Latin American Posgraduate Program in Biophysics (POSLATAM)

IX POSLATAM Course

Quantitative imaging in Biophysics

San Miguel de Tucumán, November 21 to 25, 2016

Description: This course will introduce key concepts in fluorescence microscopy. The course's goal is to provide a solid background in both the theoretical concepts and practical skills in quantitative fluorescence microscopy with applications in biological research. Topics will be introduced in lectures, and computer labs will give the student hands-on practice on different image analysis strategies.

COURSE CONTENTS

PART 1. FUNDAMENTALS

Photochemistry and fluorescence

Electronic states and transitions. Molecular orbitals. Electron spin. Transition probabilities: Beer law; Einstein coefficients; Selection rules; Frank-Condon principle. Kinetics of photochemical reactions: production and deactivation of excited states. Jablonski diagrams. Lifetimes and quantum yields. Fluorescence excitation and emission spectra. Stokes shift. Fluorescence quenching. Dipole-dipole interaction and Förster Resonance Energy Transfer (FRET). Emission anisotropy. Molecular structure and fluorescence: Common fluorophores and fluorescent probes (dyes, quantum dots, visible fluorescent proteins. Strategies for labeling proteins with fluorescent probes: intrinsic and extrinsic probes; Bioconjugation techniques; Immunostaining.

Introduction to optical microscopy

Brief description of optical-based microscopes: Ray Tracing, Imaging formation. Interference and diffraction. The Snell laws and total reflection. Interference and diffraction. Magnification and resolution. Working distance.

Basic components of optical microscopes: Sources of coherent and incoherent illumination. The microscope objective and other lens; Eyepiece, Lens tube, Condenser, etc. Detectors: photomultiplier tubes, avalanche photodiodes, micro-channel plates and Charge-coupled devices (CCD).

Types of microscopes: Fluorescence, Dark Field , Phase Contrast, Differential Interference Contrast (DIC).

Scanning Confocal Microscopy. Pixel definition. Axial resolution, lateral resolution.

PART 2 – APPLICATIONS

Förster Resonance Energy Transfer (FRET) microscopy

Theoretical principles and practical considerations for resonance energy transfer microscopy. Donnors and aceptors. Methodos for measuring FRET efficiencies. FRET microscopy. Light sources. Wavelength selectors. FRET and ratio imaging. Colocalization vs interaction. Data processing. Biophysical applications.

Fluorescence Recovery after Photobleaching (FRAP)
Basic principles of fluorophore-photobleaching. Photobleaching in fluorescence Imaging. Acquisition photobleaching. Recovery after photobleaching. Data processing. Biophysical applications.

Fluorescence Correlation Spectroscopy (FCS)
Fluctuations in fluorescence signals. The autocorrelation function. The effects of particle concentration and particle size on the autocorrelation curve. Two channel detection: Cross-correlation. Experimental concerns. Biophysical applications.

PRACTICAL ACTIVITIES

Digital image analysis. Post-processing, quantification and presentation of images and numerical data.

Suggested Textbooks

1. J. R. Lakowicz. Principles of fluorescence spectroscopy. Springer; 2nd ed. (June 30, 1999)
2. B. Valeur. Molecular fluorescence: Principles and applications. Wiley-VCH; 1st ed. (October 11, 2001)
3. Digital microscopy. Volume 72, Second Edition: A second edition of "Video Microscopy" (Methods in Cell Biology). Academic Press; 2nd ed. (December 19, 2003)
4. Hecht E. Optics. Addison Wesley; 4th ed. (August 2, 2001)
5. Fluorescence Correlation Spectroscopy. R. Rigler (Editor), E.S. Elson (Editor). Springer; 1st ed. (March 15, 2001)

Course coordinator

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Instructors

Hernan Grecco, Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

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Invited Lectures .

Enrico Gratton, Laboratory for Fluorescence Dynamics, University of California at Irvine, Irvine, USA.

Dave Jameson, University of Hawaii- John A. Burns School of Medicine- Hawaii, USA

Carlos Bustamante, University of California at Berkeley, USA

Thomas Jovin, Max Plank Institute for Biophysical Chemistry, Alemania

Fernando Stefani, Centro de Investigaciones en Bionanociencias, CONICET, Facultad de Ciencias Exactas y Naturales, Universiad de Buenos Aires, Argentina

Jerson Silva, Laboratório de Termodinâmica de Proteínas e Estruturas Virais Gregorio Weber Universidade Federal do Rio de Janeiro, Brasil

Target audience

The course is designed to be taken by both life sciences and physical sciences Ph.D. students. It is specifically designed to accommodate the different academic backgrounds of the students enrolled in the different programs. The course is also appropriate for advanced undergraduate students who have sufficient laboratory training. Undergraduates should (and Ph.D. students may) consult the instructors to check that they have adequate preparation for the course.

Grading

There will be one exam (take home, but worked on independently) at the end of the course.