This document describes the course that can be given at your site.
It also applies in large part to the public versions of the course.

Fluorescence in Drug Discovery:
Principles and Applications

John C. Owicki, Ph.D.

- Fluorescence is one of the premier technologies for drug discovery.
- Now you can bring a professional-development short course on this topic to your site, taught by an authority in the field.
- The course reviews the principles of fluorescence and fluorescence labeling, then surveys the wide variety of standard and recently developed fluorometric assays in drug discovery. *The content and length of the course can be adjusted to meet your needs*.

Why Hold the Course at Your Site?

- It will improve your ability to choose and use appropriate technologies for your analytical tasks.
- It is convenient. You provide only a room and a projector (and reproduction of course notes for courses presented outside North America). There are no travel hassles. There is a single pre-negotiated all-inclusive payment.
- It is economical compared with the travel of a group of people to off-site training.
- It can be customized to meet your needs.
- Other professionals rate the course very useful (references available).
- Continuing education for professional development is good business.

Who Should Attend?

- Scientists
- Technicians
- Engineers
- Technical managers
- Some familiarity with fluorescence is helpful but is not essential with a sound technical background.
- Special arrangements can be made for people with less technical experience, e.g., many sales representatives

Length of the Course

- The course is normally given as a one-, one-and-a-half-, or two-day presentation.
- More specialized half-day presentations can be arranged within the San Francisco Bay area.

Learning Objectives

- Master the basic concepts of fluorescence at the level required to understand fluorometric assays and instrumentation.
- Become familiar with the application of fluorometric assays to the major classes of pharmacological targets.
- Understand the basis and applications of advanced methods such as fluorescence imaging, fluorescence polarization, time-resolved energy transfer, and fluorescence-fluctuation spectroscopy.
- Learn the important types of interferences in fluorometric assays.
- Discover where to look for more advanced information, using the extensive list of references provided.

Topics and Course Customization

The topics listed below take about three days to present, so a two-day course involves some selection from among the topics and a one-day course permits presentation of about a third of the topics. Jack Owicki works with clients to customize the content to the client's needs while retaining a coherent presentation.

A list of standard topics follows on the next page. It is sometimes possible to include topics that are not on the list. If you require presentation of such material, contact Jack Owicki to discuss feasibility.

- **Review of fluorescence fundamentals**
  - Basic components of a fluorometer
  - Excitation and emission spectra
  - Lifetimes
  - Quantum yields
  - The Jablonski diagram
  - Phosphorescence
  - Quenching
- **Fluorescent labels and labeling chemistries**
  - Standard labels, such as fluorescein, coumarins, and Cy dyes
  - More exotic labels, such as lanthanides, green fluorescent proteins, phycobiliproteins, and quantum dots
  - Chemistries for labeling amino and sulfhydryl groups
  - Quenching due to multiple labeling of macromolecules
  - Biotin-avidin linkage
  - Protein-protein conjugation
  - Non-covalent labeling for detection of proteins and nucleic acids
  - Optimization of labeling
- **Instrumentation for fluorometric assays**
  - Special considerations for fluorometry in microplates
  - Homogeneous vs. heterogeneous assays
  - Characteristics of major light sources and detectors
  - Point-reading vs. imaging systems
  - Scanning vs. CCD-based imaging systems
  - Flow cytometry
  - Fluorescence in femtoliter volumes via confocal microscopy and multi-photon fluorescence
  - New alternatives to microplates, such as microfluidic chips and gel-permeation assays
- **Interferences in and limitations on fluorometric assay**s
  - Background fluorescence
  - Photon shot noise (fluctuations in photon counting)
  - Color quenching
  - Chemical quenching: dynamic and static
  - Light scattering
  - Photobleaching
  - Role of ratiometric measurements
- **Applications based simply on conventional measurements of fluorophore concentration**
  - Fluorogenic substrates to assay enzymes such as proteases and cytochrome P-450s
  - Fluorometric plate-binding assays
- **Applications based imaging the distributions of fluorophores**
  - Imaging cellular translocation of labeled proteins ("High Content Screening")
  - DNA labeling for sequencing, gel electrophoresis, FISH, and chromosome painting
  - Imaging ligand binding to cells or beads
- **Applications based on flow cytometry**
  - Alternatives to plate-binding assays, including ELISAs
  - SNP detection
- **Applications based on changes in fluorescence due to binding of fluorophores or dynamic quenching**
  - Noncovalent DNA dyes
  - Noncovalent protein dyes
  - Detection of DNA and proteins in electrophoretic gels and blots
  - Ion sensors, particularly real-time functional assays for cytoplasmic Ca^{++}
  - Membrane-potential measurements with bis-oxonol and related dyes
  - Oxygen concentrations in cell culture for metabolic and viability measurements
- **Applications based on time-resolved fluorescence (TRF)**
  - Principles of TRF
  - DELFIA
- **Applications based on fluorescence resonance energy transfer (FRET)**
  - Principles of FRET
  - FRET between identical labels (photon migration)
  - Fluorogenic substrates using quenchers
  - Molecular beacons for detecting nucleic-acid hybridization
  - The Taqman™ technology for quantitative PCR
  - Tandem dyes and application to DNA sequencing
  - Recruitment of cytosolic proteins to membranes
  - Reporter-gene assay based on beta lactamase
  - Membrane-potential sensing
  - Intracellular Ca^{++} measurements using fusion proteins of green fluorescent proteins and calmodulin
- **Applications based on time-resolved FRET (TRET)**
  - Principles of TRET (also called HTRF™ and Lance™)
  - Applications of TRET, including transcription-factor dimerization and helicase activity
  - A tour-de-force application of TRET to structure-function relationships in an ion channel, an application in basic research
- **Beyond lanthanide TRF: Applications based on measuring fluorescence lifetime**
  - Time-domain and frequency-domain methods for measuring lifetime
  - Applications based on detecting changes in fluorescence lifetime

