Two-Photon Microscope with Spectral Resolution

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Technology description

Various detection schemes for spectral properties have been implemented in most commercial confocal microscopes. However, confocal microscopes suffer from at least one of the following problems, when compared to two-photon microscopes: (1) low acquisition speeds, mostly due to use of a point-scan method; (2) photodestruction (bleaching) of fluorescent molecules due to exposure of the whole sample to excitation light (while the signal is only read from a thin layer); (3) low signal-to-noise ratio, due to the fact that excitation and emission occur at wavelengths close to one another, making filtering of the signal difficult. On the other hand, two-photon microscopes, which avoid problems (2) and (3) above, either do not present spectral resolution at all, or, when they do, they suffer from slow acquisition speed. Dr. Raicu’ s technology produces spectrally resolved fluorescence images of samples using a two-photon microscope after only one full scan of the sample, which avoids all of the aforementioned problems.
Laser scanning microscopes (such as two-photon and confocal microscopes) are widely used for acquiring images of narrow sections of cells and tissues, in which molecules of interest are tagged with fluorescent molecules. Fluorescent tags make the molecules of interest visible through light emission. By acquiring multiple such thin sections, three-dimensional images of the samples can be obtained. In experiments involving fluorescence tagging with multiple colors, such as in studies of protein colocalization or protein-protein interactions, a fourth dimension becomes critically necessary: the spectral dimension (i.e., the wavelength of the emitted light).

MARKETS

The largest market for two-photon microscopy is academia, while biotechnology companies and government laboratories make up the other main markets. Multiphoton system prices range from $250,000-$600,000. Systems are in strong demand and an annual growth rate of more than 5% is expected in the coming years. As of 2005 microscopes in general accounted for a $7.2 billion market. Sales for 2005 were expected to approach $20 million for 2-photon microscopes (Instrument Business Outlook 2005). It is estimated that about 500 multiphoton microscopy units are sold annually. With a lifetime of 5 to 10 years, the current installed base is estimated at 2,500 to 5,000 units.

Application area

Applications for two-photon microscopy with spectral resolution include creation of 3D images, live-cell imaging, deeper tissue imaging, study of multiple proteins simultaneously, and long-term imaging without compromised tissue viability. Two-photon microscopy also excels at imaging of embryos, whole organs, brain slices, and even entire animals. This technology is particularly important for the study of protein colocalization and can be used to determine protein complexes using FRET in living cells.

Advantages

Better resolution – Acquires images of at least 10 times higher spectral resolution
Faster – Theoretical acquisition speeds of at least 50 times that of current confocal microscopes
Keeps samples safer – Technique avoids photo-bleaching of fluorescent samples
More data gathered – Well-suited for co-localization studies and many proteins can be studied simultaneously (up to 200)
More precise - Can precisely determine the structure of protein complexes

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