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Multi-Photon Confocal Microscopy by Using a Femtosecond Cr Forsterite Laser

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Confocal laser scanning microscopy provides a significant improvement in axial resolution over conventional epi-fluorescence microscopy by eliminating out of focus fluorescence using a spatial filter in the form of a confocal aperture Combining two-photon induced fluorescence with laser scanning microscopy, Denk *et al.* [1] achieved high axial/depth discrimination even without a confocal aperture in front of the photodetector, due to the quadratic dependence of the two-photon absorption on the laser intensity Strong fluorescence is only induced in the vicinity of the focal point The background scattering light and autofluorescence of the sample in the two-photon excited system is also lower. The use of infrared wavelength leads to deeper penetration depth in most materials, providing the opportunity to image thicker samples Two-photon scanning microscopy have been applied extensively in the field of biology and biomedicine, taking advantage of the recent rapid development and commercial availability of femtosecond Ti:sapphire lasers However, recent studies on human skin and biological tissues have suggested that the highest penetrating infrared wavelength in most human and biological tissues exists around 1200-1250 nm, which is not available with Ti:sapphire lasers. The bio-damage induced by Ti:sapphire laser light also indicates the importance of finding alternative light source for the multi-photon scanning microscopy It is thus substantial to development the two-photon confocal scanning microscopy techniques based on 1200-1250 nm wavelength infrared laser light In this presentation, we will present our recent studies two-photon confocal scanning microscopy by using a femtosecond Cr:forsterite laser with output wavelength centered at 1220-1240 nm

The experiments were performed by using a SESAM modelocked Cr:forsterite laser at 1230 nm wavelength with a laser pulsewidth of 130 fs The average output power of the modelocked Cr:forsterite laser is usually on the order of 300 mW The output wavelength of Cr:forsterite laser can not also match the penetration window of most biological tissues, the induced two-photon luminescence can cover red to infrared wavelength and the induced three-photon luminescence can cover purple to orange wavelength Combining with its high output power and short pulsewidth (can be down to 25 fs), the Cr:forsterite lasers show much better advantage over the recent proposed Er:fiber lasers A dichroic beam splitter was employed in our experiments to reflect the Cr:forsterite laser beam to an 80X objective The laser beam was then focused onto the sample, which was hold by a microscope slide Thus induced multi-photon luminescence was collected by the 80X objective, transmitting through the dichroic beam splitter and collected into a cool-CCD based spectrometer The image for the specific luminescence wavelength was then accomplished by scanning the sample in a computer controlled XYZ stage Figure 1(left) shows the measured two-photon luminescence from Corn stems and Corn leaves The luminescence emitting at ~685 and 740 nm from the corn leaf should be contributed from chorophyll We have also checked the luminescence from several widely used labeling dyes, including SYTO for nucleic acid stains, BODIPY TR for Amine-reactive probe, Alexa Fluor 594, Mitotracker, and Lysotracker Excellent luminescent efficiency can be observed Figure 1(right) also shows the 3-photon luminescence from Mitotracker peaked at 600nm. In this presentation we will also compare the images taken by Cr:forsterite laser with those by Ti:sapphire laser

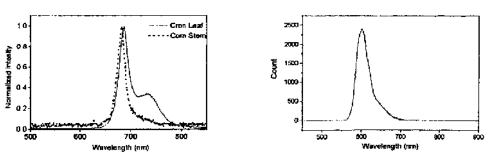

Figure 1: Multi-photn PL taken by using 1230 nm femtosecond light from a Cr:forsterite laser (left) Two-photon luminscence taken from Corn leaves and corn stems (Right) Three-photon luminescence taken from Mitotracker

Reference:

[1] W Denk, J H Strickler and W W Web, *Science* **248**, 73 (1990)

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Phase-drift suppression in heterodyne detection and its application to optical coherent tomography

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Interferometric measurement techniques have a wide range of applications from measuring distances to optical tomographies. Recently, optical coherent tomography (OCT) for biological tissues has attracted special interest[1] OCT systems utilize low-coherence interferometry with heterodyne detection Heterodyne beat signals change, due to phase drifts between the signal and reference waves, through fluctuations of wavelength, phase, and optical length. In this study, we devised a phase-drift suppression method (PDSM) that can suppress the influences of this phase drift to enhance the stability of the heterodyne beat signal and demonstrated its applications[2].

The beat signal consists of many harmonics, created by sinusoidal phase modulation (PM) We define the computed signal intensity (CSI) as the square root of the sum of the squares of each harmonic intensity as shown by:

$$I_{CS}(\phi_O,\phi_d) \equiv 2I_O\sqrt{\sum_{i=1}^{n/2}\sin^2(\phi_d)J^2_{2i-1}(\phi_O)+\sum_{j=1}^{n/2}\cos^2(\phi_d)J^2_{2j}(\phi_O)},$$

where I_O is the light intensity containing signal ; n is a finite, positive and even integer ; ϕ_d is the total phase drift ; $J_n(x)$ is the Bessel function ; and ϕ_O is the modulation index in PM. The backscattered light from biological tissues can be measured as CSI. We based our OCT system on a fiber Mach-Zehnder interferometer using a single-mode fiber. The light source was a superluminescent diode (λ_O : 840 nm, $\Delta\lambda$: 25 nm) The temperature of the light source was stabilized at 25 °C. A PZT PM controlled the modulation index by applying a sinusoidal voltage of 15 kHz. The sample arm comprises of a half mirror coupler and an optical probe using a GRIN lens. We detected the beat signal with Si-APD. The individual intensities of the higher harmonics in the beat signal were fed into a personal computer, where they were processed with an RF spectrum analyzer to obtain the CSI. The axial resolution was 17 μm, and the lateral resolution was 7.5 μm

Using a mirror for a sample, we measured variations of the CSI with a time of 10min for modulation indexes Figure 1 shows the dependence of the relative standard deviation (RSD = standard deviation of CSI / <CSI>) on the modulation index C_1 indicates conventional heterodyne detection using only the fundamental component $C_{1\text{-}n}$ indicates that the CSI was obtained using harmonics from the fundamental to the n-th component. At modulation indexes of 2.6, and 2.8 rad, the stability was increased by 6.3 times in $C_{1\text{-}2}$ and 10 times in $C_{1\text{-}4}$ We applied our PDSM to sectional imaging of the cover glass on a slide glass, using $C_{1\text{-}2}$ and a modulation index of 2 6 rad as shown in Fig.2. The noise was suppressed on both surfaces of the cover glass by the application of our PDSM.

In conclusion, the PDSM is useful for enhancing the signal stability, without complex optical and electronic feedback, and is applicable to conventional heterodyne detections. We will present further details at the conference

[1] D. Huang, et al.," Optical Coherence Tomography," Science, vol 254, pp.1178-1181 (1991)
[2] M Sato, et al. "Signal processing of optical heterodyne by detections of harmonics and biotomography", Technical report of IEICE, vol.MI99-23, pp.19-24 (1999).

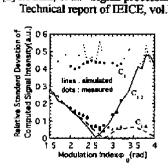
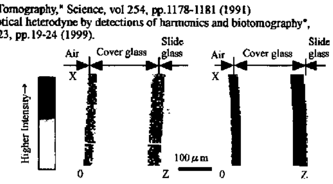


Fig. 1 Dependence of RSD on modulation index.



(a) C_1 without PDSM (b) $C_{1\text{-}2}$ with PDSM
Fig. 2 OCT images of air/cover glass/ slide glass
1 pixel = 5μm x 5μm; 60 pixel x 60 pixel.