(Preliminary agenda)

5thAnnual Workshop in Advanced Microscopy and Biophotonics

November 20th-24th of 2023

Institut Pasteur de Montevideo



Organizer: **Dr. Leonel Malacrida and MSc. Marcela Diaz** Speakers:

Dr. Enrico Gratton (UCI-USA)

Dr. David Jameson (U. Hawaii-USA)

Dr. Rafael Piestun (U. Colorado-USA)

Dr. Jay Knutson (NHLBI-NIH-USA)

Dr. Michelle Digman (UCI-USA)

Dr. Alberto Diaspro (IIT-Italy)

Dr. Fernando Stefani (CBION-Argentina)

Dr. Luca Lanzanó (U. Catania-Italy)

Dr. Hernán Grecco (UBA-Argentina)

Dr. Giuseppe Vicidomini (IIT-Italy)

Dr. Valeria Levi (UBA-Argentina)

Dr. Per Niklas Hedde (UCI-USA)

Dr. Xiaoyu Shi (UCI-USA)

Dr. Suman Ranjit (Georgetown U-USA)

Dr. Chiara Stringari (Ecole Polytechnique-France)

Dr. Belén Torrado (UCI-USA)

Dr. Lorenzo Scipioni (UCI-USA)

Dr. Melissa Birol (Max Delbruck Center-Germany)

Dr. Rupsa Datta (U. Wisconsin-USA)

Dr. Diego Presman (UBA-Argentina)

Dr. Giulia Tedeschi (UCI-USA)

Dr. Alexander Vallmitjana (UCI-USA)

Dr. Francesco Palomba (UCI-USA)

Dr. Agustin Mangiarotti (Max Planck Inst. of Colloids & Interfaces-Germany)

Bach. Bruno Schuty (UBA/IPMon-Uruguay)

Sponsors:

Chan Zuckerberg Initiative 🏵

Supported by:







UNIVERSIDAD DE LA REPÚBLICA URUGUAY

Course target and goals:

The workshop in Advanced Microscopy and Biophotonics is dedicated to disseminate cutting-edge technology and advanced methods in fluorescence microscopy and biophotonics. The target audience includes postgraduate students, postdocs, and academics from all Americas with a background in the fundamentals of fluorescence microscopy, seeking to learn about recent advances in the combination of spectroscopy with fluorescence microscopy. In the course, we will cover the fundamentals of advanced instrumentation and methods, such as FCS, FLIM, Hyperspectral imaging, Super-resolution, Non-linear microscopy, Deep tissue imaging, light-sheet microscopy, among others. Since 2020 the workshop become hybrid, with 20 in-person participants and 20-30 participants by zoom. The course is organized with lectures of fundamentals (40+10 min), application talks (20+10 min), and an optional advanced section for a virtual practical section (two section 2hs/each) and discussions. This last section is restricted to 20 students and will be given on the last day. Regional participants will be able to apply for a limited number of fellowships (5). The lectures will be given in English.

Workshop agenda:

<u>Monday November 20st</u>

08:45-09:00 - Opening and course introduction. Leonel Malacrida

09:00-10:00 – Lecture 1 (50+10). **David Jameson.** Introduction to fluorescence fundamentals and methods.

10:00-10:50 – Lecture 2 (40+10). Leonel Malacrida: Advanced fluorescence instrumentation and microscopy.

Virtual coffee break and meet with the instructors (20 min)

11:10-12:00 – Lecture 3 (40+10). **Enrico Gratton:** Introduction to fluorescence correlation spectroscopy (FCS) and the photon counting histogram (PCH).

Virtual lunch break and meet with the instructors

13:30-14:20 – Lecture 4 (40+10). **Michelle Digman:** Raster image correlation spectroscopy (RICS) and in vivo applications.

14:20 – 15:10 - Lecture 5 (40+10). **Michelle Digman:** Number & Brightness (N&B) and its applications for protein oligomerization studies in vivo.

Virtual coffee break and meet with the instructors (30 min)

15:40-16:30 – Lecture 6 (40+10). Francesco Palomba: The pair-correlation function and its applications for deciphering the occurrence of barriers and obstacles inside the cell.

16:30 – 17:00–Application talk #1 (20+10). Giulia Tedeschi: Fluorescence Fluctuation Spectroscopy enables quantification of potassium channel subunit dynamics and stoichiometry.

17:00 – 17:30–Application talk #2 (20+10). Diego Presman: Glucocorticoid receptors oligomeric state at the cell nucleus using N&B.

<u>Tuesday November 21st</u>

09:00-10:00 - Lecture 7 (50+10). **David Jameson:** Introduction to lifetime measurements and the phasor plots.

10:00-10:50 – Lecture 8 (40+10). Enrico Gratton: The phasor plots for Fluorescence lifetime Imaging Microscopy (FLIM).

