



**UNIVERSIDAD DE BUENOS AIRES**  
**FACULTAD DE CIENCIAS EXACTAS Y NATURALES**  
Departamento de Física  
Laboratorio de Electrónica Cuántica

1

**1) Denominación del Curso:**

Advanced Fluorescence Microscopy Techniques: Dynamics through the microscope:  
Fluorescence correlation spectroscopy

**1.a) Carácter del curso:** Doctorado y Postgrado

**2) Fecha de iniciación:** 3 de septiembre de 2007 **Fecha de finalización:** 7 de septiembre de 2007

**3) A dictarse en:** Departamento de Física

**4) Docentes:**

**4a) Responsable:**

Dr. Oscar Martínez, Departamento de Física, FCEyN. Profesor Titular plenario.

**4b) Auxiliares:**

Dra. Valeria Levi, investigadora adjunta de CONICET, departamento de Física, FCEyN.

Lic. Laura Estrada, ayudante de primera, Departamento de Física, FCEyN

**4c) Invitados:**

**Enrico Gratton, Professor**  
University of California, Irvine  
Department of Biomedical Engineering  
Irvine, CA, USA

Enrico Gratton was born in Merate (Como) Italy in 1946. He received his doctorate degree in physics from the University of Rome in 1969. From 1969 to 1971 he was a post-doctoral fellow at the Istituto Superiore di Sanità in Italy. He went to the University of Illinois at Urbana-Champaign (UIUC) in 1976 and began his work as a research associate in the Department of Biochemistry. In 1978, he was appointed assistant professor in the Department of Physics of the University of Illinois at Urbana-Champaign (UIUC). In 1989, he was promoted to professor. Dr. Gratton's laboratory has reached international recognition for the development of instrumentation for time-resolved fluorescence spectroscopy using frequency domain methods.

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2



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In 1986, Dr. Gratton was awarded a grant from the National Institutes of Health, National Center for Research Resources, to establish the first national facility dedicated to fluorescence spectroscopy: the Laboratory for Fluorescence Dynamics (LFD). The LFD, housed in Loomis Laboratory of Physics at UIUC, is a state-of-the-art fluorescence laboratory for use by local, national, and international scientists. It has a dual and equal commitment to research and development of fluorescence instrumentation and theory and to service in a user-oriented facility. Dr. Gratton's research interests are varied and many; they include design of new fluorescence instruments, protein dynamics, hydration of proteins, and I.R. spectroscopy of biological substances. Dr. Gratton has authored or co-authored over 400 publications in refereed scientific journals.

**Andre M.O. Gomes, Professor**  
Laboratório de Termodinâmica de Proteínas e Estruturas Virais Gregório Weber  
Instituto de Biologia Médica  
Universidade Federal do Rio de Janeiro  
Brasil

Andre M.O. Gomes was born in Rio de Janeiro, Brazil in 1975. He received his doctorate degree in Biological Chemistry from the Federal University of Rio de Janeiro (UFRJ) in 2002. He works at the Gregório Weber Laboratory of Thermodynamics of Proteins and Viruses at the UFRJ. In 2002, he got a position as Associate Professor at the Medical Biochemistry Institute at the same University and from 2004 to 2006 he joined the Laboratory for Fluorescence Dynamics (LFD) at the University of Illinois at Urbana-Champaign as a postdoctoral fellow.

Dr. Gomes' research interests are in the area of the structural biology of viruses, virus-cell interaction and protein-protein, protein-lipid and protein-nucleic acid interactions. His current projects involve the use of fluorescence spectroscopy, hydrostatic pressure and two-photon microscopy, including single particle tracking and fluorescence correlation spectroscopy (FCS), for the study of viral particles inactivation, assembly and infection of cells.

