

**Program (per 18.10.2005)**  
**3<sup>rd</sup> European Short Course on Principles & Applications of**  
**Time-Resolved Fluorescence Spectroscopy,**  
**Berlin, October 31-November 4, 2005**

<b>Monday</b>	<p><b>Joseph R. Lakowicz: „Basic definitions and principles of fluorescence“</b>          (2 h 45 min, 9:30-10:45, 11:15-12:45)</p> <ul style="list-style-type: none"> <li>■ Jablonski diagram and stokes shift</li> <li>■ Basic spectral properties</li> <li>■ Excitation and emission spectra</li> <li>■ Fluorescence anisotropy</li> <li>■ Fluorescence lifetime</li> <li>■ Energy transfer</li> </ul>
	<p><b>Rainer Erdmann: „Instrumentation (1)“</b> (1 h 45 min, 13:45–15:15)</p> <ul style="list-style-type: none"> <li>■ Overview of steady state fluorometer design</li> <li>■ Light sources: lamps, LEDs, lasers</li> <li>■ Wavelength selection: monochromators, filters</li> <li>■ Detectors: PMTs, MCP-PMT, SPAD, CCD</li> <li>■ Electronics</li> <li>■ Analog and photon counting</li> <li>■ Design rules</li> <li>■ Sources of error in fluorescence</li> <li>■ Introduction to time domain measurement</li> <li>■ Introduction to frequency domain measurement</li> <li>■ Special considerations for NIR applications</li> </ul>
	<p><b>Matthias Patting : „Introduction to data analysis“</b> (30 min, 15:45-16:15)</p> <ul style="list-style-type: none"> <li>■ Typical approaches in TCSPC data analysis</li> <li>■ Common artefacts and how to handle them</li> <li>■ Spoiled data and how to avoid them</li> <li>■ Choosing appropriate models</li> <li>■ Step by step example</li> </ul>
	<p><b>Zygmunt Gryczynski: „Introduction to Hands-on experiments“</b> (30 min, 16:15-16:45)</p> <ul style="list-style-type: none"> <li>■ Physics behind the experiments</li> </ul>
	<p><b>Companies: „Introduction to Hands-on experiments“</b> (15 min per company, 16:45-18:15)</p> <ul style="list-style-type: none"> <li>■ Instrumental aspects of the experiments</li> </ul>
	<p><b>Joseph R. Lakowicz: „Time-resolved fluorescence“</b> (1 h 45 min, 8:15-10:00)</p> <ul style="list-style-type: none"> <li>■ Resolution of complex decays</li> <li>■ Multi-exponential anisotropy decays</li> <li>■ Transient effects in quenching</li> <li>■ Time-Resolved Emission Spectra (TRES)</li> </ul>
<b>Tuesday</b>	<p><b>Joseph R. Lakowicz: „Time dependent phenomena“</b> (1 h 30 min, 10:30-12:00)</p> <ul style="list-style-type: none"> <li>■ Multi-exponential decays</li> <li>■ Time domain lifetime measurements</li> <li>■ Frequency domain lifetime measurements</li> <li>■ Quenching: static, dynamic, transients</li> <li>■ Anisotropy decays</li> <li>■ Energy transfer – distance distribution</li> <li>■ Time-dependent spectral relaxation</li> <li>■ Excited state reactions</li> </ul>
	<p><b>Zygmunt Gryczynski: „Analytical applications of fluorescence“</b> (1 h 45 min, 13:15-15:00)</p> <ul style="list-style-type: none"> <li>■ Analytical determinations by fluorescence</li> <li>■ Ratiometric determination based sensing</li> <li>■ Anisotropy-based sensing</li> <li>■ Fluorescence lifetime-based sensing</li> <li>■ Modulation based sensing</li> <li>■ Energy transfer-based lifetime sensing of metal ions</li> <li>■ Visual polarization sensing</li> <li>■ Error sources in fluorescence assays</li> </ul>

**Michael Wahl: „Instrumentation (2) for time-correlated photon counting and fluorescence lifetime imaging“** (2 h 00 min, 8:15-10:15)

- 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 TCSPC (including routers)
- Electronics for Time-Tagged Time Resolved (T<sup>3</sup>R) data acquisition
- TCSPC instrumentation for Fluorescence Lifetime Imaging (FLIM)

**Stefan Hell / Lars Kastrop: „Modern nonlinear fluorescence microscopy“** (1 h 45 min, 10:45-12:30)

1. Principles 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)

**Peter Czerney: „Fluorescent markers, probes and labels“** (1 h 30 min. 13:30-15:00)

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<sub>2</sub>, reactive oxygen species, Ca<sup>2+</sup>, Cl<sup>-</sup>, 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 00 min, 8:15-10:15)

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

Wednesday

Thursday

<b>Thursday</b>	<b>Martin Hof: "Solvent relaxation techniques: Application in studies of biomolecules"</b> (1 h 30 min, 10:45-12:15)
	<ul style="list-style-type: none"> <li>■ Solvent relaxation (SR) and steady state spectra</li> <li>■ Time-resolved emission spectra</li> <li>■ SR in biomembrane research</li> <li>■ Protein-Membrane interactions studied by SR</li> <li>■ Lipid systems for drug delivery protocols studied by SR</li> <li>■ SR in protein and DNA research</li> </ul>
<b>Friday</b>	<b>Matthias Patting: „Advanced data analysis“</b> (1 h 30 min, 8:15-9:45)
	<ul style="list-style-type: none"> <li>■ Fundamentals of TCSPC fitting</li> <li>■ Decay models</li> <li>■ Advanced error analysis</li> <li>■ Fluorescence Lifetime Imaging (FLIM) analysis</li> <li>■ Förster Resonance Energy Transfer (FRET) analysis</li> </ul>
	<b>Manfred Auer: „High throughput screening“</b> (2 h 15 min, 9:45-10:30 and 11:00-12:30)
	<ul style="list-style-type: none"> <li>■ The drug discovery process</li> <li>■ General aspects of high throughput screening</li> <li>■ Ensemble averaging fluorescence technologies in high throughput screening</li> <li>■ Single molecule spectroscopy technologies in high throughput screening</li> <li>■ Affinity selection, chemical genomics, chemical genetics in drug discovery</li> </ul>