Program (per 29.9.2004)
2nd European Short Course on Principles & Applications of Time-Resolved Fluorescence Spectroscopy, Berlin, November 1-5, 2004

Part I

Monday

Joseph R. Lakowicz: „Basic definitions and principles of fluorescence“ (2h 45 min)

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

Richard Thompson: „Instrumentation (1)“ (1 h 30 min)

- Overview of steady state fluorometer construction
- Light sources: lamps, lasers, LEDs
- Wavelength selection: monochromators, filters
- Detectors: PMTs, PD/APD, CCD, MCP-PMT
- Design features
- Sources of error in fluorescence
- Introduction to lifetime measurement
- Introduction to time domain measurement
- Introduction to frequency domain measurement

Rainer Erdmann: „Introduction to data analysis“ (30 min)

- Background and philosophy of data analysis
- Why do we need data correction?
- Nonlinear problems and data fitting
- Simple exponential fitting routines

Zygmunt Gryczynski: „Introduction to Hands-on experiments“ (30 min)

- Physics behind the experiments

Jobin Yvon / Olympus / PicoQuant / PTI / Varian: „Introduction to Hands-on experiments“
(15 min per company)

- Instrumental aspects of the experiments

Tuesday

Joseph R. Lakowicz: „Time resolved fluorescence“ (1 h 45 min)

- Resolution of complex decays
- Multi-exponential anisotropy decays
- Transient effects in quenching
- Time resolved emission spectra (TRES)

Joseph R. Lakowicz: „Time dependent phenomena“ (1 h 30 min)

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

Richard Thompson: „Analytical applications of fluorescence“ (2 h)

- 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

Part II

Wednesday

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

- 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 TCPC (including routers)
- Electronics for Time Tagged Time Resolved (T^3R) data acquisition
- TCSPC instrumentation for Fluorescence Lifetime Imaging (FLIM)

Rainer Erdmann: „Time resolved near-infrared spectroscopy“ (45 min)

- Principles and advantages of NIR spectroscopy
- Samples and probes
- Special instrumentation
- Typical applications of NIRS

Stefan Hell: „Modern nonlinear fluorescence microscopy “ (1 h 45 min)

1. Prinicples of confocal microscopy
   - Advantages of confocal microscopy
   - 2-Photon excitation
   - 3-Photon excitation
   - Pulsed excitation
2. Resolution improvement
   - 4Pi confocal microscopy
   - Stimulated Emission Depletion Spectroscopy (STED)

Joeseph R. Lakowicz / Zygmunt Gryczynski: “1. Radiative Decay Engineering: The Use of Metallic Nano-Structures to Control Emission Properties of Fluorophores. 2. Surface Plasmon-Coupled Emission: Ultra Sensitive Fluorescence Detection Technology.” (1 h 30 min)

- Abstracts are available at www.picoquant.com/_trfcourse.htm

Thursday

Otto S. Wolfbeis: „Fluorescent markers, probes and labels“ (1 h 45 min)

1. Fluorescent labels
   - Intrinsic fluorescence
   - Labels: wavelength and decay time considerations
   - Labeling biomolecules
   - Purification and characterization of conjugates
   - Specific features of protein labeling
   - Specific features of DNA labeling
   - Representative examples of labeling via reactive groups
   - Quantum dots, GFP / RFP
2. Fluorescent probes
   - Definitions
   - Probes for pH, pO_2, reactive oxygen species, Ca^{2+}, Cl', etc.
   - Features of metal ligand probes
   - Probes for sensing purposes
3. Applications of fluorescent probes and labeled species
   - in microscopy and imaging
   - in arrays and High Throughput Screening (HTS)
   - in cellular biophysics
   - in FRET studies
   - in optical fiber sensors
   - in immunoassay and hybridization assay

Jörg Enderlein: „Fluorescence fluctuation and single molecule spectroscopy” (2 h)

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

Thursday	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
	Sabato D'Auria: „Structure, stability, conformational dynamics and biotechnological applications of thermophilic enzymes” (Approx. 1 h 15 min)
	■ Effects of temperature, detergents, organic solvents on the enzyme activity / structural properties of a beta-glycosidase as monitored by circular dichroism and steady-state and time-resoved fluorescence spectroscopy ■ Use of thermophilic proteins as probes for the development of fluorescence biosensors for analytes
Friday	**Matthias Patting: „Advanced data analysis“** (1 h 15 min)
	■ Fundamentals of TCSPC fitting ■ Decay models ■ Advanced error analysis ■ Fluorescence Lifetime Imaging (FLIM) analysis ■ Fluorescence resonance energy transfer (FRET) analysis
	Dr. Auer: „High throughput screening“ (2 h 30 min)
	■ 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