Draft of the Workshop booklet

Please note that this is a draft and not the final version. This draft is distributed to allow participants to select their hands-on session preferences.

The final version will be distributed shortly before the workshop. It may contain changes to the program or other parts and additional information.

GerBI FLIM Workshop 2024

26. - 29. February 2024

The German Bioimaging FLIM Group organizes this workshop hosted at the Core Facility Bioimaging of the Biomedical Center of the Ludwig-Maximilians-Universität München.

Local organizer: Steffen Dietzel, Core Facility Bioimaging, BMC, LMU.

The GerBI FLIM group are: Ali Gheisari (Thorlabs), Gerhard Holst (PCO/Excelitas), Fabian Jolmes (Picoquant), Sebastian Karpf (University of Lübeck), Anca Margineanu (MDC Berlin), Angelika Rück (University of Ulm), Roland Thünauer (Leibniz Institute of Virology Hamburg), Cornelia Wetzker (NFDI4BIOIMAGE - Research Data Management for Microscopy and BioImage Analysis), Werner Zuschratter (Leibniz Institute for Neurobiology Magdeburg) and Steffen Dietzel.

German Bioimaging and the FLIM group

German BioImaging, or short GerBI, connects and supports microscopists and bioimage analysts as well as core facilities throughout Germany. Since the foundation of the society in April 2017 the full name is “German BioImaging-Gesellschaft für Mikroskopie und Bildanalyse e.V. (GerBI-GMB, Society for Microscopy and Image Analysis)”. It has emerged as the successor of the DFG funded German BioImaging network. GerBI is organizer of the biannual Trends in Microscopy (TiM) conference. The next one will take place 17. – 21. March 2025. Much of the current FLIM workshop was modelled after the successful hands-on-sessions as experienced at the last TiM meetings.

Within GerBI several special interest groups have formed, among them the GerBi FLIM group. We aim to raise awareness about the opportunities provided by Fluorescence Lifetime Imaging (FLIM) microscopy to the life science community. Projects towards this goal are

- Fostering communication between FLIM enthusiasts
- Creating a database of fluorescence lifetime measurements
- Developing educational material on FLIM applications and providing examples where FLIM improves the outcome of experiments or allows new insights. Benefits and limitations; background suppression; “functional imaging”.
- Developing training contents
- Organizing workshops (meetings) on FLIM microscopy and contributions to the Trends in Microscopy conferences

The first FLIM course was held in November 2020 during the COVID-19 pandemic and thus was an online only event. The lectures and “hands-on sessions” from this workshop are still online. An overview can be found here: https://www.bioimaging.bmc.med.lmu.de/learn/courses/flim2020/

The GerBI FLIM group meetings are online, typically on the first Monday of a month at 10:00. As German Bioimaging as a whole, also the FLIM group is happy to welcome new members.

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This workshop is sponsored by Toptica.

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This workshop is supported by the following companies through providing hardware and expertise:

Becker & Hickl with Toptica and ibidi

Becker & Hickl

TOPTICA

ibidi cells in focus

FLIM Labs

FLIM LABS

ISS

ISS focus and discover

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This workshop is supported by providing lab ware or reagents by the following companies:

ibidi (culture vessels)

ibidi cells in focus

Spirochrome (staining reagents)

SPIROCHROME Probes for Bioimaging

Schedule

Jennifer Lippincott-Schwartz' lecture

We are expecting a "BMC trailblazer lecture" by Jennifer Lippincott-Schwartz on Monday, 11:00. This lecture is not part of the workshop, it just happens to be on the same day in the same lecture hall, two hours before our workshop starts. In her lab, photoactivation localization microscopy (PALM) was developed, one of the first superresolution microscopy techniques. I imagine that some scientists who are interested in FLIM might also be interested in what she has to say, therefore I put this information here.

Monday's Workshop Talks, 26 Feb 2024

	Title	Speaker
Getting started:		
13:15	Welcome	
13:30-14:15	Introduction to FLIM	Stefanie Weidkamp-Peters, Düsseldorf
14:15-14:45	Tension sensors: FLIM measurement of Flipper-TR in live cells	Juan Manuel Garcia Arcos, Geneva
14:45-15:15	Break	
Label-free metabolic FLIM:		
15:15-15:45	Label-free metabolic imaging of living tissues by Fluorescence Lifetime Microscopy of endogenous biomarkers	Chiara Stringari, Paris
15:45-16:15	Metabolic FLIM (NADPH) and oxygen dependent PLIM	Angelika Rück, Ulm
16:15-16:45	Label free wide-field metabolic imaging of NADH and FAD	André Weber, Magdeburg
16:45-17:15	Break	
Advanced applications:		
17:15-17:45	Investigating HIV Maturation and Nanoparticle Stability with phasor FLIM	Don Lamb, Munich
17:45-18:15	Video rate volumetric FLIM with nanosecond pixel dwell time	Sebastian Karpf, Lübeck
18:15-18:45	Separation of fluorochrome in the same spectral channel with FLIM in confocal and STED microscopy	Mariano Gonzalez Pisfill, Munich

Tuesday's Workshop talks, 27 Feb 2024

	Title	Speaker
FLIM-FRET and standards		
9:00-9:30	FLIM-FRET data acquisition and analysis	Ali Gheisari, Munich
9:30-10:00	Recording dimerization and ligand binding by confocal FLIM-FRET with GPCRs in native lipid environments of cells, membrane vesicles and polymer-derived lipid nanodiscs	Michael Börsch, Jena
10:00-10:30	Characterizing the system: FLIM standards	Michael Börsch, Jena
10:30-11:00	FLIM standards – panel discussion	various
11:00-11:30	Break	
11:30-12:00	Imaging live-cell biochemistry by light	Alessandro Esposito
12:00-12:30	Short talks on community developed FLIM-software: FlimJ FlimFit FLUTE napari-flim-phasor-plotter: more than a plugin to visualize and phasor analyze FLIM data in napari	Sebastian Karpf, Anca Margineanu, Chiara Stringari, Cornelia Wetzker

Parallel hands-on session slots, 27. – 29. Feb. 2024

Tuesday, 27. Feb. 2024
13:30-16:00 Hands on 1
16:30-19:00 Hands on 2

Wednesday, 28. Feb. 2024
9:30-12:00 Hands on 3
13:00-15:30 Hands on 4
16:00-18:30 Hands on 5

Thursday, 29. Feb. 2024
9:30-12:00 Hands on 6

Hands-on session topics – overview

For each session, find below designation, title, conducting persons and the hardware on which it can be performed.

At BMC seminar rooms

N11-1: Analyzing data with FLIMfit
Anca Margineanu, Max-Delbrück-Center, Berlin
Participants Notebooks

N11-2: How to compute fluorescence lifetime photon-by-photon using a cuvette-based optical system
Alessandro Rossetta, FLIM LABS S.r.l.
FLIM LABS equipment

N13-1 Metabolic FLIM (NADPH) and oxygen dependent PLIM
Angelika Rück, Daniela dos Santos, Universität Ulm
Metabolic Imager, Becker & Hickl with Toptica 780 nm and 920 nm femtosecond laser and ibidi stage-top incubator

N13-2 FLIM-FRET with fluorescent proteins
Michael Börsch & Lukas Spantzel, Jena
Metabolic Imager, Becker & Hickl with Toptica 780 nm and 920 nm femtosecond laser and ibidi stage-top incubator

N14-1 How to measure FLIM, PLIM and both simultaneously
Yuansheng Sun, ISS
Q2 confocal with pulsed lasers (375, 488, 635nm) and 2 detectors (ISS Hybrid & Excelitas SPAD), ISS

N14-2 How to use the phasor plots to separate two or three species
Yuansheng Sun, ISS
Q2 confocal with pulsed lasers (375, 488, 635nm) and 2 detectors (ISS Hybrid & Excelitas SPAD), ISS

N14-3 How to use the phasor plots to process and analyze FLIM-FRET data
Yuansheng Sun, ISS
Q2 confocal with pulsed lasers (375, 488, 635nm) and 2 detectors (ISS Hybrid & Excelitas SPAD), ISS

N15-1 Introduction to Frequency Domain FLIM and FRET
Jeroen Wehmeijer, Lambert Instruments
Lambert Instruments LIFA vTAU camera-based FLIM system

N16-1 Video-rate volumetric FLIM with Nanosecond pixel dwell time (SLIDE)
Florian Sommer & Stefan Meyer, Medizinisches Laserzentrum Lübeck GmbH
SLIDE System, Medizinisches Laserzentrum Lübeck GmbH

N19-1 Widefield FLIM - Frequency Domain Fluorescence Lifetime Imaging for Widefield Microscopy
Gerhard Holst, PCO
pco.flim PRO camera w. Nikon stand, 445nm or 488nm or 636nm exc.

