Focal Modulation Microscopy (FMM): A new method to increase penetration depth in confocal fluorescence

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KEYWORDS: Confocal microscopy, fluorescence microscopy.

Confocal microscopy can image to a depth of about 200 μm in tissue. Multiphoton microscopy can image deeper, but uses an expensive laser, while optical coherence tomography (OCT) can also image deeper but does not give molecular information from fluorescence. We have developed a novel method, Focal Modulation Microscopy (FMM), that greatly increases penetration depth (to over 700 μm) in fluorescence microscopy. The illumination light is split into two parts, one of which is frequency-shifted, and interference between the two beams occurs only at the focal spot. For a specimen with a fluorescent label, only light emitted from the fluorescence at the focal region exhibits modulation at the source modulation frequency. For out of focus regions which are only illuminated by one of the beams, fluorescence will not exhibit such modulated intensity and can be simply removed through heterodyne techniques.

The images below, obtained at a depth of 160 μm into a leaf, demonstrate that FMM retains a strong optical sectioning effect and gives a substantial improvement in contrast.

![Fig 1a Chlorophyll image of a mesophyll cell in Schefflera Arboricola (Dwarf umbrella tree), 160μm deep](image1)

![Fig 1b Focal modulation microscopy image of the same area](image2)

Scale bar: 50 μm

REFERENCES: