WORKSHOP

"Exploring the molecular world by advanced fluorescence microscopy approaches"

September 12–15, 2017, "Jožef Stefan" Institute, Ljubljana, Slovenia

Program

SEMINAR AT NATIONAL INSTITUTE OF CHEMISTRY (Monday, 11 Sept, 4pm)

Lipid membrane bioactivity – new insights from advanced (super-resolution) optical microscopy (Christian Eggeling)

TUTORIALS (Tuesday, 12 Sept)

Overview of (super-resolution) fluorescence microscopy (Christian Eggeling)
Basics of optical microscopy, confocal and two-photon excitation (2PE) microscopy;
Super-resolution microscopy – localization & stimulated emission depletion (STED)

Micro-spectroscopies (Janez Štrancar)
Fluorescence microspectroscopy (FMS), fluorescence lifetime imaging (FLIM), fluorescence resonance energy transfer (FRET)

Approaches to study molecular dynamics (Erdinc Sezgin)
Fluorescence correlation and cross-correlation spectroscopies (FCS and FCCS), STED-FCS;
Image mean square displacement (iMSD), raster and spatio-temporal image CS (RICS and STICS)

Image analysis (Dominic Waithe)
Understanding basics (bit-depth, channels, look up tables, …);
Overview of software for manipulation, rendering, deconvolution

Design and synthesis of fluorescent probes (Janez Mravljak)
Excitation and emission properties, photo-stability, environment-sensitivity (spectrum, lifetime), targeting/partitioning, design and synthesis

Fluorescent labelling of biological samples (Dilip Shrestha/Katharina Reglinski)
Lipid analogues, plasmids, protein expression, antibodies, nanobodies, snap/halo tags;
Cell fixation

Optical trapping in microscopy applications (Iztok Urbančič)
Optical manipulation of biological samples by laser tweezers, cell mechanics

PRACTICAL SESSIONS (**Thursday & Friday, 14–15 Sept**)

OPT - Basic optics (Silvia Galiani/Iztok Urbančič)
Beam walk (mirrors, pinholes), expansion (lenses), fibre coupling

LABEL - Protein labelling (Katharina Reglinski/Dilip Shrestha)
Protein expression, labelling with antibodies and , snap/halo tags, cell fixation

MEM - Membrane samples (Erdinc Sezgin/Falk Schneider)
Supported lipid bilayers (SLB), giant unilamellar vesicles, giant plasma membrane vesicles (GPMV), cells

FMS/TW - Fluorescence microspectroscopy; Laser tweezers (Iztok Urbančič/Rok Podlipec)
Phase-separated GPMVs (polarity sensitive dyes); bead/cell manipulation

IMG - Laser scanning confocal imaging (Silvia Galiani/Iztok Urbančič)
1/2-colour confocal, 2D/3D imaging; beads/fixed cells

STED - STED imaging (Silvia Galiani)
2D/3D-STED imaging; fixed/live cells

FCS - STED-FCS (Falk Schneider/Erdinc Sezgin)
FCS, scanning FCS, STED-FCS, co-diffusion

2PE - Two-photon microscopy (Rok Podlipec)
Label-free autofluorescence imaging of cells

DATA AND IMAGE ANALYSIS (**Thursday & Friday, 14–15 Sept**)

IMGa - Image analysis (Dominic Waithe)
ImageJ: image basics; segmentation (thresholding), co-localization …

FMSa - FMS image analysis (Iztok Urbančič)
Shifts of polarity-sensitive probes, generalized polarization (GP), bleaching correction, linear unmixing

FCSa - FCS analysis (Falk Schneider/Erdinc Sezgin)
Fitting (transit times, concentrations), multiple components, co-diffusion, diffusion laws from STED-FCS