N20-1 FLIM-FRET measurements in plant tissue
Stefanie Weidtkamp-Peters, Universität Düsseldorf
Nikon AX with PicoQuant FLIM kit, PicoQuant

N20-2 Studying cell signaling in single living cells
Alessandro Esposito, Brunel University, London
Nikon AX with PicoQuant FLIM kit, PicoQuant

N21-1 Label free wide-field metabolic imaging of NADH and FAD
André Weber, Leibniz-Institut für Neurobiologie, Magdeburg
Lincam with Nikon stand and Omikron light enginge, Photonscore

At the rooms of the Core Facility Bioimaging at the BMC

CF43-1 FLIM measurement of Flipper-TR in live cells
Juan Manuel García-Arcos, Université de Genève
Leica SP8 STED FALCON, Core Facility Bioimaging

CF43-2 Separation of fluorochromes in the same spectral channel with FLIM in confocal and STED microscopy
Mariano Gonzalez Pisfill, Ludwig-Maximilians-Universität München
Leica SP8 STED FALCON, Core Facility Bioimaging

CF44-1 Label-free metabolic FLIM with 2 photon excitation
Chiara Stringari, Institut Polytechnique de Paris
SP8 MP WLL DM8 FALCON, Core Facility Bioimaging

CF44-2 Environmental sensing via lifetime: From TauSense to FALCON
Heike Glauner & Daniel Smeets, Leica Microsystems
Stellaris 8 FALCON, Leica Microsystems

At buildings in the neighborhood

CuP1: Fluorescence lifetime imaging of viral particles and nanocarriers using the phasor approach
Irene Gialdini, Ludwig-Maximilians-Universität München
Self-constructed system, Don Lamb group, Dept. Chemistry and Pharmacy (5 min walk)

CuP2: Structural Biology with a FLIM Microscope Using Graphene Near Field Quenching
Tim Schröder & Giovanni Ferrari
Self-constructed system, Philip Tinnefeld group, Dept. Chemistry and Pharmacy (5 min walk)

MPI1: Lifetime-based Imaging of Order Heterogeneity in in vitro membrane systems
Jan Hagen Krohn & Yusuf Qutbuddin, Max-Planck-Institut für Biochemie
PicoQuant MicroTime 200 system, Schwille lab, Max-Planck-Institut für Biochemie (10 min walk)

MPI2: Sub-resolution spatial information from FLIM
Martin Spitaler & Markus Oster, Max-Planck-Institut für Biochemie
Leica SP8 FALCON, Imaging Facility, Max-Planck-Institut für Biochemie (10 min walk)

Hands-on sessions - abstracts:

N11-1 Analyzing data with *FLIMfit*

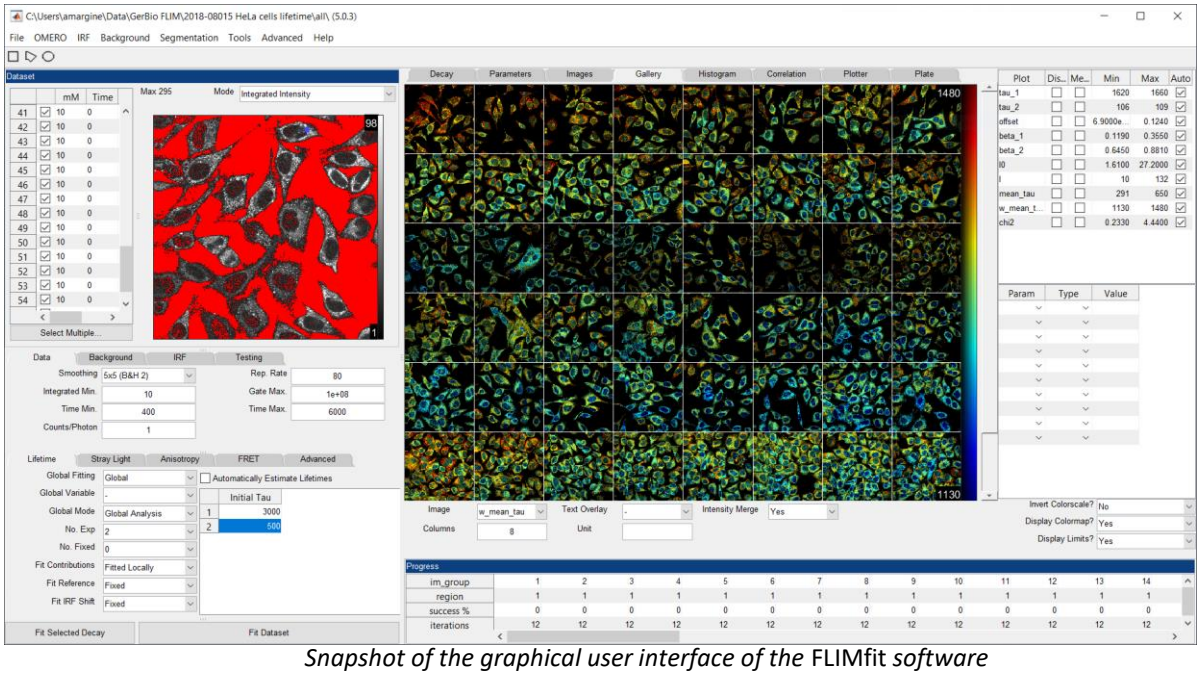
Dr. Anca Margineanu
Max-Delbrück-Centrum für Molekulare Medizin in der Helmholtz-Gemeinschaft, Berlin, Germany
E-Mail: Anca.Margineanu@mdc-berlin.de

FLIMfit is an open-source software for the analysis of confocal time-resolved single photon counting (TCSPC) and wide-field time-gated data (https://flimfit.org/, *publication*: Warren et al., (2013), PLoS ONE 8(8): e70687, doi:10.1371/journal.pone.0070687). The software is based on global analysis algorithms, providing fast fitting of complex decays with low photon counts within very long image series (e.g. z planes combined with time and/or tile acquisitions, multiwell plates).

During the workshop, I will present data handling using the software, as well as analysis concepts that can be applied when using model-based fluorescence decay fitting integrated within the software. Depending on the time and on the interests of the participants, the main points will be:

- Uploading files of various formats (Becker&Hickl .std/_uz.std, .pt3 files from Picoquant and exported from Leica Falcon format, LaVision Biotec ome.tiff, series of .tiff images acquired with custom setups and ASCII .txt, .csv);
- Fitting time-resolved fluorescence decays with exponential models using instrument response function (IRF) convolution, background correction and segmentation (incl. phasor segmentation)
- Applications of global fitting models for the analysis of FRET (Förster resonant energy transfer) biosensors, membrane tension dyes and NADH/NADH biosensors in tissues, organoids and cells;
- Examples of fitting a FRET model for donors with complex decay;
- Example of FRET screening in a multiwell plate with improved results by using segmentation based on acceptor images;
- Models for fitting time-resolved anisotropy decays.

Participants can install the software on their own laptops and are encouraged to bring and test their own images in one of the aforementioned formats, but some examples of data will also be provided.



Snapshot of the graphical user interface of the FLIMfit *software*

Note from the organizers: no notebooks can be provided by the organizers. Bring your own and best install FLIMfit before the meeting if you wish to use the software directly during the session

N11-2: How to compute fluorescence lifetime photon-by-photon using a cuvette-based optical system (FLIM LABS system)

Alessandro Rossetta | FLIM LABS <alessandro.rossetta@flimlabs.com>

Description:

Join us for an immersive 2-hour hands-on session where we delve into the intricate world of fluorescence lifetime analysis, bridging theory and practice using Python. This workshop is designed to equip you with the skills to compute fluorescence lifetime from the ground up.

Agenda:

Theoretical Foundations (35 minutes):

Understanding the fundamentals of fluorescence lifetime.

Introduction to minimum requirements of an hardware setup for fluorescence lifetime calculation.

Introduction to fluorescence intensity decay profile reconstruction.

Overview of Instrument Response Function (IRF) and its significance.

Introduction to phasors and their role in lifetime analysis.

Instrumentation Setup (25 minutes):

Exploration of the hardware components: pulsed laser, Single-Photon Avalanche Diode (SPAD) detector, and time-tagger.

Hands-on experience with a cuvette-based single-point spectroscopy setup.

Fluorescence signal acquisition.

Python Implementation (30 minutes):

Step-by-step guidance on coding for fluorescence lifetime computation.

Incorporating phasor analysis for comprehensive insight.

Utilizing IRF-deconvolved fittings for precise results.

Practical Application (30 minutes):

Integration of theoretical concepts into real-world scenarios.

Live demonstration with sample measurements and analysis.

Troubleshooting common issues in fluorescence lifetime experiments (e.g SNR).

Goal:

By the end of this session, you will have gained a holistic understanding of fluorescence lifetime analysis, hands-on experience with a cuvette-based optical system, cutting-edge hardware, and proficiency in implementing fluorescence lifetime calculation using Python.

N13-1 Metabolic NADH/FAD/FMN FLIM in bioenergetic alterations (B&H system)

Angelika Rück, Daniela dos Santos

University Ulm, Core Facility confocal and multiphoton Microscopy N24, Albert-Einstein-Allee 11, 89081 Ulm, Germany: e-mail: angelika.rueck@uni-ulm.de

Metabolic Imager, Becker & Hickl with Toptica 780 nm and 920 nm femtosecond laser and ibidi stage-top incubator

KEY WORDS: FLIM, TCSPC, multiphoton microscopy, NAD(P)H, FAD, FMN

A common property during tumor development and other diseases is altered energy metabolism, which could lead to a switch between oxidative phosphorylation (OXPHOS) and glycolysis. FLIM (fluorescence lifetime imaging) of metabolic coenzymes, as NAD(P)H and FAD, is now widely accepted to be one of the most reliable diagnostic methods to determine cell metabolism and different algorithms are actually investigated to get reproducible results. The shift of cell metabolism from OXPHOS to glycolysis, observed in tumor cells, is mostly associated with a shortening of the fluorescence lifetime of NADH (more free NADH), whereas bound FAD increases. During the hands on training, metabolic FLIM will be compared for two different cell lines, one tumor cell line, a squamous carcinoma cell (SCC4) and a normal keratinocyte cell, the so called HaCaT cell. Bioenergetic alterations will be analyzed and discussed.

N13-2 FLIM-FRET with fluorescent proteins (B&H system)

Lukas Spantzel & Michael Börsch, Jena.
Lukas.Spantzel@med.uni-jena.de, michael.boersch@uni-jena.de

Metabolic Imager, Becker & Hickl with Toptica 780 nm and 920 nm femtosecond laser and ibidi stage-top incubator

In this hands-on session, confocal FLIM-FRET microscopy and controls will be demonstrated. FLIM-FRET is a powerful technique employed to explore molecular interactions within biological samples *in vitro* and *in vivo*. The experiments utilize fixated *Escherichia coli* samples which were genetically engineered to express different fusion constructs of the bright fluorescent proteins mNeonGreen as FRET donor and mScarlet as FRET acceptor. These cytosolic fusion proteins are connected by different peptide linker lengths, i.e., mNeonGreen-5-mScarlet, mNeonGreen-17-mScarlet, and mNeonGreen-32-mScarlet, for different FRET efficiencies. These peptide linkers assure close proximities of FRET donor and acceptor fluorophores. As necessary controls for fluorescence lifetime measurements, fixated *Escherichia coli* expressing either soluble mNeonGreen or mScarlet are recorded.

