Review of in vivo imaging methods

Optical imaging of biological processes has historically been largely restricted to cells in culture, to naturally transparent organisms, to tissue slices or to the surface layers of thicker samples. In recent years, however, new or newly applied optical techniques are beginning to extend the possibilities for imaging deeper into tissue or even into living organisms. Here Ntziachristos broadly surveys the major methodologies for optical imaging of molecular reporters at microscopic, mesoscopic and macroscopic scales. In this review, he discusses the principles and performance of these methods and their existing and potential applications in biology.

Brief Communication p637

Better light sheets

The length of time required to scan a single point through a three-dimensional object limits conventional optical sectioning microscopy to small specimens. Recent advances have used a thin sheet of light to acquire entire image planes in fast succession and quickly scan through large specimens. But this approach does not resolve the problem of reduced image quality resulting from light scattering at greater sample depths. To overcome this obstacle, Keller and colleagues combined light-sheet microscopy with dynamic structured illumination. Removal of scattered light and increased image contrast allowed them to image early development in both zebrafish and fruit fly.

A multitalented fluorescent protein

Building on their previous report of tetrameric Iris fluorescent protein, Nienhaus and colleagues report a monomeric version, mIrisFP. Because it is monomeric, mIrisFP will be useful for live-cell imaging as a fusion to many cellular proteins. It can be irreversibly photoswitched from a green to a red form, permitting pulse experiments. Additionally, both green and red forms can be reversibly photoactivated, allowing imaging of structures at superresolution with photoactivation localization microscopy (PALM). The researchers used a combined pulse-chase and superresolution approach to study assembly and disassembly of focal adhesions in living cells.

Brief Communication p627

Kinetics of mammalian gene expression

To understand the transcriptional kinetics of single alleles, Yaron Shav-Tal and colleagues integrated single copies of the entire cyclin D1 gene locus under the control of a viral or the endogenous promoter in HEK-293 cells. In the 3′ untranslated region of the gene, they included binding sites for the bacteriophage coat protein MS2 that ensured that each nascent mRNA molecule interacted with MS2-GFP fusion proteins expressed in the same cells. This approach allowed exact quantification of the number of transcripts from each promoter and calculation of transcription rates by measuring fluorescence recovery after photobleaching. Interestingly, Shav-Tal and colleagues found evidence for transcriptional activity after DNA replication, which suggests an interplay between the two.

Brief Communication p631

Voltage-imaging the brain

Genetically encoded voltage-sensitive fluorescent proteins (VSFPs) allow measurements of electrical activity from selected populations of neurons, but their in vivo application had yet to be shown. Knöpfel and colleagues now demonstrate that VSFP2s provide an optical voltage report from targeted neurons in culture, acute brain slices and living mice. By expressing VSFP2s in pyramidal cells of the mouse somatosensory cortex, the authors show that these proteins can report cortical electrical signals in response to natural sensory stimuli in an anesthetized mouse. The sensitivity of VSFPs for imaging subthreshold electrical activity makes them a complementary method to calcium imaging techniques in the analysis of neuronal network activity.

Article p643, The Author File p571