A diode-pumped high power extended cavity femtosecond Yb:KGW laser: from development to applications in nonlinear microscopy

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ABSTRACT

We present the design and development of a diode-pumped high average power femtosecond laser based on a crystal of Yb-ion doped potassium gadolinium tungstate (Yb:KGW) and a semiconductor saturable absorber for passive mode-locking. The laser delivered up to 0.85 W of average power with ~200 fs pulses at a repetition rate of 14.6 MHz, corresponding to a pulse energy of 60 nJ with a peak power of ~300 kW. The developed laser system was used to visualize the structure of muscle cells from *Drosophila melanogaster* larvae *in vivo* by acquiring high-resolution images with a nonlinear multimodal scanning microscope, capable of simultaneous detection of two-photon fluorescence, second and third harmonic signals.

Keywords: Ultrafast lasers; Diode-pumped solid-state lasers; Nonlinear microscopy

1. INTRODUCTION

Yb-ion based femtosecond lasers [1] operating around 1000 nm are particularly suited for biological imaging with high-resolution multiphoton excitation fluorescence, and third and second harmonic generation microscopy. They are a good alternative to commonly used Ti:sapphire lasers emitting in the 800 nm wavelength regime. Longer excitation wavelengths are desirable due to lower scattering and higher penetration depths in the biological tissue. In addition, the autofluorescence and bleaching are also largely reduced. Furthermore, the generated third and second harmonics fall into the visible range (rather than UV), resulting in higher throughput and a simpler detection scheme. On the other hand, wavelengths in the range of a Cr:forsterite laser at around 1200-1300 nm suffer from significant water absorption. At higher excitation powers that may cause photo-damage of biological tissues. Moreover, direct diode pumping of Yb-ion doped laser gain media [1] considerably reduces the cost of a system in comparison to Ti:sapphire and Cr:forsterite lasers, which are usually pumped by expensive solid-state or fiber lasers.

Another important issue with the conventional laser sources currently used in nonlinear microscopy is associated with their high repetition rates, which are typically around 70-100 MHz. Such high repetition rates can cause accumulation of long-lived triplet states and photo-generated reactive oxygen species within the sample, resulting in tissue degradation over time. These detrimental effects can be reduced by employing low repetition rate (1-20 MHz) ultrashort pulse lasers. Operation at low repetition rates is also beneficial for applications in fluorescence lifetime measurements, helping to avoid triplet-triplet annihilation and cumulative effects.

Pulse repetition rate can be reduced by a pulse picker or a cavity dumper [2, 3], both of which require high speed and high voltage electronics, adding to the complexity and cost of the laser system. Alternatively, since the pulse repetition rate is inversely proportional to the length of a laser resonator, it can be reduced simply by extending the laser

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cavity by an appropriate distance. This can be done either using specially designed optical multi-pass cells [4,5] or intracavity telescopes constructed from the standard optics [6, 7]. Pulses as short as 43 fs were generated with energies up to 150 nJ from a Ti:sapphire based extended cavity laser [5].

Recently, our group demonstrated the first diode-pumped low pulse energy (2-10 nJ) extended cavity femtosecond Yb:KGW laser [8] using intracavity telescopes. This laser was frequency doubled into the visible wavelength range and used for optical DNA detection. In this paper we demonstrate a high pulse energy (60 nJ) version of the low repetition rate femtosecond Yb:KGW laser specifically designed for applications in nonlinear multimodal microscopy and fluorescence lifetime imaging.

2. LASER CRYSTAL

Femtosecond, high power lasers require a gain medium with a broad amplification bandwidth, relatively large laser emission cross-section, and good thermal properties to handle the heat load. The crystals of Yb-doped potassium gadolinium tungstate (Yb:KGW) [9] are known to exhibit more superior properties than the other Yb-doped solid-state laser materials [10]. The Yb:KGW crystals have broad amplification bandwidths, high emission cross sections, good thermal conductivity and are suitable for efficient diode pumping using commercially available laser diodes in the 930-980 nm region. Previously, pulse energies on the order of 12-32 nJ were demonstrated from the end-pumped Yb:KGW lasers in femtosecond mode-locked regime [11, 12].

Table 1 summarizes the main optical and physical parameters for the Yb:KGW crystal. The energy level structure of the Yb-ion in the KGW matrix is presented in Fig. 1.

Properties		**Units**
Laser wavelength	1.026	μm
Emission bandwidth	20	nm
Emission cross-section	2.8 ($E\|\|n_m$) 2.1 ($E\|\|n_p$) 0.7 ($E\|\|n_g$)	10^{-20} cm^2
Absorption wavelength	981	nm
Absorption cross-section	1.2 ($E\|\|n_m$) 0.2 ($E\|\|n_p$) 0.2 ($E\|\|n_g$)	10^{-19} cm^2
Fluorescence lifetime	~0.3	ms
Transparency intensity	0.27	kW/cm^2
Refractive index	2.023 (n_m) 1.978 (n_p) 2.042 (n_g)	-
Temperature coefficient of refractive index	0.4	10^{-6}K^{-1}
Thermal conductivity	2.6 [100] 3.8 [010] 3.4 [001]	W/(m×K)
Coefficient of linear thermal expansion	6.3 [100] 2.4 [010] 21.7 [001]	10^{-6}K^{-1}

Table 1. Properties of Yb:KGW crystal [13].