Bacterial cells will be immobilized on a coverslip coated with Concanavalin A before the treatment with methanol for fixation, ensuring the preservation of the photophysical properties of the fluorescent proteins. Subsequently, these cells will be embedded in a mounting agent, which serves to minimize light scattering and to reduce background fluorescence, thereby enhancing the precision of the confocal FLIM measurements.

Additionally, FLIM-FRET data with purified fluorescent proteins will be recorded using solutions of the fusion proteins for FRET with different linkers. Purified fusion proteins can be stored and used as FLIM-FRET standards.

N14-1: How to measure FLIM, PLIM and both simultaneously. (ISS)

Yuansheng Sun, Yuansheng.Sun@iss.com

Q2 confocal microscope with pulsed lasers (375nm, 488nm, 635nm) and two detectors (ISS Hybrid & Excelitas SPAD), ISS

Since 1984, ISS has been committed to the development and design of highly sensitive scientific instrumentation for research and clinical applications. Our fluorescence product line includes steady-state / time-resolved spectrofluorometers and time-resolved laser scanning confocal microscopes and accessories for many quantitative measurements at the single molecule detection sensitivity:

- Multi-dimension (x, y, z, t, τ, λ) confocal imaging by single-photon and multi-photon excitation.
- FLIM / FLIM-FRET / PLIM / upconversion, analyzed by both fitting and phasor plots.
- Time-resolved superresolution imaging by SPLIT-STED with pulsed excitation and pulsed depletion.
- Time-resolved superresolution imaging by a SPAD-array airy detector.
- Steady-stead and time-resolved polarization anisotropy imaging.
- FCS / FLCS / nanosecond FCS / photon antibunching / Scanning FCS / PIE-FCCS / PCH / N&B / RICS
- PIE-smFRET burst analysis with the 2D analysis of Stoichiometry vs. FRET efficiency.

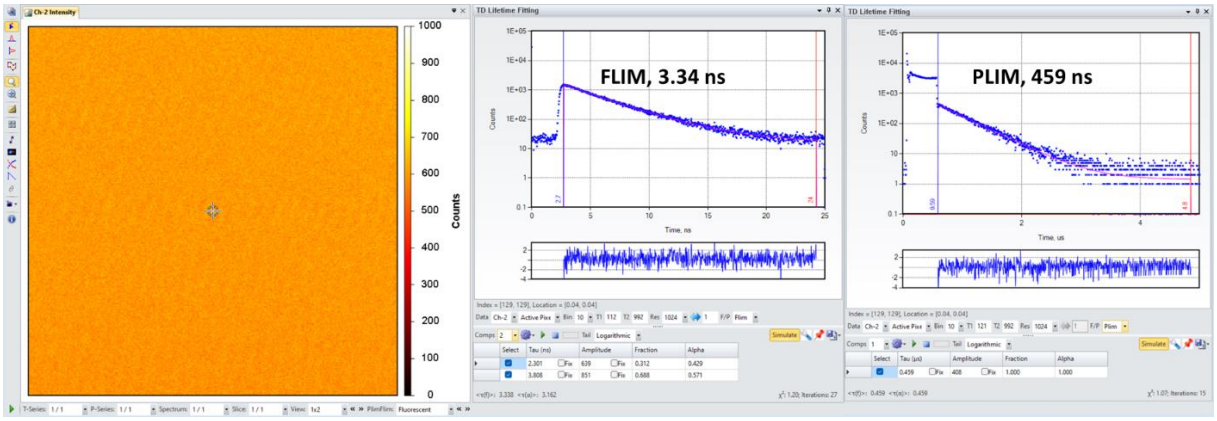
For the workshop, ISS will bring the state-of-the-art Q2 confocal microscope, equipped with three pulsed lasers (375nm, 488nm, 635nm) and two detectors (ISS Hybrid & Excelitas SPAD) covering the detection wavelengths of 300-1050nm. It is capable of measuring time-resolved data by both digital TCSPC and digital frequency domain (DFD, AKA FastFLIM™) techniques. The demonstration will be focused on lifetime imaging and data analysis by the phasor plots using the ISS VistaVision software and its powerful multi-image phasor analysis (MiPA) plugin. Other measurements (e.g. FCS, scanning FCS, N&B, smFRET, antibunching, etc.) can be carried out upon participants' requests.

For more information and options, please visit www.iss.com and contact microscopy@iss.com.

Session schedule: How to measure FLIM, PLIM and both simultaneously.

The standard fluorescent and phosphorescent dyes will be used to demonstrate FLIM and PLIM, respectively. A mixture of both fluorescent and phosphorescent dyes will be used to demonstrate the simultaneous fluorescence and phosphorescence lifetime imaging.

Both fitting and phasor analyses for FLIM and PLIM data using the ISS VistaVision software will be demonstrated and discussed.

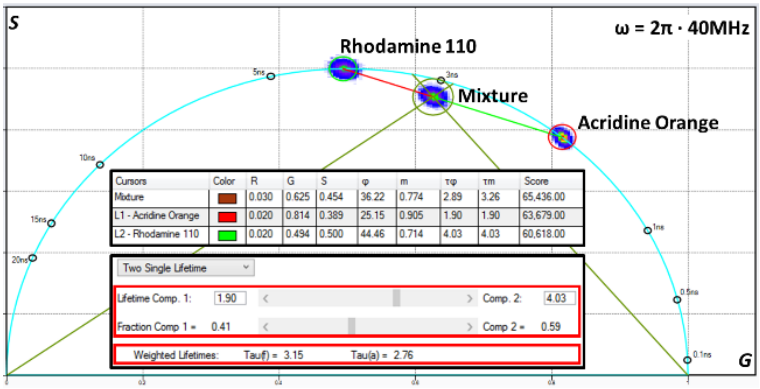


N14-2: How to use the phasor plots to separate two or three species. (ISS)

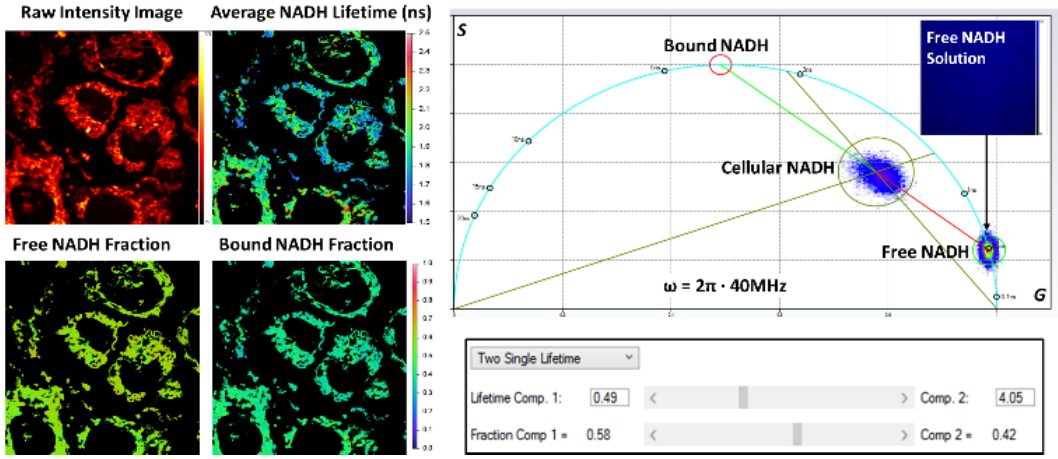
Yuansheng Sun, Yuansheng.Sun@iss.com

Q2 confocal microscope with pulsed lasers (375nm, 488nm, 635nm) and two detectors (ISS Hybrid & Excelitas SPAD), ISS

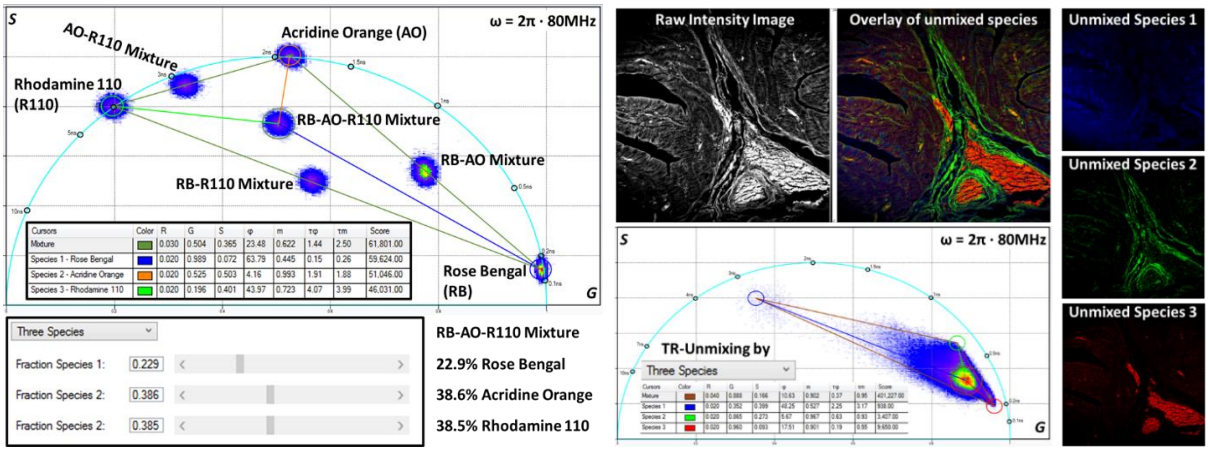
Phasor unmixing and quantitative separation of a mixture of two fluorescent dyes in solution.



Phasor unmixing and quantitative separation of Free vs. Bound NADH in unlabeled live cells.



Phasor unmixing and quantitative separation of three species in a HE-stain tissue sample.

