Lab 2: Cellular and tissue imaging with multiphoton excitation microscopy

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Summary

Two-photon microscopy (TPM) is a three dimensional incoherent imaging technique based on the nonlinear excitation of fluorophores. Two-photon excitation occurs only at the focal point by the simultaneous absorption of two photons each having half of the energy needed for the excitation transition, and it has four unique features. First, TPM uses high numerical aperture objective and a high peak mode-lock femto-second titanium:sapphire laser, and it is considered for various biological specimen imaging with sub micrometer resolution down to a depth of a few hundred micrometers using illumination of near infrared (NIR) wavelength light. Second, TPM causes little photodamage while imaging of living specimens. Third, TPM allows high-sensitivity imaging by eliminating the contamination of the fluorescence signal by the excitation light. Fourth, TPM can initiate a photochemical reaction within a sub-femtoliter volume inside cells and tissues. In contrast, confocal microscopy obtains three-dimensional information by eliminating out-of-focus light through the use of a pinhole, but the energy delivered by the excitation light causes extensive photodamage to living tissues. Further, the excitation wavelength used in confocal microscopes is typically in the UV and blue/green range which is scattered and absorbed strongly in tissues. This effect limits the depth of signal detection.

In this lab, we will briefly introduce the two-photon instruments (lasers, light source, etc) and we will image two sets of specimens: a slide containing cells with a fluorescent actin label from Molecular Probes, and a cartilage sample containing chondrocytes. We will image the specimen using a fluorophore or autofluorescence from the specimen: Chondrocytes in the cartilage specimen stained with cell tracker green and the SHG from cartilage collagen. We will use ImageJ to quickly look at the obtained image data in 3D.

Recommended Reading

P. T. C. So *et al.*, "Two-photon excitation fluorescence microscopy," *Annu. Rev. Biomed. Eng.*, **2**.