- **Applications based on fluorescence polarization (FP)**
  - Principles of FP
  - Practical equivalence of FP to fluorescence anisotropy
  - Molecular-weight considerations in constructing FP assays
  - Applications of FP to protein kinases, proteases, and receptor binding
  - Optimization of FP assays (uses simulation program)
  - Flagging interferences in FP assays
- **Applications based on confocal fluorescence fluctuation spectroscopy**
  - Principles of fluorescence fluctuation
  - Analysis of time correlation of fluctuations (fluorescence correlation spectroscopy)
  - Analysis of intensity histograms (fluorescence intensity distribution analysis, or photon counting histogram)
  - Applications to binding assays
- **Optimizing fluorometric assays**
  - Optimizing the Z factor rather than signal or noise separately
  - Optimum substrate concentration and percent conversion in enzymatic assays
  - Optimum tracer and protein concentrations in binding assays
  - How rules of thumb for assay development with other technologies may not apply to fluorometric assays
- **Summary of other optical assays in drug discovery (alternatives to fluorescence)**
  - Absorbance
  - Scintillation proximity
  - Chemiluminescence assays for reporter genes and enzyme labels
  - Calcium assays based on aequorin-catalyzed chemiluminescence
  - AlphaScreen™ luminescent oxygen-channeling assay
  - BRET™ bioluminescence resonance-energy transfer assay
  - Surface plasmon resonance
  - Electrochemiluminescence
- **Optimizing assays based on equilibrium binding**
  - Review of equilibrium-binding equations
  - Depletion of free label, in contrast to radioligand binding
  - Consequences of not being able to equate total and free concentrations
  - Effects of label depletion on controls for nonspecific binding

About the Instructor

Jack Owicki is an independent consultant with extensive experience in bioanalytical methods for drug discovery. As Vice President for Research at LJL BioSystems and Associate Technical Director at Molecular Devices, he was involved in the research and development of successful commercial fluorometric systems for high-throughput screening.

Prior to his industrial work, he was Assoc. Prof. of Biophysics at the Univ. of California, Berkeley, where he investigated intermolecular interactions in membranes, also teaching graduate and undergraduate biophysics courses. He was a post-doc in Biophysical Chemistry at Stanford Univ., holds a Ph.D. in Biophysical Chemistry from Cornell Univ., and M.S. and B.S. degrees in Biochemistry from Michigan State University.

The author of over 60 technical articles and patents, he was on the Program Committee for the 2001 Annual Meeting of the Society for Biomolecular Screening (SBS), chairing the session on Advances in Detection Technologies. He coordinated the short-course program at the Annual SBS Meeting in 2003 and again has this role in 2004.

He has been on the editorial boards of the Journal of Biomolecular Screening, the Journal of Fluorescence, the Biophysical Journal, the Annual Review of Biophysics and Biomolecular Structure, and the Journal of Biomedical Optics.

In addition to his regular consulting over the past nine years, he has successfully presented courses on methods in drug discovery at many conferences, under the auspices of the Society for Biomolecular Sciences, Select Biosciences, and IBC. He has also given the course at many industrial sites, including pharmaceutical companies, biotech companies, and companies that vend instruments and reagents for drug discovery.

Cost

The cost depends on the length of the course, the number of attendees, and travel considerations. There is an all-inclusive fee that generally ranges from $5,000 to $15,000, higher outside North America. Contact Jack Owicki to discuss your needs and obtain a quote.

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