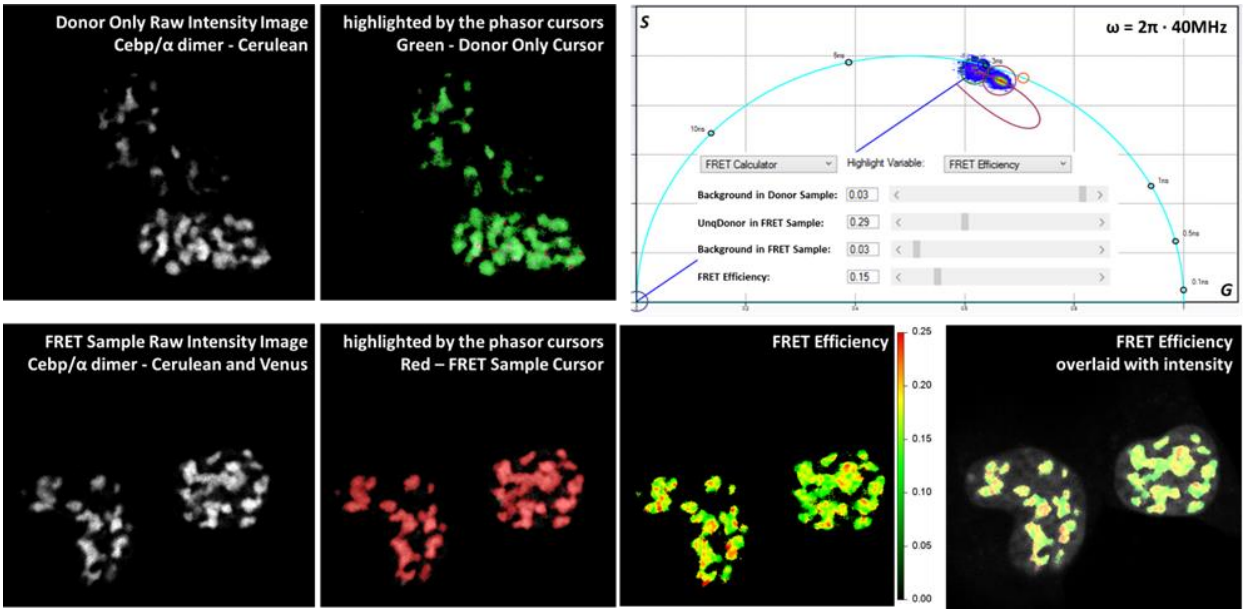


N14-3: How to use the phasor plots to process and analyze FLIM-FRET data (ISS)

Yuansheng Sun, Yuansheng.Sun@iss.com

Q2 confocal microscope with pulsed lasers (375nm, 488nm, 635nm) and two detectors (ISS Hybrid & Excelitas SPAD), ISS

One of the major applications of FLIM is to measure FRET. The FLIM-FRET method does not require the corrections for spectral bleedthrough that are necessary for intensity-based measurements of sensitized emission from the acceptor. Also, quantifying FRET by FLIM is much less affected by photobleaching and scatter / ambience noise compared to the intensity-based FRET imaging. All these factors make FLIM to be among the most accurate methods of measuring FRET [1]. Due to the non-linear quenching process in FRET, the phasors of unquenched and quenched species do not follow a linear combination relationship, making the quantitative FRET analysis more complicated using the phasor plots. Fortunately, Dr. Gratton and coworkers developed an elegant solution, called the "FRET trajectory" approach [2,3], to quantitatively calculate FRET efficiencies on the phasor plots. This FRET trajectory method is implemented as the FRET calculator in ISS VistaVision MiPA plugin [4]. This demonstration will show how to use the FRET calculator to determine the FRET efficiencies of the FRET-standard samples and localize the dimerization of the transcription factor Cebp/α binding proteins in live cells [1].



References:

[1]. Y. Sun, R.N. Day and A. Periasamy, "Investigating protein-protein interactions in living cells using fluorescence lifetime imaging microscopy," *Nat. Protoc.* 6, 1324-1340 (2011).

[2]. M.A. Digman, M. A., V.R. Caiolfa, M. Zamai and E. Gratton, "The phasor approach to fluorescence lifetime imaging analysis," *Biophys. J.* 94, L14-6 (2008).

[3]. S. Ranjit, L. Malacrida, D.M. Jameson, E. Gratton, "Fit-free analysis of fluorescence lifetime imaging data using the phasor approach," *Nat. Protoc.* 13(9): 1979-2004 (2018).

[4]. Y. Sun, U.C. Coskun, S.C. Liao and B. Barbieri, "Quantitative tools in the Multi-image Phasor Analysis (MiPA)," *Proc. SPIE 11965, Multiphoton Microscopy in the Biomedical Sciences XXII*, 119650A (2022).

N15-1: Introduction to Frequency Domain FLIM and FRET (Lambert Instruments)

Jeroen Wehmeijer jeroen@lambertinstruments.com

Lambert Instruments LIFA vTAU camera-based FLIM system

Fluorescence Lifetime Imaging Microscopy (FLIM) is gaining interest as a tool to assess the biochemical environment of fluorescent molecules/probes. Upon excitation, fluorescent molecules emit light and the fluorescence lifetime quantifies the decay rate of that emitted light. The fluorescence lifetime is a telltale signature of the molecules and their immediate environment. Among others, the decay time can be affected by pH, pressure, temperature and neighbouring fluorescent molecules (FRET)

FLIM is the technique to map the spatial distribution of lifetimes in living cells and inorganic material. A key advantage of the fluorescence lifetime over the light intensity is that fluorescence lifetime is independent of concentration, bleaching and intensity variations, making it an inherently quantitative technique.

The LIFA vTAU is a camera-based FLIM system for fast Fluorescence lifetime imaging. Through the frequency-domain detection technology offered by the modulated camera and modulated light source, it allows fast acquisition of lifetime images.

The camera makes the LIFA system highly versatile and therefore applicable for live cell imaging. The standard, widefield system includes a Multi-LED modulated light source with high-power LEDs. Using a Multi-LASER engine it can be easily combined with Total Internal Reflection Fluorescence (TIRF), with multi-beam confocal spinning disk, or with lightsheet Microscopy.

Workshop schedule:

10-15 min.: brief introduction to camera-based frequency domain FLIM

30 min. Handson on FLIM measurements from a selection of samples (plan is to demonstrate FRET, participants are encouraged to bring own samples)

15 min summary and Q&A

Remaining time will be available for users to freely test the system

For further information/questions please contact sales@lambertinstruments.com

N16-1 Video-rate volumetric FLIM with Nanosecond pixel dwell time (SLIDE)

Florian Sommer & Stefan Meyer, Medizinisches Laserzentrum Lübeck GmbH
SLIDE System, Medizinisches Laserzentrum Lübeck GmbH

Two-photon microscopy enables imaging deep within tissue with high spatial resolution and reduced photobleaching. However, real-time imaging of fast biological processes requires new strategies to increase the acquisition rate. Many commercially available two-photon microscopes are point scanning instruments that are limited to 10 - 100 frames per second, which is not sufficient for video-rate volumetric imaging.

At the workshop, we showcase a prototype for video-rate volumetric two-photon microscopy and FLIM imaging at nanosecond voxel dwell time, based on the technique Spectro-temporal Laser Imaging by Diffracted Excitation (SLIDE). The prototype is currently being developed within the framework of a Leibniz Transfer grant. SLIDE enables high speed two-photon microscopy with kHz-frame rates by angular dispersion of a pulse-modulated, rapidly wavelength-swept laser, implementing inertia-free beam steering. High-bandwidth hybrid photodetectors (HPD) allow acquisition of fluorescence life times with high sampling rates and the application of an objective scanner enables video-rate volumetric imaging with nanosecond pixel dwell times and therefore real-time 3D FLIM acquisition of fast biological processes under realistic conditions deep within tissue. The hands-on workshop will showcase the SLIDE prototype including home-built FLIM video-rate volumetric imaging using Micromanager as well as a Napari.

N19-1 Widefield FLIM - Frequency Domain Fluorescence Lifetime Imaging for Widefield Microscopy (PCO system)

Gerhard Holst & Alexander Dietz, Excelitas PCO GmbH, Kelheim, Germany

pco.flim PRO camera w. Nikon stand, 445nm or 488nm or 636nm exc.

Abstract:

Fluorescence Lifetime Imaging allows to use both parameters of photo luminescence, intensity and the decay time, which provide relevant information about bio-markers, indicators, FRET-pairs or auto fluorescence. In this workshop a camera based FD (frequency domain)-FLIM system connected to a standard inverted microscope (Nikon Ti2) is used to measure fluorescence intensities and fluorescence lifetimes in a direct and simple way.