Virtual coffee break and meet with the instructors (20 min)

11:10-12:00 – Lecture 9 (40+10). Leonel Malacrida: The spectral phasor plot and its applications for hyperspectral imaging.

Virtual lunch break and meet with the instructors

13:30-14:20– Lecture 10 (40+10). Luca Lanzanó: Fundamentals of Super-resolution microscopy and its combination with spectroscopy.

14:20-15:10 – Lecture 11 (40+10). **Michelle Digman:** Multi-component analysis on FLIM data with the phasor approach.

Virtual coffee break and meet with the instructors (30 min)

15:40-16:30– Lecture 12 (40+10). Suman Ranjit: FLIM-Phasor for cell metabolism phenotyping.

16:30–17:00– Application talk #3 (20+10). Michelle Digman: The Phasor FLIM analysis reveals metabolic modulation and cell migration through adhesion contractility.

17:00 – 17:30– Application talk #4 (20+10). Jay Knutson: FLIM and new oxygen sensor for in vivo imaging.

Wednesday November 22nd

Wednesday November 23rd

09:00-09:50 – Lecture 13 (40+10). Fernando Stefani. Far-field fluorescence nanoscopy with sub-10 nm resolution.

09:50-10:40 – Lecture 14 (40+10). Giuseppe Vicidomini: Multi-Parameter Live-Cell Observation of Biomolecular Processes with Single-Photon Detector Array.

Virtual coffee break and meet with the instructors (20 min)

11:00-11:50 – Lecture 15 (40+10). Enrico Gratton: 3D-Single particle tracking and its applications.

11:50-12:40 – Lecture 16 (40+10). Xiaoyu Shi: Expansion microscopy fundamentals and its applications.

Virtual lunch break and meet with the instructors

14:00-14:50 – Lecture 17 (40+10). Alexander Dvornikov: The DIVER microscope for deep tissue imaging.

14:50-15:40 – Lecture 18 (40+10). Rafael Piestun: Ultra-thin microendoscopes.

Virtual coffee break and meet with the instructors

16:10-17:00– Lecture 19 (40+10). Leonel Malacrida: The single plane illumination microscopy and its applications to fluorescence spectroscopy.

17:00 – 17:30 – Application talk #5 (20+10). Leonel Malacrida: Spectral phasor analysis of ACDAN fluorescence on in vivo zebrafish lens.

<u>Thursday November 23th</u>

9:00 – 9:30– Application talk #6 (20+10). Alexander Dvornikov: FLIM-Phasor for metabolic imaging.

09:30 – 10:00– Application talk #7 (20+10). Alberto Diaspro: Muller matrix and phasor analysis.

10:00 – 10:30 -Application talk #8 (20+10). Hernan Grecco: CASPAM: A Triple-Modality Biosensor for Multiplexed Imaging of Caspase Network Activity.

Virtual coffee break and meet with the instructors (20 min)

10:50 – 11:20– Application talk #9 (20+10). Bruno Schuty: Spectral phasor of skin autofluorescence.

11:20-11:50 – Application talk #10 (20+10). Melissa Birol: TBC.

Virtual lunch break and meet with the instructors

13:30 – 14:00– Application talk #11 (20+10). Rupsa Datta: Autofluorescence lifetime imaging of cell division

14:00 – 14:30 - Application talk #12 (20+10). Valeria Levi: FCS applications for protein dynamics within the nucleus.

14:30-15:00 – Application talk#13. Agustin Mangiarotti: Wetting by biomolecular condensates increases membrane lipid packing and dehydration.

Virtual coffee break and meet with the instructors (30 min)

15:30-16:00 – Application talk#14 (20+10). Leonel Malacrida: Connectivity maps with cells: the 2D-pair correlation function.

16:00 – 17:00 - Lecture 20: Workshop close and special lecture. **David Jameson:** A nano-history of fluorescence.

17:00 – 17:30 – Workshop closing, group picture and virtual coffee break with instructors

Virtual coffee break and meet with the instructors (30 min)

15:30-16:00 – **Application talk#14 (20+10). Leonel Malacrida:** Connectivity maps with cells: the 2D-pair correlation function.

16:00 – 17:00 - Lecture 20: Workshop close and special lecture. David Jameson: A nano-history of fluorescence.

<u>17:00 – 17:30 – Workshop closing, group picture and virtual coffee break with instructors</u>

Friday November 24th

09:00 -12:00 Two topics (total 10 students by subgroup)

Spectral phasor for hyperspectral imaging (Leonel Malacrida and Marcela Diaz)

- Imaging of multicomponents on fixed samples.
- Imaging of LAURDAN on in vivo cells.

Virtual lunch break and meet with the instructors

- 13:30 15:30 Computer room work and presentation preparation
- 15:30 16:30 Practical presentation and discussions
- 16:30-17:00 Workshop closing and group picture.