AuPS/ASB Meeting - Adelaide 2010

Symposium: Imaging and dynamic microscopy (imaging of biological and biophysical processes)

Monday 29th November 2010 - The Gallery - 14:30

Chair: Pierre Moens

Cadherin dynamics and the cytoskeleton

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Classical cadherin adhesion receptors are major determinants of tissue organization both in health and disease. They have long been thought to function in close cooperation with the actin cytoskeleton. Despite this, the molecular mechanisms responsible for cadherin-actin cooperation are poorly understood and lack, indeed, a clear over-arching conceptual framework. A major analytic challenge is to develop approaches to encompass both the dynamics of adhesion receptor organization and the intrinsic dynamics of actin polymer assembly/disassembly and organization. We are tackling this problem by using lentiviral shRNA systems to "replace" endogenous proteins with XFP-tagged transgenes; and then combining live cell imaging (including FRAP and photactivation) with mathematical modelling in order to quantitatively characterize cadherin and cytoskeletal dynamics. This multimodal approach is yielding a picture of functional cytoskeletal modules that cooperate, in a context-dependent fashion, to regulate the stability and turnover of cell-cell junctions.

Detecting stem cell differentiation using fluorescence lifetime microscopy (FLIM) by the phasor approach

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In fluorescence lifetime microscopy (FLIM) of live tissues a major issue is the assignment of auto-fluorescence to specific molecular components and their interactions within the physiological context. Analyzing the intensity decays with a multi-exponential fit is often not sufficient to properly describe this complexity. Here we use the phasor approach to FLIM to analyze complex decays in a live tissue. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of fluorophores and allows interpreting the FLIM images directly in molecular terms. The phasor signatures of different species have been used to separate many tissue components inside the testes of an Oct4-GFP transgenic mouse and to map the relative concentration of auto-fluorescence, GFP, collagen, retinol, retinoic acid, FAD and NADH. Furthermore the analysis of the fluorescence decay with higher harmonics of the phasor plot can separate different tissue components that have the same location in the phasor plot at one harmonic, but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique fit-free and straightforward method for interpreting complex decays in terms of molecular features the relative concentration of fluorophores. This method has the potential to become a non-invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues.

Light, x-rays or electrons for imaging malaria parasites?

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Imaging technologies have provided us with phenomenal insight into the micro- and nano-scopic domains and efforts to answer the major medical and biotechnology questions of the 21st century will be heavily reliant on the use of advanced imaging techniques. However there are limitations. Conventional light microscopy can be used with hydrated (in some cases, live) cells but has limited resolution, particularly for full-field imaging. Conventional electron microscopy offers very high resolution however the strong absorption of electrons by air and by the sample means that it can only be used with very thin, fixed, dehydrated samples. Imaging technologies that overcome some of the disadvantages of optical and electron microscopies are keenly sought.

We have used two “bridging” imaging modalities to explore sub-cellular topography. Three-dimensional structured illumination microscopy (3D-SIM) permits super-resolution fluorescence imaging of cells that are specifically labelled with fluorescent probes. Immunoelectron tomography offers high resolution imaging of individual ultrastructural features in a cellular context. Combined with serial sectioning and immunogold labeling it permits precise mapping of whole cell architecture.

The malaria parasite, *Plasmodium falciparum*, develops within human erythrocytes. As it grows the parasite establishes a membrane network outside its own limiting membrane in the cytoplasm of its host cell. These membrane structures play an important role in the trafficking of virulence proteins to the host cell surface, however their ultrastructure is only partly defined and there is on-going debate regarding their origin, organization and connectivity. Parasite endocytic processes are also poorly understood. The parasite consumes host haemoglobin in order to support its own growth. Small packets of haemoglobin are transferred from the host cell cytoplasm to a parasite digestive vacuole for haemoglobin digestion and heme detoxification however the precise mechanism for uptake is debated. Advanced imaging methods have provided novel insights into parasite cell architecture and functional cell biology.

