVs) are double-layered phospholipid vesicles having nanometric size that are rapidly gaining in popularity as biomarkers of various diseases, acting as cargoes of valuable information from the cell of origin. Despite their value, their current use in clinical practice is still limited. Among the limiting factors, one of the most critical is their isolation. In fact, conventional approaches are characterized by low purity and throughput, or poor reproducibility. Here, we propose a droplet microfluidic platform developed for EV isolation by affinity capture with magnetic beads. This platform is capable of processing large sample volumes in a relatively short time. Systematic comparison with commercial methods proves that the platform leads to an improved EV capture efficiency of 2.5-fold. This is due to the fact that EVs and magnetic beads are co-encapsulated within the same droplet, which acts promoting their mixing. The beads are extracted within the microfluidic system and collected for EV analysis. At first, the platform has been validated from the microfluidics point of view: throughput, automation and magnetic beads handling have been investigated. Then, the EV isolation capability has been performed by the most used techniques: confocal microscopy and flow-cytometry prove the presence of EVs captured on the beads, while scattering techniques and protein assays allow defining a capture efficiency. Finally, the miRNAs cargo has been quantified to verify the EV integrity. The remarkable improvements compared with monophasic microfluidic indicate how droplet microfluidics represent a suitable technology for EV isolation especially in case of clinical applications, where a few mL of starting sample is considered. To achieve this aim, preliminary validation using human plasma samples will be presented.

12.30: The use of light to guide neuronal connection in vitro and in vivo

Speaker: Prof. Cecilia Laterza (DSB, UNIPD)

The brain is the most complex and delicate organ in our body. Brain damage typically results in devastating outcomes and consequences not only for the patient's health but also for their quality of life. These effects are caused by the irreversible loss of neurons, building blocks of the brain responsible of signal transmission. Neuronal loss therefore leads to an alteration in communication between the different areas of the brain, resulting in the inability to perform specific functions. One of the most complex challenges of regenerative medicine is to find a strategy to recreate functional connections within the damaged brain, with the aim of restoring the patient's physiological activities. Currently, there is no therapy capable of regenerating lost tissue and connections between neurons. There are no drugs capable of regenerating dead cells, and cellular therapy, which exploits the use of stem cells, is unable to effectively replace lost tissue and recreate functional connections. In this project, we aim to develop an integrated approach between bioengineering and regenerative medicine capable of controlling and guiding the formation of brain connections, in order to promote the restoration of lost brain functions following damage. Specifically, to replace damaged tissue, we will exploit the use of brain organoids, small three-dimensional structures that resemble the brain in cellular composition and structural organization. To guide the formation of neuronal connections, photosensitive gels will be used, which can be manipulated with infrared light directly inside the brain of the living animal. Specifically, using multiphoton light, it is possible to create three-dimensional structures or empty channels within the biomaterial itself that guide the growth of neurons. Thanks to the ability to manufacture these structures directly within the brain in a defined anatomical site, we can then connect the neurons of the organoid implanted at the site of the lesion with the remaining neurons in the tissue of the host animal, thus reconnecting the damaged tissue. This will enable the design of a new neuronal network capable of recovering a specific function lost due to the lesion. This project therefore has the potential to create a new therapeutic strategy to promote the restoration of lost brain functions following damage (e.g., stroke, tumor resection, trauma), made possible only through the fusion of expertise from very different scientific fields: neuroscience, bioengineering, and regenerative medicine.

12.50: Detecting membrane contacts and associated Ca2+ signals by reversible chemogenetic reporters

Speaker: Dr. Riccardo Filadi (CNR, Institute of Neurosciences, Padova)

Membrane contact sites (MCSs) enable different intracellular organelles to coordinate their activities, yet the small size and the dynamic nature of these regions hinder their study by current imaging techniques. By designing a series of reversible chemogenetic reporters based on improved, low-affinity variants of splitFAST, we analysed the dynamics of different MCSs at high spatiotemporal resolution, both in vitro and in vivo. We demonstrated that these versatile reporters suit different experimental setups well and identified a hitherto unknown pathway, in which calcium (Ca2+) signalling regulates the juxtaposition between endoplasmic reticulum and mitochondria. Finally, the integration of Ca2+-sensing domains into the splitFAST technology allowed us to introduce PRINCESS (PRobe for INterorganelle Ca2+-Exchange Sites based on SplitFAST), an unprecedented class of reporters to simultaneously visualize MCSs and the associated Ca2+ dynamics by a single biosensor.

