UNIVERSITÀ DEGLI STUDI DI PALERMO

DIPARTIMENTO DI FISICA E CHIMICA
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DiFC

DiFC ON LINE SEMINAR

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Phasor S-FLIM: A nanocamera system for fast, spectrally resolved lifetime imaging in living cells

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Fluorescence microscopy is a powerful imaging technique yielding high contrast and high specificity to subcellular targets of interest by labelling them with fluorescent markers (fluorophores), the fluorescence signal of which is excited by a laser pulse. In a typical configuration, the signal is integrated to form the image, averaging important information about the distribution of wavelengths emitted by the fluorophore (emission spectrum) and the probability of emission (fluorescence lifetime). Access to this information can yield important insights about the characteristics of the nano-environment surrounding the fluorophores and allow for the simultaneous acquisition of multiple fluorophores at the same time.
We developed Phasor Spectral and Fluorescence Lifetime Imaging Microscopy (Phasor S-FLIM), a novel microscopy technique which combines electronic, computational and technological developments to obtain spectral and lifetime information simultaneously and process them in real time. Phasor S-FLIM uses a diffraction grating to chromatically separate the light, that is then collected by a detector array of 32 photomultipliers, each of which has been made capable of acquiring the lifetime information. Processing of the data relies on the phasor approach for a fast and precise quantification of the results. We demonstrate that our system is capable of achieving a comprehensive and photon efficient characterization of Forster Resonant Energy Transfer (FRET) measurement of biosensors, blind unmixing of multiple fluorophores and parallel, 4-colors lifetime imaging in living cells and tumor spheroids.

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