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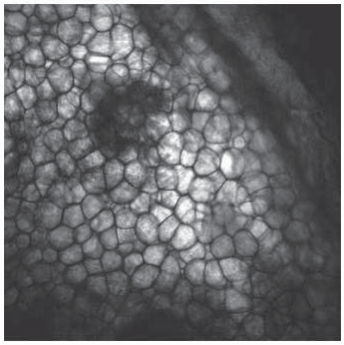
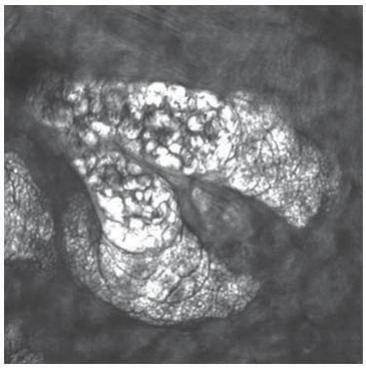


FPPO-fs

Fiber laser pumped, femtoseond optical parametric oscillator

Application Notes:

CARS and two-photon imaging



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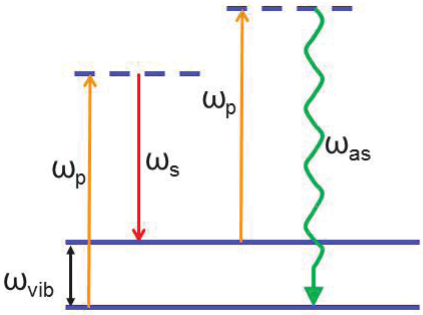
FPPO-fs applications

Application Notes:

Imaging techniques such as CARS (coherent anti-stokes Raman scattering) imaging and two-photon microscopy are powerful methods for imaging biological samples, but require the high peak power from ultrafast pulses to efficiently drive the nonlinear process. The FPPO-fs fiber laser pumped OPO provides a flexible solution for use in both techniques.

CARS imaging is label free, as probes the vibrations inherent to a molecule. In figure 1, the energy level diagram for a CARS process is shown. Two syncronized pulses (pump and Stokes pulses) of different wavelengths are incident on the sample. If the difference in pulse energy matches the vibrational frequency, a molecular vibration is excited. A second interaction with the pump pulse interacts with the molecules, causing a photon a blue shifted photon to be emitted (the Anti-Stokes photon). By using the inherent vibration present in the molecule, CARS offers a label free imaging method that offers inherent sectioning capabilities.



$$\omega_p\text{-}\omega_s = \omega_{vib}$$

$$\omega_{as} = 2\omega_p\text{-}\omega_s$$

Fig. 1. CARS energy diagram

For chemically selective imaging, pulses with linewidths less than the width of the vibrational resonance are desireable. For many molecules this is < 10 cm^{-1}, for which picosecond pulses are optimal. However, the CH_2 stretch in lipids has a width of ~60 cm^{-1}, allowing the use of femtosecond pulses for many biological applications.

The FPPO-fs provides 300 fs pulses with nearly continuous spectral coverage from 730nm-1000nm for the signal pulse and 1150nm - 200 nm for the idler pulse. Signal and idler pulses are automatically synchronized, without the need for complicated electronic need to two separate lasers. Figure 2 shows a possible experimentla implemantation of the FPPO-fs for use in CARS imaging. The signal and idler pulses are tuned to the desired vibration and colinearly incident on the sample. The anti-Stokes signal is filtered from the excitation pulses adn focused onto a detector. By used of a scanning mirror, a full image can be obtained.

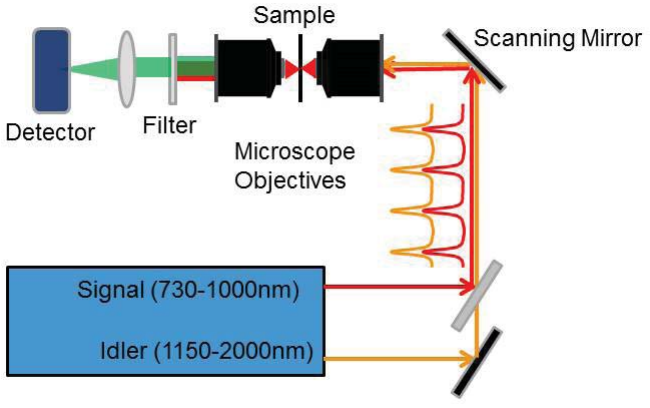


Fig. 2. Experimentla digram showing the use of the FPPO-fs for CARS imaging.

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FPPO-fs

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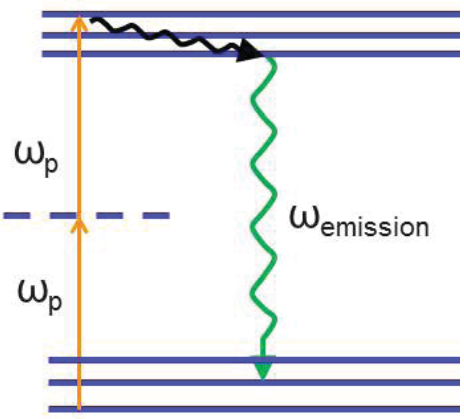
Application Notes

ITwo photon microscopy provides a versatile complement to CARS imaging when flourescent labels can be used. As shown in the energy level diagram of figure 3, two photons with energy less than the electronic transition energy are simultaneously absorbed. The resulting emssion is signifigantly blue shifted from the excitation light.



When compared with single photon fluorescence imaging, two photon imaging has several advantages.

- Localized excitation volume provides inherent 3D sectioning
- Longer wavelength excitation is scattered less
- Better imaging depth
- Emission is shifted far from excitation light for higher signal to noise

Fig. 3. Energy level diagram for 2-photon fluorescence.