---------- 13.10-14.00: LUNCH ----------

SESSION 3 – ADVANCED TECHNIQUES FOR MICROSCOPY

Chair: Dr. Nicoletta Plotegher (DiBio, UNIPD)

14.00: Towards personalized medicine: investigating Parkinson's disease by patient-derived midbrain organoids

Speaker: Prof. Mario Bortolozzi (DFA, UNIPD)

One of the most exciting advancements in stem cell research of the last few years has been the development of human brain organoids. This in vitro system consists of multiple cell types that can self-organize in three-dimensions representing a brain region able to recapitulate physiological and pathological relevant aspects. Compared to animal models, patient-derived organoids provide emerging prospects for testing new drugs and developing precision medicine. Human midbrain organoids (hMBOs) can mimic the sunstantia nigra, the brain region that degenerates in Parkinson's disease (PD), including the complex interaction of dopaminergic neurons with other types of neurons and glial cells. In this work, we characterized hMBOs derived from healthy subjects and PD patients carrying monogenic mutations identified to cause PD in a highly penetrant manner. In order to address the complexity of hMBOs, we combined patch-clamp, multielectrode arrays (MEA) and two-photon microscopy, testing potential therapeutic compounds.

14.20: Astrocyte-mediated phagocytosis: identification of novel players using a genetic screening

Speaker: Prof. Laura Civiero (DiBio, UNIPD)

Astrocytes participate in the clearance of obsolete or unwanted neuronal synapses. However, the molecular machinery recruited for the recognition of synapses is not fully clarified, especially in pathological conditions. Here, we investigated the phagocytic process through individual gene silencing using a druggable gene library. Our study demonstrates that astrocyte-mediated synapse engulfment is regulated by the Atypical chemokine receptor 3 (Ackr3). Mechanistically, we have shown that Ackr3 recognizes phosphatidylethanolamine (PE)-bound Cxcl12 at synaptic terminals both in vitro and in cells, thus serving as a novel marker of synaptic dysfunction. The removal of synapses by astrocytes, dependent on Ackr3, occurs prominently in brains affected by Alzheimer's disease (AD). Notably, both the receptor and its ligand are overexpressed in post mortem AD human brains, and downregulation of the receptor in AD mouse models (5xFAD) significantly diminishes astrocyte-mediated synaptic elimination. Overall, this work unveils a novel, possibly targetable mechanism of astrocyte-mediated synaptic engulfment implicated in the most common neurodegenerative disease.

14.40: Highlighting mitochondria-ER contact sites

Speaker: Prof. Marta Giacomello (DiBio, UNIPD)

In recent years it has become clear that intracellular organelles are not isolated entities, but rather they interact to coordinate their function. Organelles crosstalk occurs at points of proximity between their surfaces, which are kept together by proteinaceous tethers. These closely juxtaposed membrane subdomains are known as membrane contact sites (MCS). The most studied MCS are those between the endoplasmic reticulum and the mitochondria (MERCs). MERCs play an important role in many physiological and pathological subcellular processes, including lipid and Ca2+ homeostasis, mitochondrial dynamics and response to stress stimuli: altered MERCs structure and

function contribute to severe pathological conditions including Alzheimer's and Parkinson's disease. Hence, understanding which conditions or treatments modulate the structure of MERCs could be of relevance for both basic and translational research. To this end, we developed a FRET-based mitochondria-ER proximity probe (FEMP) for the study of MERCs dynamics in living and fixed cells, and we miniaturized it to make it suitable for high-throughput screenings.