**Luis Felipe Aguilar Cavallo, Professor**  
Departamento de Biotecnología  
Escuela de Ciencias Agropecuarias  
Universidad de Viña del Mar  
Chile

Luis Aguilar was born in Chile and received his PhD in Chemistry from the Catholic University of Valparaiso. In 1999, he was awarded a postdoctoral grant from CONICYT (The National Committee of Sciences and Technology), Chile. In 2000 he was a post-doctoral fellow at the Crocker Nuclear Laboratory, University of California, Davis in USA. Since 2001 he has been the Director and principal investigator of the Laser Laboratory at the Catholic University of Valparaiso. In 2003, he was appointed full professor in the School of Agriculture Sciences at the University of Viña del Mar, Chile, where he is the Director of the Biotecnology program.

Dr. Aguilar's research interests are protein-lipid interactions and enzymatic kinetics. His current projects involve the use of fluorescence spectroscopy for the study of influence of the lipids domains in the membrane proteins conformation and function.

**Susana Sanchez, PhD**  
University of California, Irvine  
Laboratory for Fluorescence Dynamics  
Irvine, CA, USA

Susana Sanchez was born in Chile and received her doctorate degree in Chemistry from the Catholic University of Valparaiso. In 1997, she joined the Laboratory for Fluorescence Dynamics (LFD) at the

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3

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University of Illinois at Urbana-Champaign as a post doctoral fellow. Since 2001 she has been the User Coordinator of the LFD.  
Dr. Sanchez research interests are in the area of protein-protein and protein-lipid interactions. Her current projects involve the use of two-photon microscopy, including FCS and Landau GP imaging, for the study of cholesterol removal by HDL particles from artificial systems such as Giant Unilamellar Vesicles (GUVs) and cells.

**Andrea C. Oliveira, Professor**  
Laboratorio de Termodinámica de Proteínas e Estructuras Virais Gregorio Weber  
Instituto de Bioquímica Médica  
Universidade Federal do Rio de Janeiro  
Brasil

Andrea C. Oliveira was born in Rio de Janeiro, Brazil in 1972. She works at the Gregorio Weber Laboratory of Thermodynamics of Proteins and Viruses at the UFPRJ. In 2001, she got a position as Associate Professor at the Medical Biochemistry Institute at the same university, and from 2004 to 2006 she joined the Laboratory for Fluorescence Dynamics (LFD) at the University of Illinois at Urbana-Champaign as a postdoctoral fellow.

Dr. Oliveira's research interests are in the area of the structural biology of viruses, virus-cell interaction and protein-protein, protein-lipid and protein-nucleic acid interactions. Her current projects involve the use of fluorescence spectroscopy, hydrostatic pressure and two-photon microscopy, including single particle tracking and fluorescence correlation spectroscopy (FCS), for the study of viral particle inactivation, assembly and infection of cells.

**5) Cantidad de horas totales de duración del curso:**

50 horas (Se incluye las horas de teóricos, laboratorios, discusión general y las horas requeridas para el examen que se tomará posteriormente al curso)

Horas semanales teóricas y problemáticas: 27 hs

Horas semanales de clases de trabajos prácticos: 18 hs

**6) Condiciones de ingreso:**

El curso está dirigido a estudiantes de posgrado en las áreas de Biología, Química, Física, Medicina, Farmacia, Bioquímica y Biotecnología.

**7) Número de alumnos: 40.**

**8) Modalidad del dictado del curso. Forma de evaluación**

La evaluación se realizará con un examen escrito final que se tomará en la semana siguiente al dictado del curso.



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El curso será teórico-práctico y contará con sesiones de discusión general. Además, se realizará una sesión de posters para que los alumnos expongan sus proyectos de investigación. Buscamos que en esta sesión los alumnos puedan discutir con los docentes, aplicaciones de las técnicas aprendidas en el curso a sus temas específicos de investigación.  
El idioma será inglés.

**9) Puntaje solicitado para las Carreras de Doctorado de esta Facultad**

Se solicita para las Carreras de Doctorado de esta Facultad un puntaje de tres puntos.

**10) Arancel propuesto**

No se propone arancel para el curso.

**11) Honorarios:**

No se solicitan honorarios para los docentes.