Molecular transport in cells by the pair correlation fluctuation method

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Traditional fluctuation spectroscopy methods are employed to determine molecular diffusion in cells. While these methods provide information about a local dynamics in a specific point in the cell, they do not respond to the question of the path taken by a molecule to travel from one point to another. This kind of question is generally addressed by techniques such as single particle tracking. However, in single particle tracking the same molecule must be observed for an extended period of time and the molecule must be isolated from others. Also, many single particle trajectories must be recorded before we have sufficient statistics to delineate the path following by the particles. These conditions are difficult to achieve and the collection of many trajectories takes some time, especially if the volume to be interrogated is small. As a consequence, single particle tracking is used with large and bright particles. We developed a method in which we can follow relatively dim molecules in the presence of many other molecules, and statistically follow the flow of many molecules at a time. We have applied this method to the traffic of molecules inside the nucleus of cells and among the cytoplasm and the nucleus.

In vivo pair correlation analysis of enhanced green fluorescent protein (EGFP) intra nuclear diffusion

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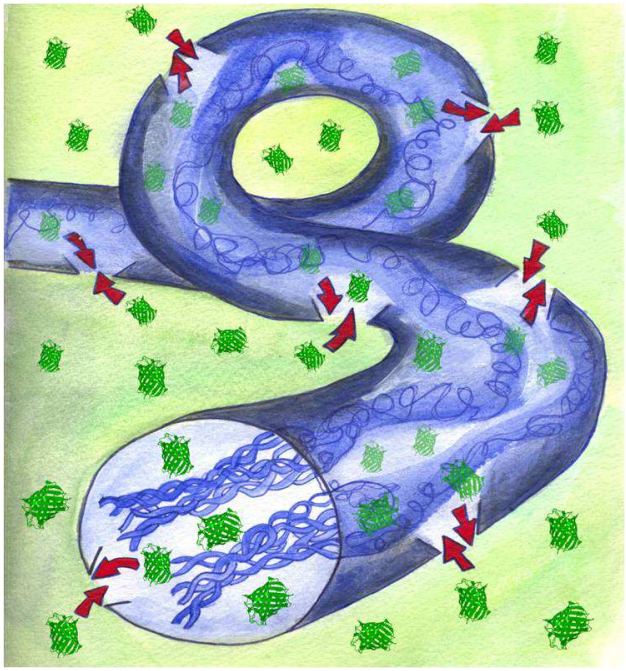
Intra nuclear diffusion is fundamental to enabling crucial cellular processes like gene transcription, DNA replication, DNA repair and epigenetic regulation to take place. The diffusion of molecules within the nucleus is obstructed by the steric constraints imposed by the nuclear environment. The extent to which nuclear architecture directs the diffusive route taken by these molecules is of significant interest. No methods proposed thus far have the capability to measure overall molecular flow in the nucleus of living cells. Here we apply the pair correlation function analysis (pCF) to measure molecular anisotropic diffusion in the interphase nucleus of live cells. In the pCF method we cross correlate fluctuations at several distances and locations within the nucleus, enabling us to define migration paths and barriers to diffusion. We use monomeric EGFP as a prototypical inert molecule and measure its flow in and between the different nuclear environments, marked by Hoechst 33342 as a reference of DNA density.



As schematically shown in the figure above our results suggest that there are two disconnect molecular flows throughout the nucleus, associated with high and low DNA density regions. We observe that the different density regions of DNA form a networked channel that allow EGFP to diffuse freely throughout, however with restricted ability to traverse the channel barriers. Upon more detailed analysis in time, rare bursts of EGFP molecules were detected entering and exiting the channel, with a characteristic time of approximately 300ms. The intermittent nature of this transit suggests an intrinsic localized change in chromatin structure which periodically turns on and off. Preliminary results obtained during mitosis, suggest the chromosomes to impart a markedly different mechanism toward regulating the equivalent transit. This is the first *in vivo* demonstration of the intricate chromatin network showing channel directed diffusion of an inert molecule with high spatial and temporal resolution.