15.00: A powerful model swims in the light blue: using the zebrafish to study neurodegeneration

Speaker: Dr. Francesca Terrin (DiBio, UNIPD)

The zebrafish (Danio rerio) emerged as a powerful tool for scientific research starting from the beginning of the 1980s. Its remarkable characteristics such as the high fecundity, the external fertilization and development, the elevated similarity with the human genome and the possibility to easily manipulate it, made the zebrafish an optimal animal model for basic research and translational applications. The optical clarity of the embryo during its whole development constitutes another fundamental feature that promoted the generation of multiple transgenic lines aimed at deciphering the mechanisms underlying cellular processes, tissues morphogenesis and organs functionality. The targeted expression of fluorescent proteins, indeed, allows direct visualization and scoring of manifold processes to shed light on different aspects of cellular and developmental biology and physiopathology of diseases. In our research, that we propose as an example of zebrafish use in the study of neurodegenerative processes, we exploited the optical accessibility of larvae and fluorescent transgenic zebrafish lines to unravel the impact of increased levels of glucosyl-sterols on the development and function of motor neurons and neuromuscular junctions. Unbalance in the amount of these molecules in the organism, indeed, has been associated with the occurrence of neurodegenerative outcomes similar to Parkinson's disease and Amyotrophic Lateral Sclerosis, that involve neuronal loss and impaired functionality.

15.20 – 16.00 FLASH PRESENTATIONS SESSION (3 min – 2 slide)

Chair: Dr. Letizia Mariotti (CNR, Institute of Neurosciences, Padova)

Evaluation panel: L. Mariotti, M. Bruzzone, C. Cecchetto, N. Plotegher, F. Pisano, A. Vogliardi, and G. Ruffato

Speaker 1: Dr. Sebastian Cremaschini (DFA, UNIPD)

Trifurcated Splitting of Water Droplets on Engineered Lithium Niobate Surfaces

Controlled splitting of liquid droplets is a key function in many microfluidic and industrial applications. In recent years, various methodologies have been used to accomplish this task. In this work, I present an optofluidic platform based on an engineered surface formed by coating a z-cut iron-doped lithium niobate (Fe:LiNbO3) crystal with a lubricant-infused layer (LIS), which guarantees hydrophobicity and provides a very slippery and robust surface for prolonged use. Illuminating the crystal with a laser light spot creates surface charges of opposite signs on the two crystal faces because of the photovoltaic effect. In this way, sessile water droplets having volume of microliters (which correspond to millimeters in size) can be easily actuated, guided, merged and even split due to dielectrophoretic force; in particular in this work I focus my attention on the splitting of water

droplets. If the illumination intensity is enough, a water droplet placed near the illuminated area can be split into two charged fragments: one fragment remains trapped in the illuminated area, while the other moves away from it. The presence of the lubricant layer with a proper thickness is crucial to observe the splitting of water droplets. The second fragment does not move randomly but rather follows one of three well-defined trajectories separated by 120°, which reflect the crystallographic anisotropy typical of Fe:LiNbO3. Numerical simulations explain the behavior of the splitting phenomenon of water droplets in the framework of the forces induced by the interplay of pyroelectric, piezoelectric, and photovoltaic effects, which originate simultaneously inside the LiNbO3 crystal, when illuminated. Such a synergetic effect studied in the proposed optofluidic platform can provide a valuable feature in applications that require splitting and coalescence of droplets, such as chemical microreactors, biological encapsulation and screening.

Speaker 2: Dr. Erva Bayraktar (DFA, UNIPD and VIMM)

Biophysical characterization of a novel gene mutation associated with the X-linked Charcot-Marie-Tooth disease using light-based techniques

Connexin-32 (Cx32), encoded by the GJB1 gene, play a crucial role in communication between living cells. Mutations in this gene lead to the X-linked form of Charcot-Marie-Tooth (CMT1X) disease, a progressive neuropathy that damages peripheral nerves. We identified a novel gene mutation (H73L) located at the border between the Cx32 protein's extracellular loop and the transmembrane domain. This site is hypothesized to be critical for the assembly and docking of connexin hemichannels, as well as for their voltage and calcium-dependent gating. Functional experiments were performed at the populational level by a luciferase/luciferin assay based on luminescence, revealing a decrease in both calcium-dependent opening and closure and a leakier hemichannel in cells with the H73L mutation. These results were confirmed by fluorescent dye uptake, and IP3 flash photolysis at the single-cell level using an ATP biosensor. Molecular dynamics simulations of the mutant hemichannel supported our experimental findings, revealing that the H73L mutation weakens electrostatic interactions in a critical area for calcium-dependent gating, accompanied by a global widening of the pore, potentially leading to increased channel permeability. Overall, these findings expand our understanding of GJB1 gene mutations associated with CMT1X and suggest that dysfunction of Cx32 hemichannels may contribute to the disease.