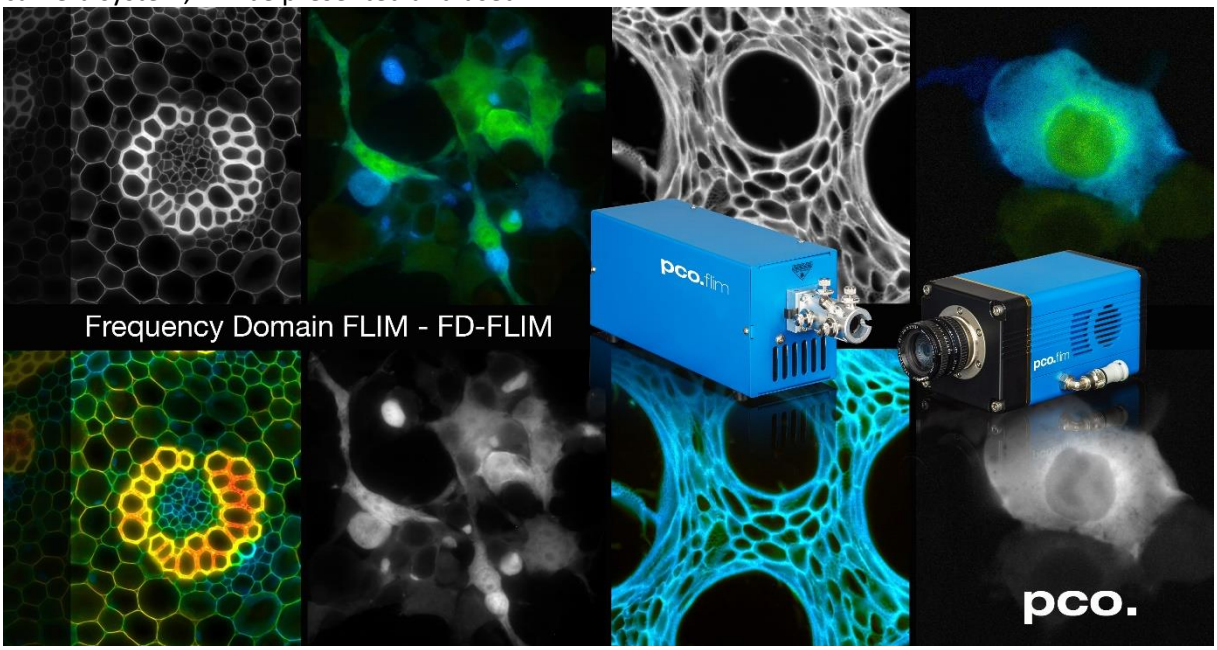
FD-Fluorescence Lifetime Imaging (FD-FLIM) provides access to the nanosecond fluorescence excited state decay kinetics, i.e. the "fluorescence lifetime", of fluorophores. FLIM allows to use the fluorescence lifetime as additional parameter which enables

- A fast, sensitive and quantitative measurement of Förster Resonance Energy Transfer (FRET) for example for the use of bio-sensors or the detection of protein interactions
- Additional spectroscopic information for the discrimination of multiple fluorophores in samples (for example tissue differentiation by auto-fluorescence)
- Better calibration of optical chemical sensors like oxygen or pH optodes
- Detection of microplastic particles in the environmental samples

Overview of Workshop:

- The basics of FD-FLIM based on a camera system will be explained and a variety of applications will be presented.
- A hands-on session will be given, which shows the process of a complete widefield FLIM measurement (including referencing / bracketing, FLIM measurements and storage of data).
- If participants bring fixed samples on microscope slides with fluorophores, which can be excited with either: **445nm or 488nm or 636nm**, it is possible to try to measure the corresponding fluorescence lifetimes. Available objective magnifications are 20x and 40x air or 40x water immersion.
- If there are no samples from participants some more measurements from different plant samples will be made.

For the first time the new pco.flim PRO camera system, which is an improved version of the pco.flim camera system, will be presented and used.



N20-1 FLIM-FRET measurements in plant tissue (Picoquant System)

Stefanie Weidtkamp-Peters, Universität Düsseldorf

Nikon AX with PicoQuant FLIM kit, 440, 485, 510, 560, 640 nm exc., MultiHarp150 TCSPC, PicoQuant

In many biological research projects, a high spatial and temporal resolution for the observation of in vivo protein interaction is needed, e.g. in order to follow changes of interactions and complex formation over time. In vivo Fluorescence or Förster resonance energy transfer (FRET) measurements allows for detailed analyses of interacting molecules in their natural environment at a subcellular level. Especially FRET-FLIM (Fluorescence lifetime imaging microscopy) measurements provide an extremely powerful and reliable tool meeting the demands for investigating in vivo protein interaction also quantitatively and with high precision.

During the workshop we will introduce in detail how to practically perform in vivo FRET measurements in living plant tissue and discuss potential pitfalls and points of consideration.

N20-2 Studying cell signaling in single living cells (Picoquant System)

Alessandro Esposito, Brunel University, London

Nikon AX with PicoQuant FLIM kit, 440, 485, 510, 560, 640 nm exc., MultiHarp150 TCSPC, PicoQuant

FRET and FLIM offer the possibility to make biochemistry in single living cells. However, FLIM has been hindered by huge limitations in speed and accessibility. The most accurate FLIM techniques (TCSPC) was slow and inefficient often limiting FLIM applications to cell biochemistry in acquiring single snapshots. Moreover, the costs of FLIM systems and the expertise required to operate them, further limited their use. Over the last decade, two factors have vastly changed the field.

First, technological innovations have significantly mitigated these limitations permitting us to make biochemistry in single living cells with high spatiotemporal resolution, with more cost-effective and user-friendly instruments and analytical tools.

Second, single-cell "omics" have refocused the interest of the biomedical community to single cell biology or, in other words, to decode the vast heterogeneity biological system exhibits. The high demand for single cell biochemical techniques and the availability of high-end affordable commercial systems are now driving the development and application of fast high resolution FLIM.

In this session, we'll learn how to use fast FLIM techniques to detect biochemical events using calcium imaging as an example. We will focus on the practical use of fast FLIM and requirements for sample preparation, and provide tips for how these techniques can be extremely useful to multiplex biosensors, and their application to 3D cultures.

N21-1 Label free wide-field metabolic imaging of NADH and FAD (LINCamPhotonscore)

André Weber, Leibniz Institute for Neurobiology, Magdeburg
Yury Prokazov, Photonscore GmbH, Magdeburg

A major problem in imaging under low illumination conditions is the noise of current detection systems. In this workshop, we present an alternative imaging method that enables time-resolved wide-field imaging with diffraction-limited spatial resolution using a pixel-free sensor without readout noise.

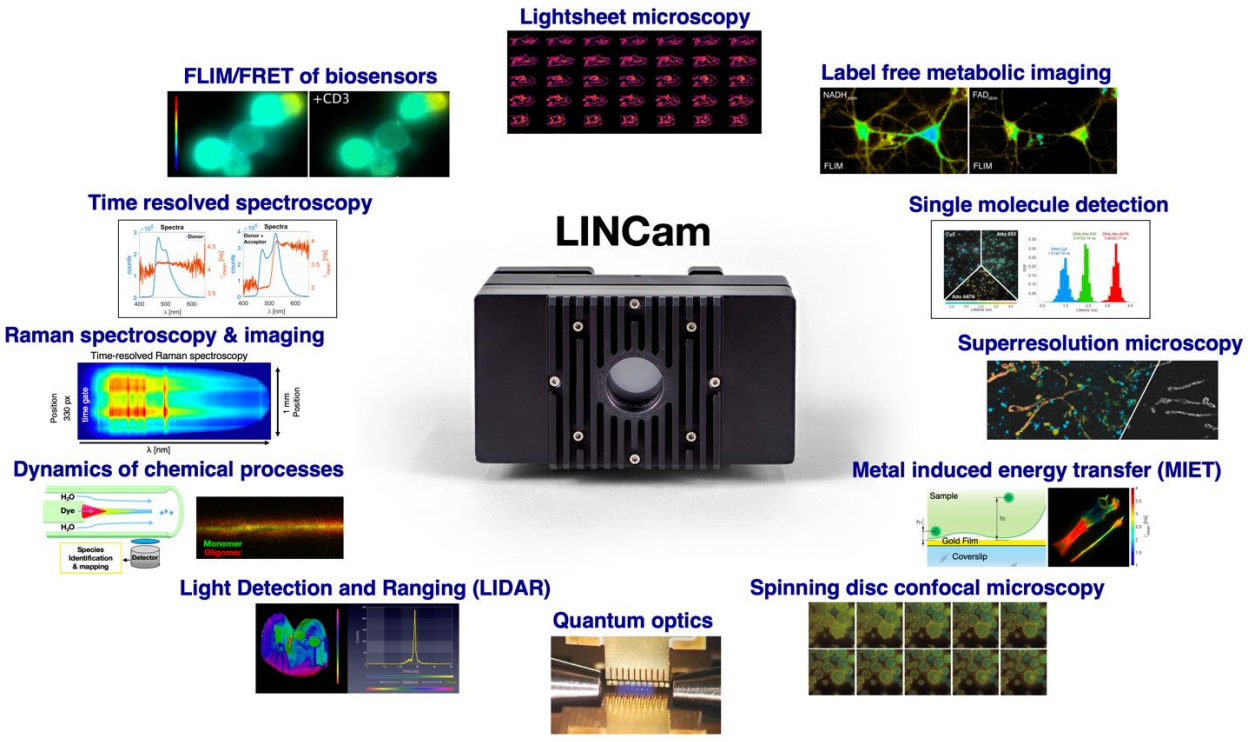
The FLIM imaging system is based on the commercially available photon counting camera (LINCam, Photonscore GmbH) that uses patented positional-sensitive photomultiplier tubes with unique features. The LINCam operates under extreme low light conditions (typically < 50 mW/cm^2) without pixel elements and enables high image quality (equivalent to a 1000 x 1000 pixel CCD) in combination with high temporal resolution (< 40 ps).

Workshop Topics

- Operation principle of wide-field single photon counting LINCam system
- Introducing high-throughput single photon counting systems LINPix and LINTag
- Applications of LINCam in different fields with focus on label free NADH imaging
- Ease of assembling including coupling of pulsed laser sources
- Ease of operation by hands-on sessions with stained and label free samples
- LINCam data analysis toolbox

Participants are encouraged to bring their own samples for NADH imaging (excitation 355nm) or any GFP-like or synthetic dyes excitable with 488 nm.

Hardware: LINCam (Photonscore), Microscope (Nikon); Objectives: 100x, 40x, 10x, 4x; Picosecond pulsed lasers: Omicron Laserage (488nm); PicoQuant (355nm)



CF43-1 FLIM measurement of Flipper-TR in live cells (Leica SP8)

Juan Manuel García-Arcos, Université de Genève
Leica SP8 STED FALCON of the Core Facility Bioimaging

Mechanosensitive small-molecule fluorescent probes, such as Flipper-TR, have revolutionized the imaging of physical forces within living systems. First introduced in 2018, these probes have since been applied across a diverse array of membrane-related research topics. Notably, they report membrane tension changes induced by osmotic shocks, investigate the impact of membrane tension on endocytosis, and uncover the presence of a membrane tension gradient during cell migration.