**12) Programa del curso**

**Programa teórico resumido**

**1. Principles of Fluorescence**

- \* Basic Spectral Properties
- \* Jablonski Diagram and Stokes' shift
- \* Excitation and Emission Spectra
- \* Polarization/Anisotropy
- \* Fluorescence Lifetime
- \* Fluorescence Quenching and Resonance Energy Transfer (FRET)

**2. Introduction to Microscopy**

- \* Overview of Optical Microscopy
- \* Instrumentation
- \* Fluorescence Microscopy
- \* Confocal and Multiphoton Microscopy



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### 3. Fluorescence Correlation Spectroscopy (FCS)

- \* Theoretical Aspects of FCS
- \* Photon Counting Histogram
- \* Point and Scanning FCS
- \* Image Correlation Spectroscopy

### 4. Introduction to Fluorescence Lifetime Imaging Microscopy (FLIM)

#### Laboratorio

- \* Fluorescence Microscopy: the basics of widefield and confocal microscopies.
- \* Point FCS: determination of diffusion coefficients, microviscosity and association of proteins.
- \* Scanning FCS
- \* Raster Image Correlation Spectroscopy
- \* Simulations of FCS and RICS data and analysis of experimental data.

### 13) Bibliografía

#### Artículos en revistas y libros

1. Elson EL. Quick tour of fluorescence correlation spectroscopy from its inception. *J Biomed Opt.* 2004 Sep-Oct;9(5):857-64.
2. Berland KM. Fluorescence correlation spectroscopy: a new tool for quantification of molecular interactions. *Methods Mol Biol.* 2004;261:383-98.
3. Bulsico DA, Wolf DE. Fluorescence correlation spectroscopy: molecular complexing in solution and in living cells. *Methods Cell Biol.* 2003;72:465-98.
4. Thompson NL, Lieto AM, Allen NW. Recent advances in fluorescence correlation spectroscopy. *Curr Opin Struct Biol.* 2002 Oct;12(5):634-41.
5. Medina MA, Schwille P. Fluorescence correlation spectroscopy for the detection and study of single molecules in biology. *Bioessays.* 2002 Aug;24(8):758-64.
6. Enrico Gratton, Sophia Y Breusegem, Nicholas P Barry, Qiaoqiao Ruan, and John S Eild. Fluctuation correlation spectroscopy in cells: Determination of molecular aggregation. In *Biophotonics: Optical Science and Engineering for the 21st Century* (Vol. XVIII). By X Shen and R van Wijk (Editors). Springer Verlag. pp. 1-14, 2006. ISBN 0387249958.

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6



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7. Michelle A Digan, Claire M Brown, Parijat Sengupta, Paul W Wiseman, Alan R Horwitz, and Enrico Gratton. Measuring fast dynamics in solutions and cells with a laser scanning microscope. *Biophys J* 89(2):1317-27, 2005.
8. Susana A Sanchez and Enrico Gratton. Lipid-protein interactions revealed by two-photon microscopy and fluorescence correlation spectroscopy. *Acc Chem Res* 38(6):469-77, 2005.
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10. Qiaoqiao Ruan, Melanie A Cheng, Moshe Levi, Enrico Gratton, and William W Mantulin. Spatial-temporal studies of membrane dynamics: scanning fluorescence correlation spectroscopy (SFCS). *Biophys J* 87(2):1260-7, 2004.
11. Susana A Sanchez, Juan E Brunet, David M Jameson, Rosalba Lagos, and Octavio Monasterio. Tubulin equilibrium unfolding followed by time-resolved fluorescence and fluorescence correlation spectroscopy. *Protein Sci* 13(1):81-8, 2004.
12. Joachim D Müller and Enrico Gratton. High-pressure fluorescence correlation spectroscopy. *Biophys J* 85(4):2711-9, 2003.
13. Joachim D Müller, Yan Chen, and Enrico Gratton. Fluorescence correlation spectroscopy in Biophotonics, Part B (Methods in Enzymology, Vol. 361). By G Marriott and I Parker (Editors). Academic Press. pp. 69-92, 2003. ISBN 0121822648.
14. Yan Chen, Joachim D Müller, Qiaoqiao Ruan, and Enrico Gratton. Molecular brightness characterization of EGFP in vivo by fluorescence fluctuation spectroscopy. *Biophys J* 82(1):133-44, 2002.
15. Ettore Bismuto, Enrico Gratton, and Don C Lamb. Dynamics of ANS binding to tuna apomyoglobin measured with fluorescence correlation spectroscopy. *Biophys J* 81(6):3510-21, 2001.
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17. John S Eild, Joachim D Müller, and Enrico Gratton. Data acquisition card for fluctuation correlation spectroscopy allowing full access to the detected photon sequence. *Rev Sci Instrum* 71(2):361-368, 2000.
18. Yan Chen, Joachim D Müller, Peter T So, and Enrico Gratton. The photon counting histogram in fluorescence fluctuation spectroscopy. *Biophys J* 77(1):553-67, 1999.
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20. Claus Czeslik, R Jansen, M Baltauf, A Wittenmann, Catherine A Royer, Enrico Gratton, and Theodore L Hazlett. Mechanism of protein binding to spherical polyelectrolyte brushes

