Lecture Program - per 30th May 2006 (subject to change)

4th European Short Course on Principles & Applications of Time-Resolved Fluorescence Spectroscopy, Berlin, October 30-November 3, 2006

Monday

Joseph R. Lakowicz: „Basic definitions and principles of fluorescence (1 and 2)“
(2 h 45 min, 9:30-10:45, and 11:15-12:45)

- Jablonski diagram and stokes shift
- Basic spectral properties
- Excitation and emission spectra
- Fluorescence anisotropy
- Fluorescence lifetime
- Energy transfer

Rainer Erdmann: „Instrumentation (1)“ (1 h 30 min, 13:45–15:15)

- Overview of steady state fluorometer design
- Light sources: lamps, LEDs, lasers
- Wavelength selection: monochromators, filters
- Detectors: PMTs, MCP-PMT, SPAD, CCD
- Electronics
- Analog and photon counting
- Design rules
- Sources of error in fluorescence
- Introduction to time domain measurement
- Introduction to frequency domain measurement
- Special considerations for NIR applications

Matthias Patting : „Introduction to data analysis“ (30 min, 15:45-16:15)

- Typical approaches in Time-Correlated Single Photon Counting (TCSPC) data analysis
- Common artefacts and how to handle them
- Spoiled data and how to avoid them
- Choosing appropriate models
- Step by step example

Zygmunt (Karol) Gryczynski / Andreas Bülter: „Introduction to Hands-on experiments“
(30 min, 16:15-16:45)

- Physics behind the experiments

Companies: „Introduction to Hands-on experiments“ (15 min per company, 16:45-18:15)

- Instrumental aspects of the experiments

Tuesday

Joseph R. Lakowicz: „Time-resolved fluorescence“ (1 h 45 min, 8:30-10:15)

- Resolution of complex decays
- Multi-exponential anisotropy decays
- Transient effects in quenching
- Time-Resolved Emission Spectra (TRES)

Michael Wahl: „Instrumentation (2) for time-correlated photon counting and fluorescence lifetime imaging“ (1 h 30 min, 10:45-12:15)

- Advantages and difficulties of the TCSPC method
- Modern excitation sources
- Specifics of sample compartments and detection optics
- Detectors for TCSPC
- Compact photon counting electronics incl. multi-photon counting
- Electronics for multidimensional TCSP C (including routers)
- Electronics for Time-Tagged Time Resolved (T³R) data acquisition
- TCSPC instrumentation for Fluorescence Lifetime Imaging (FLIM)

Zygmunt (Karol) Gryczynski: „Analytical applications of fluorescence“ (1 h 45 min, 13:30-15:15)

- Analytical determinations by fluorescence
- Ratiometric determination based sensing
- Anisotropy-based sensing
- Fluorescence lifetime-based sensing
- Modulation based sensing
- Energy transfer-based lifetime sensing of metal ions
- Visual polarization sensing
- Error sources in fluorescence assays

Wednesday

Joseph R. Lakowicz: „Time dependent phenomena“ (1 h 30 min, 8:30-10:00)

- Multi-exponential decays
- Time domain lifetime measurements
- Frequency domain lifetime measurements
- Quenching: static, dynamic, transients
- Anisotropy decays
- Energy transfer – distance distribution
- Time-dependent spectral relaxation
- Excited state reactions

Johan Hofkens: „Modern fluorescence microscopy“ (1 h 30 min, 10:30-12:00)

1. Hardware aspects:
   - Introduction to microscopy
   - Basics of (fluorescence)-microscopy (lightpath inside a microscope, l ight collection (lenses, objectives, aberration), resolution, focus properties, working distances, transmission / epi-illumination, excitation sources / possible detectors / general setup)
   - Widefield microscopy
   - Total Internal Reflection Fluorescence (TIRF)
   - Confocal and Multi-photon microscopy
   - High resolution microscopy (Stimulated Emission Depletion Spectroscopy (STED), 4-PI)
   - Spinning disk microscopy
   - Laser Scanning microscopy
2. Applications:
   - General imaging
   - Spectral mixing / unmixing
   - FLIM (phase, TCSPC, gating, ...)
   - Förster Resonance Energy Transfer (FRET) / Imaging FRET
   - Fluorescence Correlation Spectroscopy (FCS)
   - Deconvolution microscopy

Thursday

Axel Dürkop: „Fluorescent markers, probes and labels“ (1 h 30 min. 8:30-10:00)

1. Intrinsic fluorophores
   - Aromatic amino acids (Tyr, Trp, Phe)
   - Enzyme co-factors (NADH, FAD,...)
   - Tissues
2. Extrinsic fluorophores
   - Protein probes (Fluorescein, Rhodamines, Dansyl, BodiPy,...)
   - Membrane probes (DPH, TMA-DPH, Parinaric acid, Fatty acids,...)
   - DNA probes (Ethidium bromide, DAPI, Acridine Orange, Adenine, Guanine,..)
3. Chemical sensing probes
   - Ion indicators (MQAE, FURA, Calcium Green,...)
   - Fluorogenics probes (Fluorescamine, NBD-Cl,...)
4. Fluorescent Proteins
   - Phycobiliproteins
   - GFP, CFP, YFP,...
5. Lanthanides
6. Metal-Ligand complexes
7. Protein sensors
8. Luminescenct nanoparticles aka Quantum Dots

Jörg Enderlein: „Fluorescence fluctuation and single molecule spectroscopy”
(1 h 30 min, 10:30-12:00)

1. Physical principles of single molecule fluorescence spectroscopy
   - General properties of molecular light absorption and emission
   - Fluorescence lifetime and polarization
   - Single-pair Förster Resonance Energy Transfer (spFRET)
2. Fluorescence fluctuation spectroscopy
   - Confocal epi-fluorescence microscopy
   - Time-Tagged Time-Resolved photon counting
   - Fluorescence Correlation Spectroscopy (FCS)
   - Fluorescence Intensity Distribution Analysis (FIDA)
   - Single molecule burst analysis
3. Single Molecule Imaging
   - Wide-field fluorescence imaging microscopy
   - Single molecule tracking
   - Imaging single molecule orientations
   - Monitoring the interaction between individual molecules
   - Stoichiometry of molecular complexes

	Martin Hof: ”Solvent relaxation techniques: Application in studies of biomolecules“ (1 h 30 min, 13:00-14:30)
	■ Solvent relaxation (SR) and steady state spectra ■ Time-resolved emission spectra ■ SR in biomembrane research ■ Protein-Membrane interactions studied by SR ■ Lipid systems for drug delivery protocols studied by SR ■ SR in protein and DNA research
Friday	**Matthias Patting: „Advanced data analysis“** (1 h 30 min, 8:30-10:00)
	■ Fundamentals of TCSPC fitting ■ Decay models ■ Advanced error analysis ■ Fluorescence Lifetime Imaging (FLIM) analysis ■ Förster Resonance Energy Transfer (FRET) analysis
	Manfred Auer: „High throughput screening“ (2 h 15 min, 10:00-10:45 and 11:15-12:45)
	■ The drug discovery process ■ General aspects of high throughput screening ■ Ensemble averaging fluorescence technologies in high throughput screening ■ Single molecule spectroscopy technologies in high throughput screening ■ Affinity selection, chemical genomics, chemical genetics in drug discovery