Comprising two dithienothiophene (DTT) groups capable of rotational movement around a carbon bond, Flippers are hydrophobic and spontaneously integrate into lipid membranes. The lateral forces that pack lipids into a bilayer exert pressure onto Flipper and planarize the DTT groups, which are thus molecular sensors for compressive forces. These groups serve as molecular sensors for compressive forces. The resulting conformational changes impact the duration of the excited state, measured through fluorescence lifetime imaging microscopy (FLIM). In living cells, an increase in membrane tension corresponds to an extended lifetime in FLIM images, and vice versa. Today, commercially available Flipper probes enable tension imaging in various cellular compartments, including plasma membranes, mitochondria, endoplasmic reticulum (ER), and lysosomes. Additionally, HaloFlippers, which covalently attach to HaloTags expressed in the membrane of interest, allow for targeted imaging in specific cellular locations.

In this presentation, we aim to address three key aspects: i) an overview of different Flipper variants and their applications in research, ii) an exploration of limitations encountered both in vivo and in vitro, and iii) practical considerations and protocols, encompassing experimental procedures and lifetime analysis methodologies

CF43-2 Separation of fluorochromes in the same spectral channel with FLIM in confocal and STED microscopy (Leica SP8)

Mariano Gonzalez Pisfill, Ludwig-Maximilians-Universität München
Leica SP8 STED FALCON of the Core Facility Bioimaging

With the development of sensitive and highly resolving instruments, fluorescence microscopy has become an essential tool of biological imaging. Its best-known feature is the specificity of its emission signal (fluorescence) that allows structures of interest to stand out against an otherwise dark background. In addition to its spectral properties for excitation and emission, a fluorophore is defined by a third property: the lifetime of its excited state before the return to the ground state by fluorescence emission. Since the emission of a fluorescence photon normally happens in the range of nanoseconds after excitation, dedicated instrumentation is needed for lifetime measurements. Fluorescence lifetime can add significant information that helps to understand the sample better. It is affected by the environmental conditions in the sample. Spectrally overlapping fluorochromes can be separated if their lifetime is sufficiently different, allowing a better understanding of complex samples and the removal of background autofluorescence. Changes in de-excitation pathways (e.g. by FRET or STED) change the lifetime and can be used for distance measurements at the molecular level and for improved resolution in a simple and elegant way.

For example, several years ago, the common approach in multi-color STED (Stimulated emission depletion microscopy) was to multiply depletion laser wavelengths. However, alignment of depletion lasers requires highest precision in the nanometer range. It is thus preferable to apply a single depletion wavelength for several fluorochromes. But this limits the number of color channels that can be used. And this is the area where FLIM can shine.

Technical progress and the development of phasor based analysis of STED-FLIM data nowadays permit easy and fast fluorochrome separation. Moreover phasor based separation requires much smaller photon numbers compared to curve fitting when there is background present.

We here present a labeling scheme that can be applied in life science environments, e.g. on cultured human cells, with common staining protocols, commercially available fluorochromes and a turn-key FLIM-STED microscope. We describe that the number of usable fluorochromes in STED or confocal microscopy can generally be doubled by phasor based fluorescence lifetime separation of two dyes with similar emission spectra but different fluorescent lifetimes.

Reference:
Stimulated emission depletion microscopy with a single depletion laser using five fluorochromes and fluorescence lifetime phasor separation. Gonzalez Pisfil M, Nadelson I, Bergner B, Rottmeier S, Thomae AW, Dietzel S. Sci Rep. 2022 Aug 18;12(1):14027. doi: 10.1038/s41598-022-17825-5.

CF44-1 Label-free metabolic FLIM with 2 photon excitation (Leica SP8 MP)

Chiara Stringari, Institut Polytechnique de Paris, (chiara.stringari@polytechnique.edu)

SP8 MP WLL DM8 FALCON of the Core Facility Bioimaging

Two-photon microscopy of endogenous biomarkers has shown important potential for label-free and non-invasive monitoring of cellular metabolic processes in cells and living tissues. NAD(P)H and FAD are the most important ubiquitous metabolic cofactors of *redox* (reduction/oxidation) reactions in the cell and central regulators of mitochondrial ATP production and antioxidant defense. The fluorescence lifetimes of NAD(P)H and FAD are exquisitely sensitive to enzyme binding during the cycling of the electron transport chain. Therefore, two-photon fluorescence lifetime microscopy (2P-FLIM) imaging of the metabolic coenzymes NAD(P)H and FAD can provide functional information on cellular redox ratios and on the complexity of several metabolic pathways (glycolysis, oxidative phosphorylation, oxidative stress, fatty acid oxidation and synthesis).

In this workshop, we will discuss the requirements for 2P-FLIM of NAD(P)H and FAD in live cells and tissues. An *in vitro* sample of unlabeled HeLa cells will be used for imaging. The conditions for FLIM imaging and analysis will be discussed and the participants will have the opportunity to do the acquisition and adjust imaging conditions. During the workshop we will demonstrate how 2P-FLIM of NADH and FAD has the spatio–temporal resolution required to characterize metabolic cellular heterogeneity and intracellular compartmentalization (mitochondria, cytoplasm and nucleus). Finally we will demonstrate the connection of the FLIM readout with metabolic pathways, by measuring changes in lifetime induced by pharmacological treatments directly in cells (oxidative phosphorylation inhibition and electron transport chain inhibition using cyanide; glycolysis inhibition using 2-deoxyglucose (2DG) and oxidative stress using hydrogen peroxide). The workshop has a focus on the data acquisition, but aims to explain and show the data analysis and visualization as well (using Phasor analysis of FLIM and the open source software FLUTE https://doi.org/10.1017/S2633903X23000211). The outcome of the experiment is a set of FLIM images with the distribution of NAD(P)H and FAD lifetimes of HeLa cells in different metabolic states.

CF44-2 Environmental sensing via lifetime: From TauSense to FALCON (Leica Stellaris)

Heike Glauner & Daniel Smeets, Leica Microsystems
Stellaris 8 FALCON Leica Microsystems, at the Core Facility Bioimaging

Leica Microsystems' TauSense technology is a set of imaging tools based on fluorescence lifetime. Found at the core of the STELLARIS confocal platform, TauSense gives you access to this additional information and expands the potential of your research with the possibilities provided by different TauSense modes. The Leica FALCON technology is a fast and completely integrated fluorescence lifetime imaging microscopy solution complementing the information obtained by TauSense. In the FALCON module, phasor plot and exponential curve fitting based tools allow complex analyses of fluorescence lifetimes.

By studying fluorescence lifetime, quantitative information on the microenvironment of fluorophores can be obtained. Using an exemplary membrane-bound probe we will show in this workshop how exogenous fluorophores can be used to sense spatial and temporal pH changes within the endo-lysosomal system. We will explore in which way fast measurements and different analysis approaches can be realized using Leica Microsystem's TauSense technology based on average photon arrival time or using the phasor plot of the FALCON FLIM module. In addition, we will discuss the options to analyse environmental sensing experiments using FRET-based sensors.

Leica Mikrosysteme Vertrieb GmbH · Ernst-Leitz-Straße 17-37 · 35578 Wetzlar
www.leica-microsystems.com

CuP1 Fluorescence lifetime imaging of viral particles and nanocarriers using the Phasor approach

Irene Gialdini, irene.gialdini@cup.lmu.de

Self-constructed system

Thanks to pulsed lasers and time-correlated single-photon counting (TCSPC) detection electronics, fluorescence lifetime imaging microscopy (FLIM) data in the temporal domain can be easily implemented on confocal microscopes. One way of extracting the fluorescence lifetime information is by exponential fitting the decay of each pixel. However, when the sample is a mixture of different fluorescent species with a complex decay behavior, exponential fitting becomes more difficult and computationally challenging. This is especially true considering the low number of photons available per pixel when imaging at scan rates of ~ 1 s per image or faster. A more convenient way to analyze FLIM data is to use the phasor approach, a fast and fit-free graphical method1 in which TCSPC data are transformed in the Fourier space.

In this hands-on, we will illustrate the key parameters and processes involved in acquiring and analyzing FLIM data by measuring the fluorescence lifetime of free mVenus proteins and mVenus-mCherry tandem dimers in transfected HeLa cells. Additionally, we will image non-infectious mVenus-labelled HIV-1 particles produced by transfected HeLa cells2. The data will be acquired in the time domain using a home-built laser scanning confocal microscope3 and analyzed via the home-written software PIE Analyses with MATLAB (PAM)4. We will then compare the lifetime values of mVenus in the three different conditions and in different regions of the cells.

For a second experiment, we will use the phasor approach to analyze the fluorescent lifetime of previously recorded data of fluorescently labelled nanoparticles diffusing in artificial mucus. Here, we will see how the use of FLIM-phasor enabled us to discriminate between the properly formed nanoparticles versus their cargo released in solution5 in order to monitor the nanoparticles' stability overtime.

