Multiphoton laser scanning microscope

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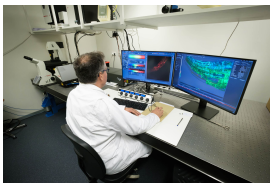

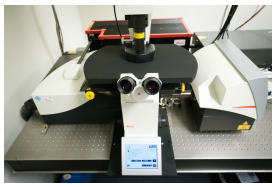

Brand

Leica

Type

SP8 DIVE with FALCON (FLIM) and LIGHTNING (deconvolution)

Contact

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Description

Multiphoton or 2-photon microscopy (MPM / 2PM) combines confocal resolution with in depth imaging. Typically, it is used to image multicellular systems like the roots / leaves of plants, or other strongly scattering media. As special features, it is equipped with spectral detectors, resonant scanners, fluorescence lifetime imaging (FLIM) detection and image deconvolution.

Technical Details

When femtosecond pulsed near infrared (NIR) light is focused to a diffraction limited spot of ~200 nm diameter, there is a probability that the energy of the photons is combined to excite a molecule. For example, two NIR photons of 800 nm can be simultaneously absorbed for a transition that would normally require the energy of a single 400 nm photon.The MPM has a Ti:sapphire laser (tunable from 700-1080 nm) for excitation. A confocal scanhead (Leica SP8) scans the beam through the sample, like a confocal laser scanning microscope (CLSM). The imaging depth can be changed by adjusting the height of the objective with respect to the sample.For detection, the layout is different from CLSM. As multiphoton excitation is only possible in the focal spot of the objective, there is no need to place a pinhole before the detector (and descan the optical beam). Therefore, a dichroic mirror is placed just below the objective to reflect the emission directly to one of two Hybrid detectors. The optical configuration and the size of the detectors is such that the loss of photons due to scattering (i.e. by changes in refractive index) is minimized.Multiphoton microscopy is combined with fluorescence lifetime imaging (FLIM). FLIM monitors the distribution of the fluorescence lifetimes of a fluorophore at the different locations within the sample. The fluorescence of a sample is monitored as a function of time after excitation by a flash of light. The lifetime may be sensitive to environmental factors like ion concentration, pH and polarity but is independent of dye concentration or light path length. The lifetime can be significantly reduced if excited state processes like FRET occur. Moreover, it can be used to measure the ultrafast dynamics of photosynthesis in leaves, algae or bacteria.

Applications

MPM is ideally suited for imaging turbid / opaque samples, including tissues (plant roots and leaves) or food samples. The absence of out-of-focus excitation improves imaging depth and minimizes photobleaching, which makes it well suited for 3D imaging. The nondescanned layout (i.e. the absence of a pinhole) reduces the loss of fluorescence due to scattering in the sample.The combination with FLIM allows for Förster Resonance Energy Transfer (FRET) imaging, as well as pH / viscosity / polarity imaging.Note that the use of an MPM requires more training than a regular CLSM. High energy pulsed laser light (CLASS 4) is used that is almost invisible but can cause severe damage to the human eye. The detectors are very sensitive and less well protected than in a regular confocal. You can contact Arjen Bader or Jan Willem Borst for an introduction.

Complementary Techniques

Confocal laser scanning microscopy