The main disadvantage is the need for short pulses in order to acheive efficient two-phton absroption. A popular source for femtosecond pulses is the Ti:sapphire laser. However, the spectral coverage of Ti:S lasers typically doesn't go beyond 1000nm. Since longer wavelength excitation experiences less scattering, allowing for deeper penetration into thick samples. The idler beam of the FPPO-fs covers the spectral range of 1150mn -2000nm, with significant power at 1200 nm (the longest wavelength for which most microscope optics are transmissive).

In figure 4 a digram showing the experimental design for a 2-photon microscope. Excitation light is focused onto a sample, where a two pho-tons are abaorbed. The emitted fluouescence is collected in the backward direction, seperated from teh exciteation light with a dichroic mirror, and focused onto a detector. The use of a scan-ning mirror enables the contruction of an image.

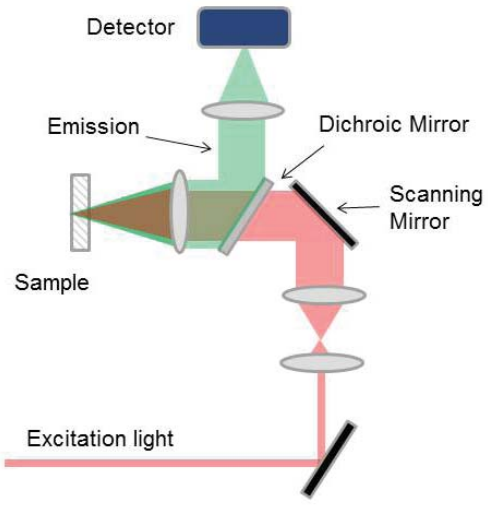


Fig. 4. Experimental diagram of a 2-photon microscope.

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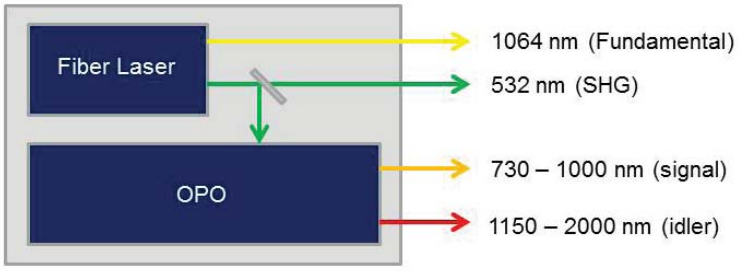
Application Notes

The FPPO-fs is a fiber laser pumped, femtosecond optical parametric oscillator that can provide a light source for researchers intersted in nonlinear imaging techniques. The system is all imclusive, with pump laser and OPO in one compact box. Up to four outputs are available: the laser fundamental at 1064 nm, the laser second harmonic at 532 nm, and the tunable signal and idler outputs from the OPO. All pulses are derived from the same fundamental pulse, so all repetition rates are inherently locked at 110 MHz.



In figure 5, the tuning rnage of the OPO signal and idler outputs is shown. The broad spectral coverage make this an idela source for optimizing CARS and two-phpoton signals. In particular, the higher power in the idler beam at wavelengths above 1100 nm allow access to excitation light not available in Ti:S lasers.

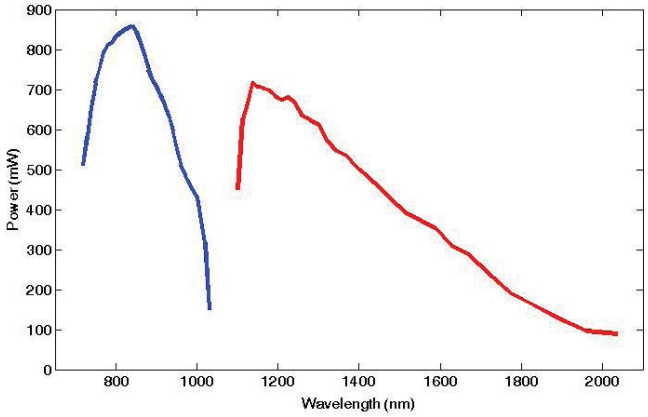


Fig. 5. Tuning curve of OPO, showing typical signal (blue) and idler (red) powers available at each wavelength.

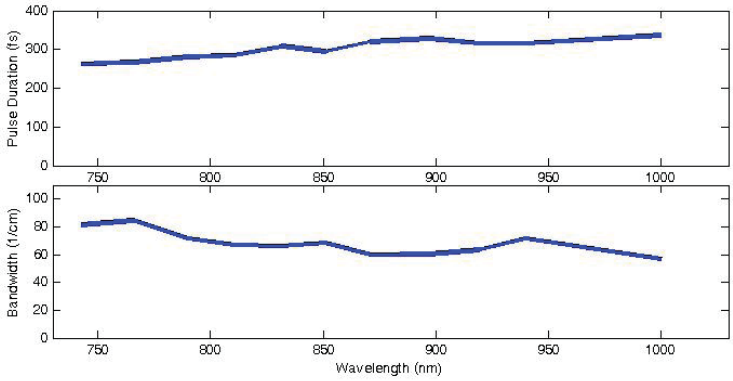


Fig. 6. Pulse duration (top) and bandwidth (bottom) for the signal pulse across its tuning range.

For both CARS and 2-photon imaging, short pulses are necessary for efficient signal. The FPPO-fs provides pulses of approximately 300 fs in duration, with a bandwidth between 50 and 80 cm^{-1} across the entire tuning range of the signal, as seen in figure 6. The pulse bandwidth roughly matches the width of the lipid resonance, which makes the higher peak intensities of femtosecond pulses attractive for CARS imaging in biological samples.

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