1 Digman, M. A., Caiolfa, V. R., Zamai, M. & Gratton, E. The phasor approach to fluorescence lifetime imaging analysis. Biophys J 94, L14-16 (2008). https://doi.org:10.1529/biophysj.107.120154

2 Müller, B. et al. Construction and characterization of a fluorescently labeled infectious human immunodeficiency virus type 1 derivative. J Virol 78, 10803-10813 (2004). https://doi.org:10.1128/JVI.78.19.10803-10813.2004

3 Hendrix, J. et al. Live-cell observation of cytosolic HIV-1 assembly onset reveals RNA-interacting Gag oligomers. J Cell Biol 210, 629-646 (2015). https://doi.org:10.1083/jcb.201504006

4 Schrimpf, W., Barth, A., Hendrix, J. & Lamb, D. C. PAM: A Framework for Integrated Analysis of Imaging, Single-Molecule, and Ensemble Fluorescence Data. Biophys J 114, 1518-1528 (2018). https://doi.org:10.1016/j.bpj.2018.02.035

5 Gabold B., Gialdini I., Khalin I., Greco A., Kromer A., Wang X-, Barlang L., Popp A., Ried C. L., Merdan T., Plesnila N., Lamb D. C., Merkel O. M. Utilizing Chitosan Nanoparticles for the Delivery of Biologics to the Brain via Intranasal Route, submitted

CuP2: Structural Biology with a FLIM Microscope Using Graphene Near Field Quenching

Giovanni Ferrari, Tim Schröder, Philip Tinnefeld

Department of Chemistry and Center for NanoScience, Ludwig-Maximilians-Universität München, 81377 München, Germany

Giovanni.Ferrari@cup.lmu.de, Tim.Schroeder@cup.lmu.de, Philip Tinnefeld phtipc@cup.uni-muenchen.de

Self-constructed system

The fluorescence lifetime of an organic dye contains valuable information about its local environment. Non-radiative decay pathways can be affected e.g. by contact interactions of the dye with a protein resulting in protein induced fluorescence enhancement (PIFE). The reduction of thermal dissipation of the excited state energy due to sticking of the dye leads to a longer fluorescence lifetime. On the other hand, Förster Resonance Energy Transfer (FRET) reduces the fluorescence lifetime of a donor dye. In this process, the excitation energy is non-radiatively transferred from the donor dye to an acceptor dye. The energy transfer rate is strongly distance dependent and the fluorescence lifetime information yields distances between the FRET-pair in the range of 2-10 nm. In our hand-on-session, we will demonstrate the non-radiative energy transfer from a donor dye to graphene, which acts as a two dimensional dark-FRET acceptor with a constant absorption cross-section in the visible range. Compared to FRET, the dynamic range extends to 5-25 nm, which makes Graphene Energy Transfer (GET) suitable to tackle open question in cell and structural biology.

On our home build confocal point scanning microscope with PicoQuant hardware, we will demonstrate how to employ GET to study the position of the actin-myosin motor of the apicomplexan parasite *Toxoplasma Gondii* within the Inner Membrane Complex (IMC) of the cell. The motor plays a crucial role in the parasite migration and host cell invasion, but its exact structure, localization and working mechanism are still not completely understood.

Additionally, we recently discovered that double stranded DNA stands vertically on DNA when immobilized with a single stranded DNA anchor. Thereby, DNA protein interactions can easily be studied via GET measurements with this construct. In this session, single molecule FLIM will be used to monitor the real-time bending of a DNA sequence containing an abasic site (AP site) due to a (mutant) bacterial enzyme, Endonuclease IV.

The participants will analyze the acquired data.

MPI1: Lifetime-based Imaging of Order Heterogeneity in in vitro membrane systems (PicoQuant Microtime 200)

Jan Hagen Krohn & Yusuf Qutbuddin,
krohn@biochem.mpg.de, yusufqq@biochem.mpg.de

Max-Planck-Institut für Biochemie, Schwille lab, Max-Planck-Institut für Biochemie.

PicoQuant MicroTime 200 system of the Schwille lab

Biological membranes consist of countless different lipid and protein components. Thus, it is not surprising that biological lipid membranes tend to demix into phases of different composition and physical properties. FLIM can be used as a powerful tool to study such membrane phase separation when combined with environment-sensitive membrane dyes. In this workshop, we will use FLIM to visualize membrane phase separation in giant unilamellar vesicles and/or supported lipid bilayers. Such artificial membrane models facilitate biophysical studies of phase separation through their reduced complexity in comparison to biological cells. Note that this workshop is complementary to that of Juan Manuel García-Arcos, who will demonstrate the FLIM of the same membrane packing sensitive dye on live cells.

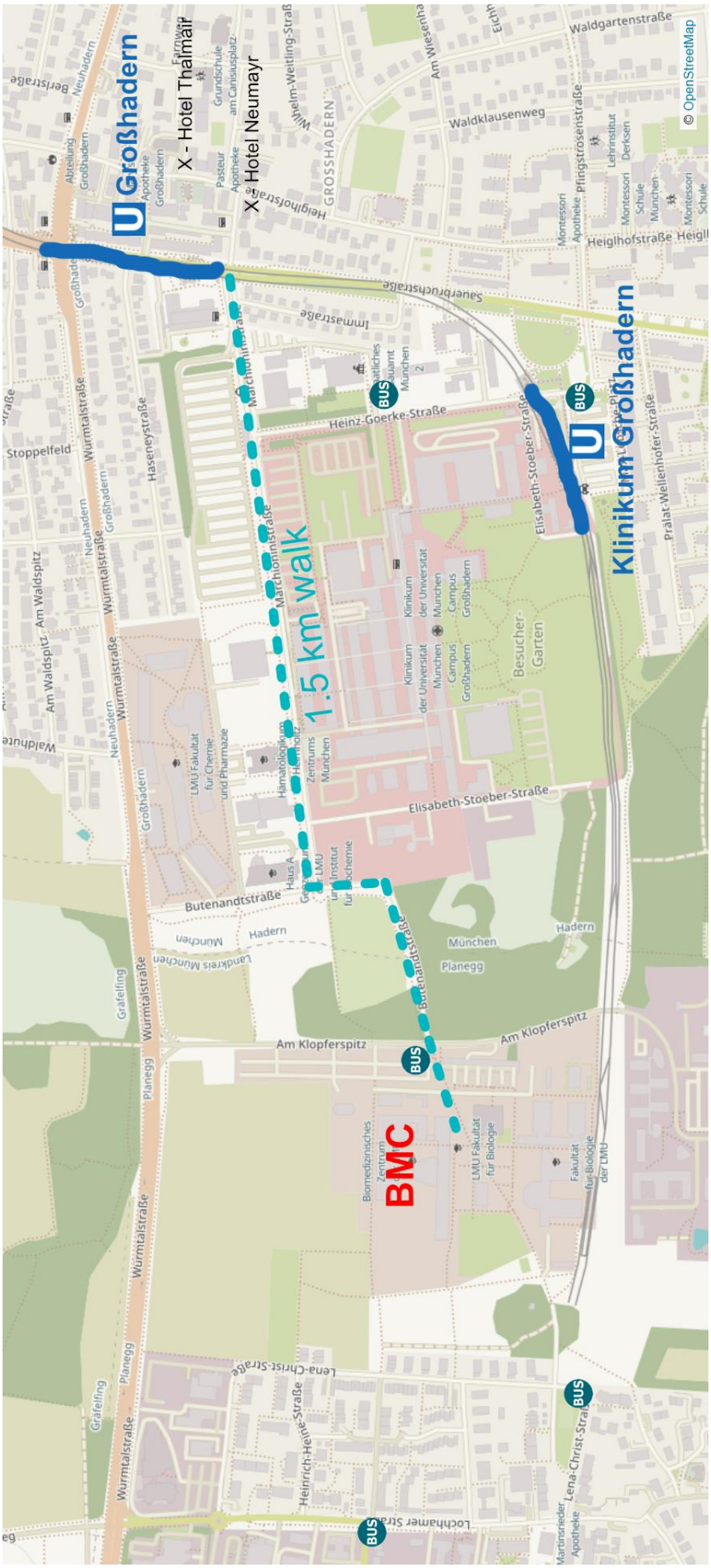
MPI2: Sub-resolution spatial information from FLIM (Leica SP8)

Martin Spitaler & Markus Oster, Max-Planck-Institut für Biochemie

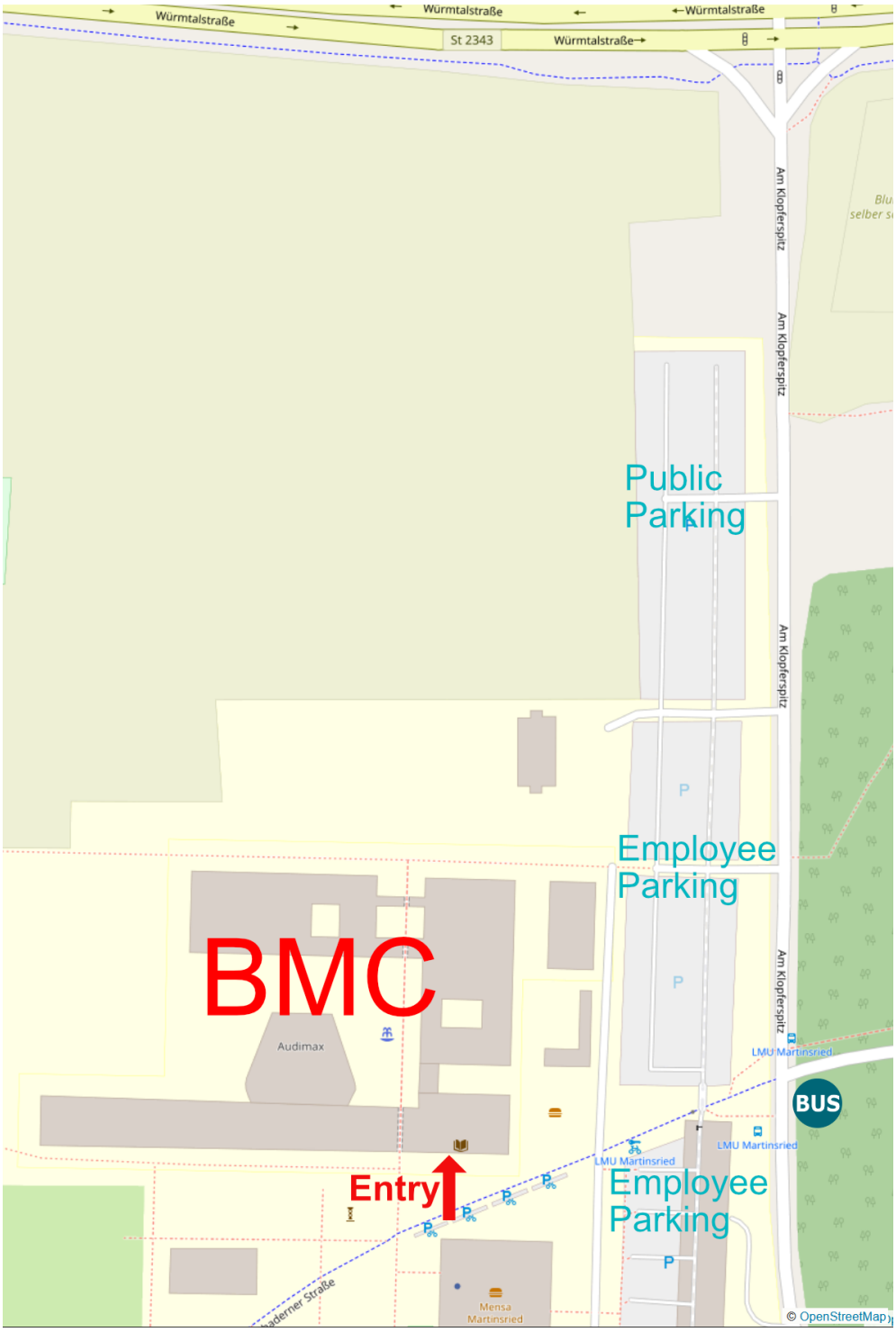
Leica SP8 FALCON, Imaging Facility, Max-Planck-Institut für Biochemie (10 min walk)

