

A simple, cost efficient fiber amplifier wavelength extension unit for broadly tunable, femtosecond pulse Ti-sapphire lasers for CARS microscopy

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Abstract: An inherently synchronized Yb-fiber amplifier based extension unit for femtosecond pulse, broadly tunable Ti-sapphire lasers is introduced, which is well suited for coherent anti-Stokes Raman scattering microscopy.

OCIS codes: (140.7090) Ultrafast lasers; (180.4315) Nonlinear microscopy

1. Introduction

Nonlinear 3D microscopy, such as two- or multi-photon absorption fluorescence microscopy, has the main advantage of imaging deeply in a biological sample with minimum perturbation or damage in the tissue. Coherent Raman scattering (CRS) methods such as coherent anti-Stokes Raman scattering (CARS) [1] and stimulated Raman scattering (SRS) [2] allow for label-free imaging of tissue with chemical contrast and high spatial and temporal resolution. In CRS, laser beams at two frequencies, called the pump, ω_p , and Stokes, ω_s , frequencies are used to illuminate the sample. When the difference frequency between the two beams is tuned to match an intrinsic molecular vibrational frequency in the sample, ω_{vib} , several nonlinear interactions occur. New light is generated at the anti-Stokes frequency, $\omega_{\text{as}} = 2\omega_p - \omega_s$, by the CARS process, and some intensity is transferred from the pump to the Stokes beam by the SRS process. Both processes offer chemical selectivity, high spatial resolution, and video-rate imaging speeds *in vivo* in living animals and humans.

For single wavelength nonlinear microscopy, such as multi-photon absorption fluorescence (TPF), second-harmonic generation (SHG) and fluorescence lifetime imaging (FLIM) microscopy, broadly tunable, femtosecond pulse Ti-sapphire lasers [3] are used typically. Recent years brought revolutionary progress in development of femtosecond pulse, all-fiber laser oscillators [4] and amplifiers being suitable for nonlinear microscopy. For instance, a novel all-fiber, multimodal (TPF+SHG+CARS) microscopy system has been presented in the same year [5]. Fiber lasers are also of interest because of the ease with which they can be combined with endoscopy [6], which would greatly increase the utility of CARS microscopy for pre-clinical applications and tissue imaging. However, there is a large number of nonlinear microscope systems all around the world, in which broadly tunable Ti:sapphire lasers are used as a pulsed light source, and they long for a simple, cost efficient wavelength extension unit that makes the microscope system capable for CARS imaging with minimum modification on the laser and microscope setups.

In this paper, we present a simple, cost efficient wavelength extension unit for these lasers comprising a two stage Yb-fiber amplifier unit, which allows CARS measurements practically on the same microscope and lasers setup that is used for single wavelength 3D microscopic imaging. In order to demonstrate the potential of the new, dual wavelength upgraded laser system for CARS imaging, we provide a few images recorded for ... at the end of the paper.

2. Experimental setup

The experimental setup is shown in Fig. 1. A broadly tunable, femtosecond pulse Ti-sapphire laser (*FemtoRose 100TUN NoTouch*, product of R&D Ultrafast Lasers Ltd [7]) generates nearly transform limited, $\tau_{\text{FWHM}} \sim 190$ fs pulses for our measurements. A Faraday isolator (FI) placed in the beam path assures that there is no back reflection to the laser oscillator from the different optical elements, such as the optical fiber ends. An achromatic beam-splitter (BS) divides the laser power into two parts: the reflected beam is focused into a small core area photonic crystal fiber (NL-1.4-775, product of NKT Photonics) by a high NA, achromatic focusing lens, which generates the seed pulses for the two-stage Yb-amplifier unit (*FemtoFiber*, product of R&D Ultrafast Lasers Ltd. [7]) operating at around 1030 nm. The transmitted beam, which plays the role of a tunable pump beam for CARS measurements, directly goes into the nonlinear microscope passing through a precision delay stage, which assures the zero delay

difference between the tunable pump and amplified, compressed Stokes pulses, the latter one is being generated by the two-stage Yb-amplifier unit.