Speaker 3: Dr. Michela Rossini (DSB, UNIPD)

Dynamic chemogenetic reporters to investigate organelle coupling

The close proximity between intracellular organelles is critical to regulate fundamental cell pathways, such as Ca2+ signaling, lipid metabolism, cell death and ER stress. Notably, alterations of organelle contacts have been reported in several pathologies. Therefore, defining the regulation of organelle juxtaposition and its contribution to different cellular functions is critical. However, efforts aimed at visualizing organelle contacts have been hindered by the lack of dynamic reporters allowing to follow membrane contact sites with sufficient spatiotemporal resolution. We hence developed and characterized a set of reversible fluorescent probes to detect the juxtaposition between endoplasmic reticulum (ER), mitochondria and the plasma membrane. These sensors are based on splitFAST, a chemogenetic reporter originally developed to detect dynamic protein-protein interactions in the green, red or far-red spectrum. By virtue of the intrinsic reversibility of splitFAST, these probes allowed to detect the dynamics of contact sites after different cellular treatments at high spatiotemporal resolution. Using confocal airyscan or lattice lightsheet microscopy, we followed ER-mitochondria contact sites in HeLa and Cos7 cells. We observed that the probe correctly marks and follows the transient docking of the two organelles. Moreover, we found that contacts are extremely

dynamic, moving along the mitochondrial and ER networks and undergoing themselves continuous remodeling based on several fusion and fission events. By taking advantage of the dynamicity of the splitFAST-based probes, our future investigations will focus on the study of the mechanisms governing the plasticity of organelle contacts.

Speaker 4: Dr. Diego Lopez-Pigozzi (DFA, UNIPD and VIMM)

3D reconstruction of neural networks in human brain organoids

Worldwide, there are estimated to be fifty million people with neurodegenerative diseases. This number is expected to double every twenty years as the population ages. The powerful combination of light-based techniques and human in vitro models has recently opened unprecedented opportunities for studying disease pathogenesis and performing drug screening. Organoids are 3D in vitro models derived from human induced pluripotent stem cells (hiPSCs) that self-organize into complex and functional mini organs, including specific brain regions, such as cortex and midbrain. In this work, we performed functional and morphological investigation in midbrain organoids (MBOs) using biophysical and molecular biology techniques. As for the morphological study of MBOs, we explored the possibility to fully reconstruct in 3D the neural network of a whole organoid (having 2-3 mm diameter) by combining advanced optical microscopy and clarification techniques to overcome the imaging limitations of confocal and 2-photon microscopes. The breakthrough of clarification techniques is dehydrating the sample and refilling it with a solvent that matches the refraction index of the imaging medium. The nearly full transparency of the clarified sample permits to reconstruct it up to several mm or even cm depth. Light sheet microscopy was used to visualize MBOs, allowing faster acquisition of large fields of view at sub-cellular resolution for final 3D reconstruction.

Speaker 5: Dr. Daniele Bonaldo (DEI, UNIPD)

Fabrication of metalenses: fundamentals and key aspects

In recent years metalenses have shown great potential in many applications such as optical tweezing and microscopy. Their power stems from sub-wavelength-sized nanopillars, the so-called meta-atoms, which allows control of light in all its degrees of freedom. Given their small size and tight tolerances, meta-atoms must be fabricated with precise semiconductor processing, such as Electron Beam Lithography. Here I will present the processes involved in the fabrication of metalenses and how their functionality is affected by fabrication constraints.