The effect of the local environment on the fluorescence lifetime of fluorophores is taking place on the nanometre scale. Therefore, the FLIM signal contains information about molecular interactions far beyond the scale of optical resolution. Examples of such information are measurements of molecular density (auto quenching), protein interactions (FRET) or special constraints (rotational freedom) of the fluorophore. In this hands-on session, we apply FLIM to measure sub-resolution events like the density of fluorophores in solution or proteins in aggregates, and the effect of spatial constraints when a DNA-binding dye intercalates into DNA.

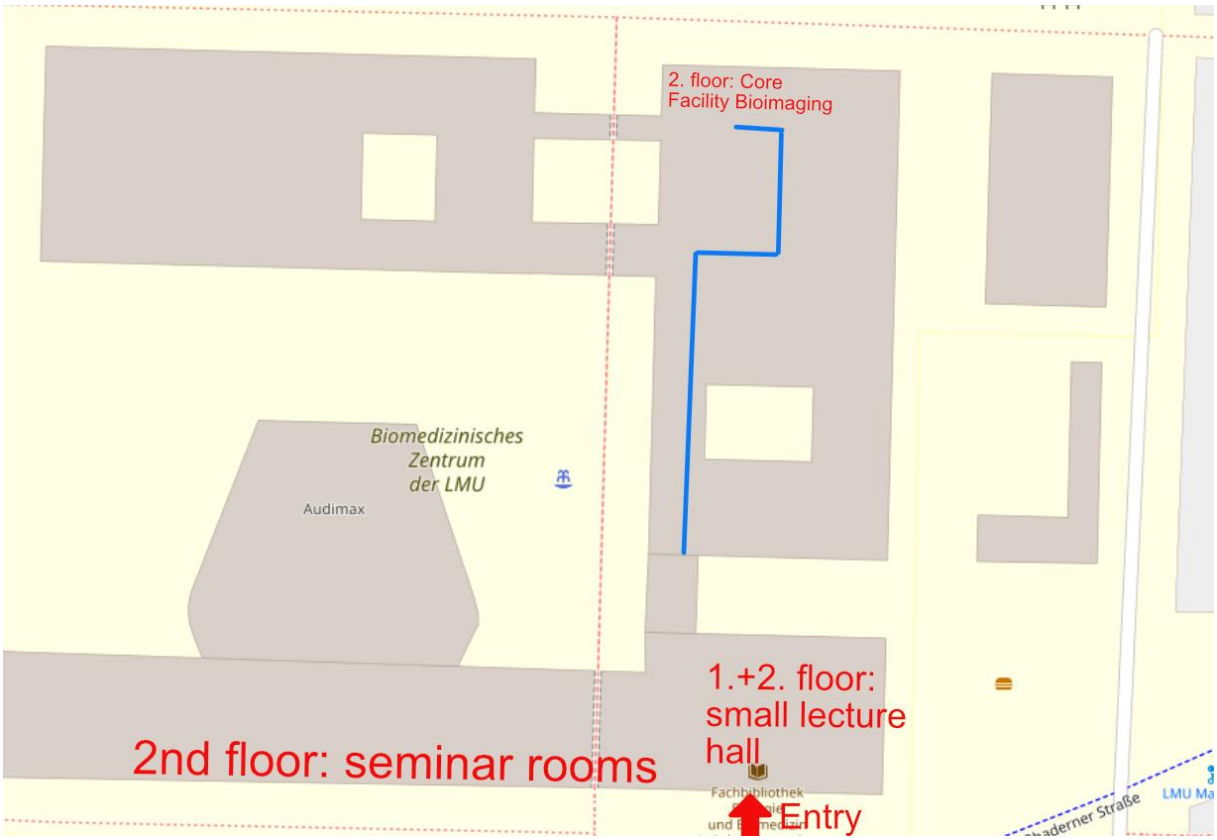
Maps & Venue Information



Large area map



Local area map



Building map

Address

Biomedical Center (BMC),
Großhaderner Straße 9
82152 Planegg

We are located in Martinsried which belongs to Planegg, hence the address. Please note that this is not within Munich city limits, but a few hundred meters outside. There is also a street of that name in Munich itself. Sometimes people confuse this when setting up their navigation system. Also, please do not confuse the Biomedical center with our neighbor to the south, the Biocenter.

How to get to the BMC

The English homepage of the local transportation system is at
http://www.mvv-muenchen.de/en/home/index.html

You also might want to try Google Maps for public transportation connections.

Local public transportation ticket: Your train ticket may include local transportation or you might have a Deutschland-Ticket. Else, you will have to buy the ticket at a blue vending machine before you enter the S-Bahn or U-Bahn platforms or use the app. Most paper tickets from the vending

machines, you have to stamp to validate it. Do it in the blue boxes before entering U- or S-Bahn platforms. With the bus, you can stamp inside. Alternatively, you can use the "MVG Fahrinfo" app on your smart phone to buy tickets and to validate them if required. Don't forget your login details. It happens that the app forgets them.

From Munich central train station (Hauptbahnhof) to U6

Take the U-Bahn U1 (direction Mangfallplatz) or U2 (direction Messestadt Ost) to the next station, "Sendlinger Tor". Switch to the U6 direction Klinikum Großhadern. See below how to continue.

Travel time to Großhadern: approx. 25 minutes (plus U6-BMC)

From train station München-Pasing

If your train is coming in from the West, over Stuttgart, Ulm, Augsburg, or from Lindau, then it very likely will stop at München-Pasing before reaching the central train station. Typically you reach the BMC faster if you exit here and take a bus. This may be an option if you do not plan to visit your hotel first.

Option 1: Bus line 259, from Pasing Bahnhof to Martinsried. This line does not run so often, you have to be a bit lucky. Exit "Martinsried Nord". From there walk through the Bertha-von-Suttner-Weg (a walkway, not a street). At its end continue straight ahead (unpaved gravel path) and walk to the BMC (Google maps does not know the latter path, but it's there!). If you miss the bus stop, after 5 min (and a tour through Martinsried) you reach the stop "Martinsried". Walking distance from here is actually somewhat shorter. With luggage this might be the better option.

Option 2: From Pasing, take the Express Bus line 56 (every 10 min, direction Fürstenried West) to "U-Bahn Großhadern" and walk from there (see below, U6 to BMC). That would be the preferred option if you wish to reach a Hotel in nearby Heiglhofstraße first. If it is raining and you wish to go to the BMC directly, exit a stop later, at Klinikum Großhadern Ost, walk to the other side of the street and wait for the bus 266 (see below, U6 to BMC).

Option 3: If you feel disoriented but wealthy, take a taxi from Pasing. According to a taxi calculator that would be ~ €25 (no warranties given).

From the Airport to U6

The airport S-Bahn station is at the central area, S-Bahn symbol is a green circle with a white S. Take S1 or S8 to the station Marienplatz. The "Marienplatz" is the central square in Munich, with the city hall.

At Marienplatz change to the subway (U-Bahn; Symbol: Blue square with white U), line U6 direction Klinikum Großhadern. See below how to continue.

Travel time from airport: approx. 1 hour (plus U6-BMC).

From U6 to the BMC

Option 1: Walk to the BMC, about 20 min. Exit the U6 at the second to last station, "Großhadern" (without "Klinikum"). Leave the station at the south end (direction of travel of your U-Bahn) and turn right (west) entering Marchioninistraße. At the end of the street continue walking next to the soccer field (both sides are ok). On the other side of the small forest you reach a street, cross it to arrive at the BMC.

Option 2: Bus transport (rainy day option). Exit the U6 at the last station, Klinikum Großhadern. Find the bus stop for the line 266. In about 3 minutes it will bring you to the station "Martinsried, LMU Martinsried" which is directly in front of the BMC. You may have to wait a bit for the bus, though.

Hotels

For hotels there are generally two options: either you try to find something within walking distance or you try to find something close to the U-Bahn line U6. For both options you will find opportunities on the typical booking web sites. Here are some close by examples:

Hotel Thalmair and Hotel Neumayr are in München-Großhadern (near U6), about 20 min walking distance from the BMC. The “Campus at Home” is even closer to the BMC, (https://www.campusathome.de/en/) between the BMC and the Max-Planck-Institutes, but typically somewhat more expensive. It is also not in the city of Munich (but in Planegg, ~11000 inhabitants) which may or may not irritate your travel expense department when the rate is rather high for a small municipality.

Internet access

Wifi is available throughout the building. Eduroam is active for academic users. Make sure you get it to work at your home institution according to local instructions! In addition there is BayernWLAN, open to everybody. You will have to accept usage conditions and then can go online.

Lists of faculty, company representatives, and participants

To be completed