Training on Advanced optical microscopy methods in Biomedicine

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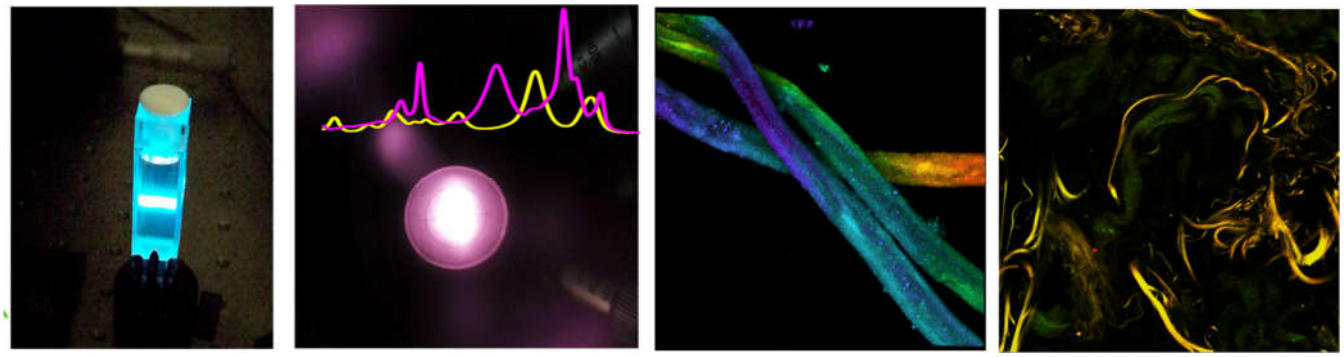

Fluorescence microscopy methods are among the most widely used in life science and medical research. This short course will provide the participants with an overview of fundamental concepts of fluorescence, working principles of confocal and multiphoton imaging, with special emphasis possibilities and limitations of these methods. Furthermore, key note lectures on advanced methods will be given by outstanding invited speakers which will discuss the latest development of fluorescence imaging.

Comitato Scientifico

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Program:

27-06-2018: *Advanced fluorescence imaging techniques: keynote lectures.*

Aula Seminari – Viale delle Scienze ed. 18

9.30:10.30 **Prof. Alberto Diaspro** *Department of Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy , Department of Physics, University of Genoa, Genoa, Italy*

"Towards a new paradigm in Microscopy: a liquid tunable microscope."

11.00-11.40 **Dr. Luca Lanzanò** *Department of Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy":*

"Nanoscale imaging of the spatial organization of transcription and replication sites in single cells"

11.40-12.20 **Dr. Martino Calamai** *European Laboratory for Non-Linear Spectroscopy (LENS), Florence, Italy.*

"Alzheimer's disease from a single molecule/single cell perspective"

Abstracts:

Towards a new paradigm in Microscopy: a liquid tunable microscope.

Taking inspiration from the philosophical and sociological speculation by Zygmunt Bauman (Liquid Modernity, Polity Press, Cambridge, 2000) a new paradigm for optical microscopy is proposed regarding its design and implementation. Advances in optical microscopy at the nanoscale in living systems make of super-resolved microscopy, label-free approaches, time and space encoding and decoding strategies, single molecule imaging and tracking approaches a powerful melting pot of different mechanisms for image formation. We have a continuous growth of variations on the theme towards image formation (Diaspro A. and van Zandvoort M.A.M.J. (eds) 2016. Superresolution Imaging in Biomedicine. CRC Press) within the scenario of unlimited spatial resolution (Diaspro A. 2014. Il Nuovo Saggiatore). Moreover, label-free methods (Bianchini and Diaspro, J.Biophotonics, 2008). (Mazumder et al. 2017 J.Optics) (Diaspro et al. 1990 IEEE Trans.Biomed. Eng.) will be outlined as integrated mechanisms of contrast in the liquid tunable microscope. Now, a "conventional" microscope architecture follows a well-established series of rules when interacting with a known or (better) unknown sample and related open questions. The microscope of the future, combining different converging technologies pixel by pixel, is a liquid tunable microscope. It is liquid because it overlaps in an optimized way different mechanisms of contrast and it is tunable because it offers a real time tunability regarding spatial and temporal resolution. It will adapt its "image formation process" to the specific light-matter interaction output. No limits to variations of the theme. We like to call this "liquitopy".

