Two-photon Imaging: Bringing Molecular Imaging into the Organism

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The triumph of reductionistic approaches in biomedical research has yielded unprecedented knowledge of the components involved biological processes, and now poses the challenge of integrating this knowledge into a complete understanding. Recent developments in the chemical or genetically encoded dyes needed to tag defined molecular components, have allowed dramatic insights from experiments performed in the test-tube or the culture dish. The next critical step is advancing such analyses to biological processes within intact, functioning organisms.

Laser scanning confocal microscopes (LSCM) have been used extensively to move molecular imaging to more challenging preparations. The LCSM scans a focused spot of light across a specimen, and detects only that light that emerges from the same point in the specimen (through the use to two "confocal" apertures). Any light scattered by the specimen or from planes other than the focal plane is rejected by the detector aperture. As a result, the microscopes have the ability to "optically section" even thick specimens, as well as offering resolution within the focal plane theoretically about 1.4 times better than a conventional microscope. The practical resolution is limited by the dramatic loss in sensitivity as the aperture is closed. This loss of sensitivity and the dramatic photobleaching created by the laser intensities to move the focal plane into tissue are dramatic complications of LSCM that make true molecular imaging unlikely or impossible by this technique.

Two-photon laser scanning microscopy (TPLSM) offers a means to minimize these concerns. TPLSM uses an intense red or infra-red ultra-fast laser to excite fluorochromes in the UV or visible wavelength range through the concurrent absorption of two photons. Because the statistics of two photons being absorbed depends on the square of the intensity and the intensity drops by the square of distance from the focal plane, the technique selectively excites fluorochromes at the focal plane. Light scattering and optical aberrations decrease the probability of the excitation, but decrease the resolution in the image much less than in other light based microscopies. TPLSM has generated images of structures deeper than 500um into biological tissue. Based on its performance to date, two-photon microscopy appears to offer the best means for molecular imaging in intact tissues.