

# FLIM not only for biologists

## Practical aspects with hands-on experience

SIS code: MB100P03

Nov 26. – Nov 28., 2018  
BIOCEV, Průmyslová 595, Vestec

Organized by:

Imaging Methods Core Facility at BIOCEV, Faculty of Sciences, Charles University  
J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences  
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences  
PicoQuant, Germany

## Mission

Fluorescence Lifetime Imaging Microscopy (FLIM) explores the additional information offered by fluorescence probes and sensors to get deeper insights into biological systems and processes.

The course focuses on explaining the principles of nano-second time-resolved fluorescence detection, on demonstration of different hardware realizations, on trying various applications of FLIM in biological imaging and on testing of several ways of FLIM data analysis.

The aim is to uncover the richness of information hidden in multiparametric fluorescence imaging and to inspire the participants to use the easily obtainable extra contrast in their imaging applications. An important part is to make the FLIM data analysis understandable.

The course will cover the most widespread FLIM realization – acquisition in time domain based on raster scanning combined with pulsed lasers and time-correlated single photon counting (TCSPC). Participants will hands-on three different systems, covering pulsed diode lasers, white-light laser source as well as two-photon excitation, SPAD, HyD and GaAsP NDD detectors and three different TCSPC systems and software.

A focus will be given on common pitfalls, instrumental artefacts and ways of avoiding them.

The demonstrated applications will include FLIM-FRET, environmental sensing, NAD(P)H imaging and pattern unmixing.

Testing of your own samples is offered any time after the course and shall be discussed directly with IMCF staff. The testing may be a subject to instrument fees, our assistance during the tests is for free ☺

# Programme

## Monday, Nov 26.

### 08:45 – 09:00 Registration

09:00 – 09:05 Welcome and organization details

Aleš Benda

09:05 – 09:50 Principles of fluorescence and point scanning microscopes

Radek Macháň

09:50 – 10:00 Introduction about PicoQuant

Frank Birke

10:00 – 10:35 Basic information about fluorescence and the PicoQuant Fluorescence spectrometer FluoTime 300

Frank Birke

### 10:35 – 10:50 Coffee break – Hands-on group assignment

10:50 – 11:30 Short presentations of participants – 3 minutes each

11:30 – 12:10 Introduction to FLIM instrumentation

Peter Kapusta

### 12:10 – 13:00 Lunch

13:00 – 14:00 Hands-on – 4 stations - Instrument introduction - First round

14:00 – 15:00 Hands-on – 4 stations - Instrument introduction - Second round

### 15:00 – 15:15 Coffee break

15:15 – 16:15 Hands-on – 4 stations - Instrument introduction - Third round

16:15 – 17:15 Hands-on – 4 stations - Instrument introduction - Fourth round

## Tuesday, Nov 27.

09:00 – 10:00 Principles of lipid membrane micro-environment sensing

Piotr Jurkiewicz

10:00 – 10:45 Principles of FLIM-FRET

Marie Olšínová

### 10:45 – 11:00 Coffee break

11:00 – 11:20 Background for NAD(P)H imaging

Aleš Benda

11:20 – 12:00 Different ways of FLIM data analysis

Radek Macháň

### 12:00 – 13:00 Lunch

13:00 – 15:00 Hands-on – 4 stations – NAD(P)H imaging, FLIM-FRET, Laurdan Imaging, Data Analysis including Pattern Matching - First round

### 15:00 – 15:15 Coffee break

15:15 – 17:15 Hands-on – 4 stations – NAD(P)H imaging, FLIM-FRET, Laurdan Imaging, Data Analysis including Pattern Matching - Second round

## Wednesday, Nov 28.

09:00 – 10:00 How to avoid FLIM acquisition artefacts

Peter Kapusta

10:00 – 10:45 Protein-protein interactions visualized by FLIM-FRET: considerations needed for reliable experiments.

Jana Humpolíčková

10:45 – 11:00 Coffee break

11:00 – 11:30 Laurdan imaging uncovered

Piotr Jurkiewicz

11:30 – 12:00 Examples of applications of FLIM-FRET sensors

Aleš Benda

12:00 – 13:00 Lunch

13:00 – 15:00 Hands-on – 4 stations – NAD(P)H imaging, FLIM-FRET, Laurdan Imaging, Data Analysis including Pattern Matching - Third round

15:00 – 15:15 Coffee break

15:15 – 17:15 Hands-on – 4 stations – NAD(P)H imaging, FLIM-FRET, Laurdan Imaging, Data Analysis including Pattern Matching - Fourth round

The course is supported by the National Infrastructure for Biological and Medical Imaging (Czech-BioImaging, Ministry of Education, Youth and Sports – Large Research Infrastructure, LM2015062).



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