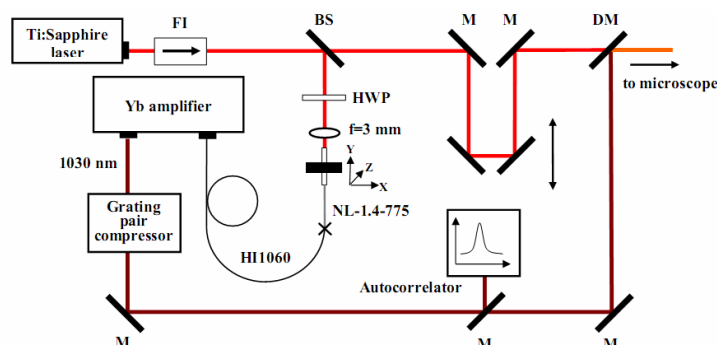


Fig. 1 Setup of the CARS extension unit

The pump and Stokes pulses are inherently synchronized, since the ~ 1030 nm seed pulses of the amplifier are generated by the Ti:sapphire laser pulses by efficient nonlinear wavelength conversion in a 109 mm long photonic crystal fiber (PCF).

3. Results

In Fig. 3, the spectral intensity vs. wavelength functions, which were measured directly after the PCF, are plotted for different pump wavelengths at coupled power levels of ~ 100 mW. For seeding the Yb-fiber amplifier, we typically use the chirped optical pulses of an all-fiber, all-normal dispersion ytterbium ring oscillator [4] delivering an average output power of 2-5 mW at ~ 80 MHz repetition rate. In this setup, we use that portion of the optical spectra of the continuum generated in the PCF, which are within the gain bandwidth (~ 1020 - 1080 nm) of the Yb-doped optical fiber amplifier.

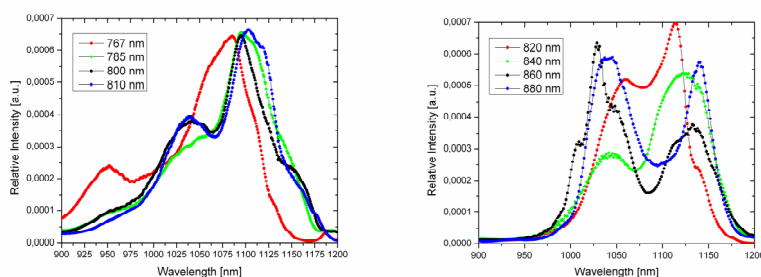


Fig. 2 Measured spectral intensity distribution vs. wavelength functions at different pump wavelengths at a coupled power level of ~ 100 mW

After the two-stage Yb-amplifier, we obtain optical pulses with central wavelength of ~ 1028 nm and a FWHM bandwidth of 11 nm. The pulse duration was measured after a transmission grating pair compressor (1200 lpmm @ 1032 nm, Wasatch Photonics) at an optimum grating distance of 19 mm, resulting in a nearly transform limited pulse duration of ~ 276 fs. The pump and the Stokes beams are combined by a dichroic beamsplitter (DM), and then transmitted to the nonlinear microscope (Axio Examiner LSM 7 MP, product of Carl Zeiss). Both the pump and the Stokes laser provide nearly transform limited optical pulses, hence we expected relatively strong anti-Stokes signals in our nonlinear microscope. The dual wavelength, inherently synchronized laser system has been tested for label-free, coherent anti-Stokes Raman scattering (CARS) imaging in the microscope. Different samples including murine ear and white adipose tissue, mineral oil and carbon nanotubes were used for testing our novel dual wavelength laser setup. For biomedical imaging applications, we show a few images recorded for murine ear and white adipose tissue. In Fig. 3, we show CARS-images of a murine ear surface with and without mineral oil treatment. The CARS image revealed the presence of the externally applied mineral oil as it penetrates into the stratum corneum. The image was taken on the surface of the skin directly after oil addition. The "CARS-delayed" image, in which the pump and Stokes pulses are delayed relative to each other in order to avoid temporal overlapping of the pulses, proves that the red signal originates from the mineral oil.

