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LECTURES

Abstract no. LO1

LINKING THE PAST WITH THE FUTURE IN BIOLOGICAL PROCESSES: DISSECTING THE CELL CYCLE

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The cell can integrate internal and environmental signals into molecular responses that become the recognisable descriptors of a combination of pathways. Not surprisingly, the live eukaryotic cell is becoming an increasing point of focus for drug discovery efforts and for research applications using reporter probes to track dynamic changes in discrete pathways. Flow cytometry and time-resolved live cell imaging are powerful approaches for tracking the origins of specific phenotypic responses and the interrelationships within cell complex populations. The expanding availability of molecular and nanoscale reporters, compatible with live cells, has given new impetus to the application of these technologies. The challenges are to link molecular events with phenotypic responses at the single cell level and to overcome several bottlenecks including: the restricted throughput potential of image acquisition platforms, the compromise between the temporal resolution of events and data stack size, the efficient analysis of images for event recognition/quantification and the need for bioinformatics tools to mine multiparameter databases. This keynote presentation will address the reporter, data acquisition and mining tools which can be used to inform predictive models capable of producing in-silico cell response fingerprints for use in drug screening, experimental therapeutics and cancer biology hypothesis-testing. There will be a focus on applications of new eGFP stealth reporters and quantum dot technologies with specific reference to understanding the action of anticancer drugs on the cell cycle. The presentation will address aspects of these challenges and the prospects for predictive modeling of the cell cycle (Supported by RC UK, BBSRC, AICR and MRC).

Abstract no. LO2

BIOLOGICAL APPLICATIONS OF AN LCoS-BASED PROGRAMMABLE ARRAY MICROSCOPE (PAM)

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Applications and development of a new generation, commercial, programmable array optically sectioning fluorescence microscope (PAM) for rapid, light efficient 3D imaging of living and fixed specimens will be presented. The stand-alone module, including light source(s) and detector(s), features an innovative optical design and a ferroelectric liquid-crystal-on-silicon (LCoS) spatial light modulator (SLM) instead of the DMD used in the original PAM design[1]. The LCoS PAM (developed in collaboration with Cairn Research, Ltd.) can be attached to a port of a(ny) unmodified fluorescence microscope. The major advantages of the PAM are: (1) simple, inexpensive design with no moving parts; (2) increase in speed of optical sectioning due to an illumination duty cycle of each pixel of up to 50%; (3) optimal detection sensitivity, e.g. using electron multiplying CCD cameras; (4) continuously programmable, arbitrary, and adaptive optical sectioning modes between and/or within images using libraries of dot, line, or pseudo-random (Sylvester) sequence patterns; (5) maximally efficient and sensitive optical sectioning due to simultaneous detection and processing of both conjugate (in-focus) and non-conjugate (out-of-focus) light (a patented concept); (6) generation and detection of patterned polarized states; (7) minimal photobleaching; and (8) compatibility with multiphoton illumination, strategies for superresolution, and other emerging techniques.

The prototype system currently operated at the MPI incorporates a 6-position high-intensity LED illuminator, a 120 W metal halide lamp (X-Cite 120, EXFO), laser sources, an Andor iXon emCCD camera, and is mounted on an Olympus IX71 inverted microscope. Further enhancements recently include: (i) point- and line-wise spectral resolution and (ii) lifetime imaging (FLIM) using phase-modulation[2]. Using quantum dot coupled ligands we show real-time single molecule binding and subsequent trafficking of ligand-growth factor receptor complexes on live cells with greater speed and higher sensitivity in the PAM than conventional CLSM systems[3]. The combined use of a 405 nm laser and LED inputs allows for photoactivation and rapid kinetic analysis of cellular processes with the photoswitchable visible fluorescent protein, DRONPA. Other applications in signal transduction studies will be presented.

References

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