Speaker 6: Dr. Lara Piemontese (DFA, UNIPD)

Tuning the properties of glasses through light-generated defects

Glasses flee from a simple classification within the traditional states of matter such as solids, liquids, or crystalline structures. Their distinctive lack of long-range order sets them apart from crystalline solids on the one hand, while their extremely high viscosity distinguishes them from common liquids on the other. The disordered atomic arrangement of glasses sets off several interesting properties and makes glasses both scientifically captivating and technologically relevant. For instance, worldwide nowadays communication systems massively use optical fibers for data transport, exploiting the physical peculiarities of amorphous glasses for light transmission. It is well known that many properties of amorphous materials are tunable via appropriate protocols involving, for example, the temperature: a well annealed glass has generally an improved transparency compared to a highly-quenched one. The objective of precisely tuning the properties of a glass can be achieved in alternative ways: the generation of defects through irradiation represents an intriguing one. As

photons penetrate the glass matrix, they trigger the formation of defects and the emergence of novel structural motifs. Among all the different parameters playing a role in this complicated game, the wavelength and polarization of the incident beam give interesting insights on the physics of such structural rearrangements. The choice of the wavelength is crucial, as it determines the penetration depth and energy deposition within the glass matrix on the one side, and influences the type and density of generated defects on the other. Controlling the polarization of the incident light allows for precise manipulation of the orientation and symmetry of the defects. The goal of tailoring the properties of glasses through light thus begins with the understanding of the mechanism generating such defects born from the interplay of photons with the glass matrix atoms.

Speaker 7: Dr. Alessio Meggiolaro (DFA, UNIPD)

Manipulation of water droplets by optical patterns imprinted on engineered LiNbO3 surfaces

Controlled actuation of liquid droplets on a surface has important implications in many industrial applications, such as heat transfer, water harvesting, energy generation, and even in clinical diagnostics. In recent years, various strategies have been used to control the motion of droplets, either in an active or passive manner. In this work, an optofluidic platform that performs the basic droplet handling operations required in a common microfluidic device is presented. In detail, it is based on z-cut, iron-doped lithium niobate crystals (Fe:LiNbO3) that, upon appropriate laser illumination, generate surface charges of opposite sign at the two main faces due to the photovoltaic effect. This provides an evanescent electric field extended outside the active optical material. The face of the crystal in contact with the droplets is coated with a lubricant-infused layer, which guarantees hydrophobicity and, more importantly, a very slippery and robust surface for prolonged use. In this way, sessile water droplets having volumes of up to a few microliters, corresponding to millimeters in size, can be easily actuated, guided, and merged by projecting on the crystal suitable static or dynamic light patterns, which act as virtual electrodes. The design of light patterns is provided by a spatial light modulator (SLM) capable of producing circular spots or linear stripes. In particular, the light intensity used for this purpose is of at least one order of magnitude lower than that reported in previous studies. The actuated droplets can cover distances of centimeters within a timescale of a few seconds. Furthermore, the resulting platform is highly flexible and reconfigurable after proper discharge and does not require the addition of moving parts.

Speaker 8: Dr. Juan Fernandez de Velasco (DSB, UNIPD)

Temporal Dynamics of Calcium Signaling: Clustering Adrenal Cells Producing Aldosterone through Ca2+ FURA-2 with the use of functional statistical analysis

The intricate regulation of calcium signaling plays a pivotal role in cellular processes, particularly in adrenal cells responsible for aldosterone production. Using calcium sensitive fluorescent ratiometric dye fura-2, this study investigates Angiotensin II (Ang II) induced intracellular calcium oscillations in adrenocortical cells, from primary normal tissue and aldosterone-producing adenomas. The aim is to understand the levels of cellular coordination between cell types and to provide proof of the existence of "functional" clusters where high levels of synchronicity in intracellular calcium oscillations are observed. With the use of a Functional ANOVA, the study ascertains the presence of clusters with a substantial level of synchronization and coordination among the cells within the same cluster, reflecting a more profound functional connection that leads to similar responses to Ang II stimulus. Additionally, a correlation-based hierarchical clustering algorithm and linear mixed-effects models find no statistically significant difference in the degree of synchronicity among cells categorized as primary normal tissue compared to those categorized as aldosterone-producing adenomas. Through the exploration of calcium bursts in adrenal cells, this study is able to provide

evidence for the presence of “functional” clusters where cells exhibit high levels of temporal synchronicity in response to Ang II.

---------- 16.00 – 16.30: COFFEE BREAK ----------

16.30 ROUND TABLE: Where are we going next?

With the participation of Prof. Filippo Romanato (DFA), Prof. Fabio Mammano (DFA), Prof. Marco Dal Maschio (DSB), and Dr. Giuseppe Vicidomini (IIT)

17.00 CLOSING REMARKS and BEST FLASH PRESENTATION AWARD