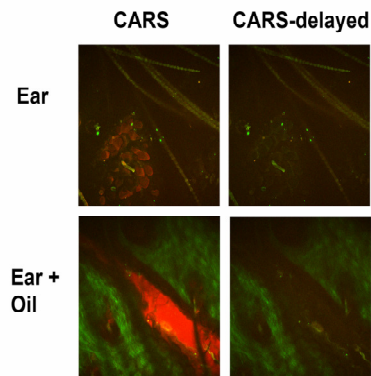


Fig. 3. CARS-images of mouse ear surface with and without mineral oil treatment.

The two images were recorded at an average power level below ~ 24 mW directly measured on the sample. In accordance with the data listed in the literature [1], the mineral oil has a strong vibration resonance at $\omega_{\text{vibr}} = 2845$ cm^{-1} , corresponding to the CH_2 symmetric stretching vibration resonance of saturated fatty acids. For the CARS measurements, the pump laser had to be tuned to 796 nm (12563 cm^{-1}) in order to satisfy the $\omega_{\text{vibr}} = \omega_p - \omega_s$ condition. Since the Stoke beam is centered at 1028 nm (9727 cm^{-1}), the anti-Stokes signal should be detected on the “red” channel of our nonlinear microscope at the wavelength of 649 nm. For the CARS measurements, the original dichroic beamsplitter and the laser blocking filter in the microscope has been replaced by new ones having a longer cut off wavelength of 760 nm. In Fig. 4, CARS-images of murine white adipose tissue are shown. These images revealed the internal homogenous distribution of saturated fatty acids inside of white adipocytes. Images of “time delayed” CARS and “wavelength detuned” CARS images proved specific signal derived from CH_2 bounds.

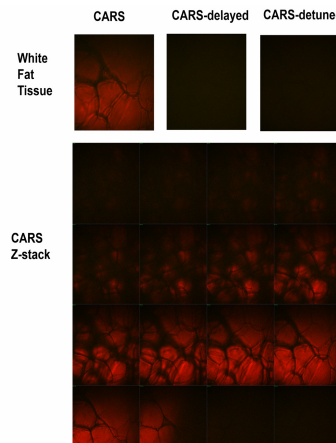


Fig. 4 CARS-images of murine white adipose tissue.

Based on our measurements, we are convinced that the novel, simple, cost efficient, inherently synchronized Yb-fiber laser based extension unit reported here is well suited for upgrading most of the existing single wavelength nonlinear microscope setups for dual wavelength, label-free, CARS imaging.

4. References

- [1] C. L. Evans and X. S. Xie, "Coherent Anti-Stokes Raman Scattering Microscopy: Chemical Imaging for Biology and Medicine", *Annu. Rev. Anal. Chem.* **1**, 883-909 (2008).
- [2] B. G. Saar, C. W. Freudiger, J. Reichman, C. M. Stanley, G. R. Holtom, and X. S. Xie, "Video-Rate Molecular Imaging in Vivo with Stimulated Raman Scattering", *Science* **330**, 1368-1370 (2010).
- [3] E.J. Mayer, J. Möbius, A. Euteneuer, W. Rühle, R. Szipöcs, "Ultrabroadband chirped mirrors for femtosecond lasers" *Optics Letters* **22**, 528-530 (1997).
- [4] J. Fekete, A. Cserteg, R. Szipöcs, "All-fiber, all-normal dispersion ytterbium ring oscillator", *Laser Physics Letters* **6**, 49-53 (2009).
- [5] A.F. Pegoraro, A. Ridsdale, D. J. Moffatt, J.P. Pezacki, B. K. Thomas, L. Fu, L. Dong, M. E. Fermann, and A. Stolow, "All-fiber CARS microscopy of live cells", *Opt. Exp.* **17**, 20701-20706 (2009).
- [6] B. G. Saar, R.S. Johnston, Ch.W. Freudiger, X.S. Xie, and E. J. Seibel, "Coherent Raman scanning fiber endoscopy" *Opt. Lett.* **36**, 2396-2398 (2011).
- [7] www.fslasers.com/products/