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7



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studied in situ using two-photon excitation fluorescence fluctuation spectroscopy. *Phys Rev E* 69(2):021401, 2004.

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Universidad de Buenos Aires  
Facultad de Ciencias Exactas y Naturales

Referencia Expte. N° 490.227/2007

Buenos Aires, 30 JUL 2007

**VISTO:**

la nota presentada por el Dr Oscar Martínez y Dra. Valeria Levi del Departamento de Física, mediante las cuales elevan, la Información y el Programa del Curso de Posgrado **ADVANCED FLUORESCENCE MICROSCOPY TECHNIQUES. DYNAMIS THROUG THE MICROSCOPE: FLUORESCENCE CORRELATION SPECTROSCOPY**, por el Dr Oscar Martínez como docente responsable, Dra. Valeria Levi y Lic. Laura Estrada como docentes auxiliares y como docentes Invitados: Enrico Gratton (University of California, Irvine), Andre M. O. Gomes (Universidade Federal do Rio de Janeiro), Luis Felipe Aguilar Caballo (Universidad de Viña del Mar), Susana Sánchez (University of California, Irvine), Andrea C. Oliveira.

**CONSIDERANDO:**

lo actuado por la Comisión de Doctorado,  
lo actuado por la Comisión de Enseñanza, Programas, Planes de Estudio y Posgrado.  
lo actuado por este cuerpo en Sesión Ordinaria realizada en el día de la fecha,  
en uso de las atribuciones que le confiere el Artículo N° 113° del Estatuto Universitario,

EL CONSEJO DIRECTIVO DE LA FACULTAD DE CIENCIAS EXACTAS Y NATURALES  
**RESUELVE:**

**Artículo 1°:** Autorizar el dictado del curso de Posgrado **ADVANCED FLUORESCENCE MICROSCOPY TECHNIQUES. DYNAMIS THROUG THE MICROSCOPE: FLUORESCENCE CORRELATION SPECTROSCOPY** de 50 hs. de duración.

**Artículo 2°:** Aprobar el Programa del Curso de Posgrado: **ADVANCED FLUORESCENCE MICROSCOPY TECHNIQUES. DYNAMIS THROUG THE MICROSCOPE: FLUORESCENCE CORRELATION SPECTROSCOPY**

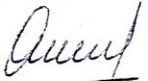
**Artículo 3°:** Aprobar un Puntaje de dos (2) puntos para la Carrera del Doctorado.


**Artículo 4°:** Aprobar un Arancel de 20 Módulos.

**Artículo 5°:** Comuníquese a la Dirección del Departamento de Física, a la Biblioteca de la FCEyN y a la Subsecretaría de Postgrado (con fotocopia del programa incluida).

**Artículo 6°:** Comuníquese a la Dirección de Alumnos (sin fotocopia del programa analítico).

Resolución CD N° 1562

  
Dra. NORA CEBALLOS  
SECRETARIA ACADÉMICA

  
Dr. JÓRGE ALIAGA  
DECANO