Short Biography of Prof. Alberto Diaspro:

Alberto Diaspro is Director of the Department of Nanophysics at the Istituto Italiano di Tecnologia (IIT), Deputy Director of IIT, Chair of the Nikon Imaging Center at IIT, Professor of Applied Physics at the Department of Physics of University of Genoa. He was President of OWLS (Optics with Life Sciences), EBSA (European Biophysical Societies Association) and Appointed Vice President of ICO (Interational Commission of Optics). AD founded the Nanoscale Biophysics Subgroup of the Biophysical Society and LAMBS (Laboratory for Advanced Microscopy, Bioimaging and Spectroscopy) - www.lambs.it.
Today, AD coordinates the IIT Nanobiophotonics research program and the Nanoscopy research line. He published more than 300 papers, 9500 citations, H=46 (source Google Scholar). He is Editor in Chief of the Wiley international journal Microscopy Research and Technique, NYAS (New York Academy of Sciences) member, IEEE senior member, OSA senior member and SPIE fellow. He received the Emily M.Gray Award of the Biophysical Society in 2014. AD is President of the Scientific Council of “Festival of Science” (www.festivalscienza.it).

Nanoscale imaging of the spatial organization of transcription and replication sites in single cells

Genome-scale mapping methods represent a powerful approach for the analysis of DNA replication and transcription but, unfortunately, they don't provide the underlying spatio-temporal organization in the cell nucleus. On the other hand, recent developments of super-resolution fluorescence microscopy techniques combine high specificity, sensitivity and less-invasive sample preparation procedures with the sub-diffraction spatial resolution required to image chromatin at the nanoscale.
Here I will present the application of optical super-resolution imaging to the investigation of the spatial organization of transcription and replication sites at the single cell level. I will present a general method to enhance the spatial resolution of a stimulated-emission depletion (STED) microscope based only on the modulation of the STED intensity. This modulation induces variations of the fluorescence emission that can be analyzed in the phasor plot in order to improve and quantify the effective spatial resolution of the STED image. I will apply this method to the direct visualization of transcription and replication foci within intact nuclei of eukaryotic cells. I will also show an analysis of the spatial distribution of the foci aimed at quantifying the proximity between replication and transcription foci. Finally, I will present preliminary results on the nanoscale alterations observed in an in vitro model of oncogene activation.

Short Biography of Dr. Luca Lanzanò:

Luca Lanzanò studied physics in Catania, Italy, where he earned his PhD in Physics in 2008. He then joined the Laboratory for Fluorescence Dynamics, University of California, Irvine, as a Postdoc under the supervision of Enrico Gratton. During his postdoctoral training he developed and applied advanced fluorescence methods to the investigation of the molecular mechanisms of renal phosphate regulation in epithelial cells. He also worked on the molecular mechanisms of translocation of cargos through the nuclear pore complex. In 2013 he joined the Nanoscopy group directed by Alberto Diaspro at the Istituto Italiano di Tecnologia in Genoa, Italy, to merge his expertise on biomedical fluorescence spectroscopy with cutting-edge super-resolution microscopy. His research is now focused on the development of fluorescence microscopy methods and their application to the investigation of chromatin alterations in cancer.

Alzheimer's disease from a single molecule/single cell perspective

We are applying advanced fluorescence microscopy and single molecule tracking techniques to investigate the molecular basis of neurodegenerative diseases, in particular Alzheimer's disease. This kind of approach has allowed discovering new features normally not accessible with standard methods based on data averaging. By studying the dynamic behaviour of toxic Abeta oligomers on the plasma membrane of living cells, and their interaction with specific membrane components, a new potential mechanism of toxicity based on loss of function has been postulated. Results similar to those obtained in the case of Abeta oligomers were found for aggregates formed by other proteins or peptides, such as amylin (involved in the development of type II diabetes) and beta2-microglobulin (associated with a familial form of systemic amyloidosis), supporting the hypothesis that amyloid diseases share similar mechanisms of toxicity. Single molecule tracking has also been used to evaluate the impact of cholesterol on the mobility of the transmembrane proteins involved in the production of Abeta peptide. In parallel, we have developed a fluorescence based bioassay to directly measure the proteolytic process preceding the release of toxic Abeta in single living cells, and to evaluate the factors influencing it.

Short Biography of Dr. Martino Calamai

After earning his bachelor's degree in Biology from the University of Florence, Martino Calamai moved to the lab of Prof. Chris Dobson at the University of Cambridge for a doctorate in Chemistry funded through a Marie Curie Training Network, and then to the group of Prof. Antoine Triller at the Ecole Normale Supérieure of Paris for a 2-year post-doctorate in Neuroscience funded by FEBS. In 2009 he joined the Biophysics Group of Prof. Francesco Pavone at LENS with a Marie Curie IEF fellowship. After a brief period as head of the research unit within the Italian ministerial program FIRB-Futuro in Ricerca2010, in 2012 he obtained a position as a permanent researcher at the National Research Council (CNR), initially at the Neuroscience Institute and then at the National Institute of Optics.