Program – TRF Course

Part I (Monday & Tuesday)

Dr. Lakowicz: „Basic definitions and principles of fluorescence“ (3 hours)
- Jablonski diagram and stokes shift
- Basic spectral properties
- Excitation and emission spectral
- Fluorescence anisotropy
- Fluorescence lifetime
- Energy transfer

Dr. 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

Mr. Erdmann: „Introduction into data analysis“ (1 hour)
- Background and philosophy of data analysis
- Why do we need data correction?
- Nonlinear problems and data fitting
- Simple exponential fitting routines

Dr. 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)

Dr. Lakowicz: „Time dependent phenomena“ (1 h 15 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

Dr. Thompson: „Analytical applications of fluorescence“ (approx. 2 h 15 min)
- 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 - Friday noon)

Dr. 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)

Mr. Erdmann: „Time resolved near-infrared spectroscopy“ (45 min)
- Principles and advantages of NIR spectroscopy
- Samples and probes
- Special instrumentation
- Typical applications of NIRS

Dr. Wolfbeis: „Fluorescent markers, probes and labels“ (approx. 3 h 15 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
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

Dr. Hell: „Modern nonlinear fluorescence microscopy “ (2 hours)
- Confocal microscopy
- Multiphoton excitation microscopy: foundations and applications
- Resolution improvement (4Pi and stimulated emission)

Dr. Enderlein: „Fluorescence fluctuation and single molecule spectroscopy” (approx. 3 hours)

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

Mr. 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“ (1 h 45 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