CASE STUDIES

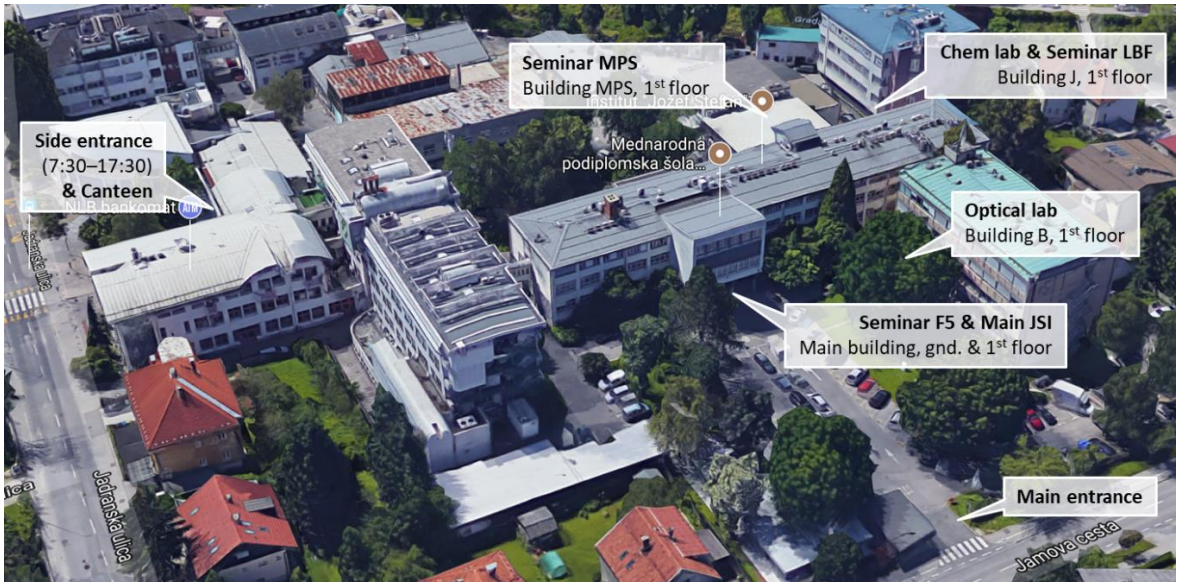
DISCUSS - Individual discussions of participants with demonstrators
Participants can discuss with demonstrators potential applications of the presented techniques to their own research problems

CASE - Interaction of cells with nanoparticles (Janez Štrancar)
Demonstration of how the presented experimental approaches can be applied to study biologically relevant mechanisms at molecular level

Timeline & locations (see schedule on the next page):

Tutorials: freely accessible, Practical sessions: for selected participants divided into groups A, B, C

Main JSI	– the main JSI lecture hall
Optical Lab	– Optical lab LBF/F5
Chem Lab	– Chemical lab LBF
Seminar LBF	– Seminar room LBF
Seminar MPS	– Seminar room of JSI International Postgraduate School
Seminar F5	– Seminar room F5



	Tuesday, 12 Sept	Thursday, 14 Sept					Friday, 15 Sept					
Act.	**Tutorials**	**Practical sessions**					**Practical sessions**					
Loc.	*Main JSI*	*Opt Lab*	*Chem Lab*	*Sem LBF*	*Sem F5*	*Sem MPS*	*Opt Lab*	*Chem Lab*	*Sem LBF*	*Sem F5*	*Sem MPS*	*Main JSI*
09:00	Reception	TW	MEM			OPT						CASE
		FMS										(PRESS)
09:30	Welcome											
	Overview -	IMG	LABEL			(discuss)						
10:00	microscopies						(PRESS					
							PHOTO)					
10:30		Coffee Break					Coffee Break					
		(*gallery below Main JSI*)					(*gallery below Main JSI*)					
11:00		TW	MEM			OPT	STED	LABEL	FCSa			
	Coffee break	FMS										
11:30	Micro-											
	spectroscopies	IMG	LABEL			(discuss)	FCS		DISCUSS			
12:00												
12:30				Lunch time					Lunch time			
13:00	Lunch time											
13:30		STED	MEM			IMGa	STED		FCSa		IMGa	
14:00	Dynamics											
		FCS	LABEL			FMSa	FCS		DISCUSS		FMSa	
14:30												
	Image analysis											
15:00		Coffee Break										
		(*gallery below Main JSI*)										
15:30	Coffee break	STED			IMGa	OPT						
16:00	Probes											
		FCS				(discuss)						
16:30												
	Labelling				FCSa							
17:00		2PE				DISCUSS						
17:30	Tweezers	2PE				DISCUSS						
18:00	Intro to practicals											