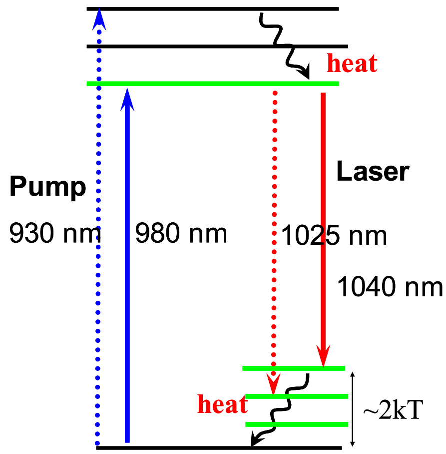

Figure 1. Energy levels of Yb-ion in KGW matrix.

3. HIGH PULSE ENERGY LASER DESIGN

A previously demonstrated high average power femtosecond Yb:KGW laser operating at a repetition rate of 97 MHz [14] was used as a platform for the development of the high pulse energy extended cavity Yb:KGW laser oscillator. The experimental extended cavity laser set-up is shown in Fig. 2.

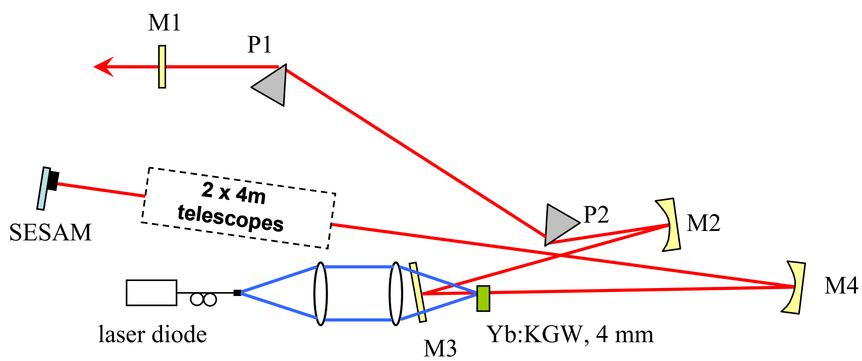

Figure 2. Experimental laser layout: M1 - output coupler, 6%; M2, M4 – curved mirrors;
M3 – dichroic mirror; P1, P2 – SF10 Brewster prisms.

To ensure a high power laser operation, the pump source used for the experiments was a 25W fiber-coupled laser diode at ~980 nm with a 200 µm (0.22 NA) fiber core diameter (Apollo Instruments Inc.). Depending on the diode output power, the central wavelength of the pump radiation changed between 975 and 981 nm [14] and resulted in the ~70-80% of the pump absorption inside the crystal.

Pumping the Yb:KGW laser crystal at 980 nm provides a smaller quantum defect and higher absorption efficiency, than, for example, using the laser diodes operating at 930 nm (see Fig. 1). Therefore, the amount of heat generated inside the crystal during laser operation will also be smaller, reducing thermal effects such as thermal lensing.

The output of the laser diode was focused by two antireflection (AR) coated spherical lenses of 40 and 50 mm focal lengths into a 4-mm-long and 2-mm-thick flat/flat AR-coated Yb:KGW crystal with 5% Yb-ion doping (Eksma). The crystal was cut for beam propagation along the N_p-axis to access the high-gain polarization parallel to the crystallographic N_m-axis. Since the pump radiation was not polarized, a flat/flat geometry of the crystal was used rather than more common Brewster/Brewster which results in higher pump loses at the crystal's interface.

The pump spot was chosen to be ~250 µm in diameter and corresponds to a confocal parameter of ~2.9 mm. The mode size diameter inside the crystal was closely matched to that of the pump and was equal to ~240 µm in diameter. The crystal was wrapped in indium foil and held between thermoelectric coolers which kept it at a constant temperature of about 10 degrees C.

The pump radiation was delivered to the crystal through a dichroic mirror M3, which was coated for high transmission (HT >95%) at the pump wavelength and high reflection (HR >99.9%) in the 1020-1080 nm region. HR mirrors, denoted by M2 and M4, were curved and each had a radius of curvature of 500 mm. The output coupler mirror M1 had a 6% transmission in the 1020-1080 nm wavelength range. All mirrors were custom designed by Layertec and Laseroptik GmbH.

One end of the cavity was terminated with a semiconductor saturable absorber mirror (SESAM, Batop GmbH) with 1% modulation depth to initiate and sustain passive mode-locking [15]. No additional curved mirror was required to focus the beam to a 420 µm spot size diameter onto the SESAM. To enter into the soliton mode-locking regime, a pair of Brewster-angled SF10 prisms (CVI Inc.) separated by 44 cm were inserted into the cavity for dispersion compensation.

Cavity length extension was realized with two identical intracavity telescopes. Each telescope had a 1:1 imaging ratio and was constructed from a HR concave mirror with radius of curvature of 2 m and an HR plane folding mirror [6, 8]. When both telescopes were incorporated inside the cavity, the pulse repetition rate changed from 66 to 14.6 MHz. Since the telescopes provided a 1:1 imaging ratio, their use did not change any laser beam parameters inside the cavity.

4. MODE-LOCKED LASER OPERATION

With 15W of pump power incident onto the crystal (~80% absorbed by the crystal), we achieved 0.85W of average output power in stable train of ~200 fs soliton-like pulses. At 14.6 MHz repetition rate this corresponded to 60 nJ of pulse energy or 300 kW of peak power. To the best of our knowledge, this is the highest pulse energy achieved in the mode-locked regime from the end-pumped Yb:KGW laser using a single fiber-coupled diode pump module.

The pulse spectrum and the pulse autocorrelation trace are displayed in Fig. 3. The pulse duration was measured with a second order autocorrelator based on two-photon absorption in a red light-emitting diode. The time-bandwidth product was calculated to be 0.34, indicating that the pulses were almost transform-limited with a $sech^2$ temporal profile.

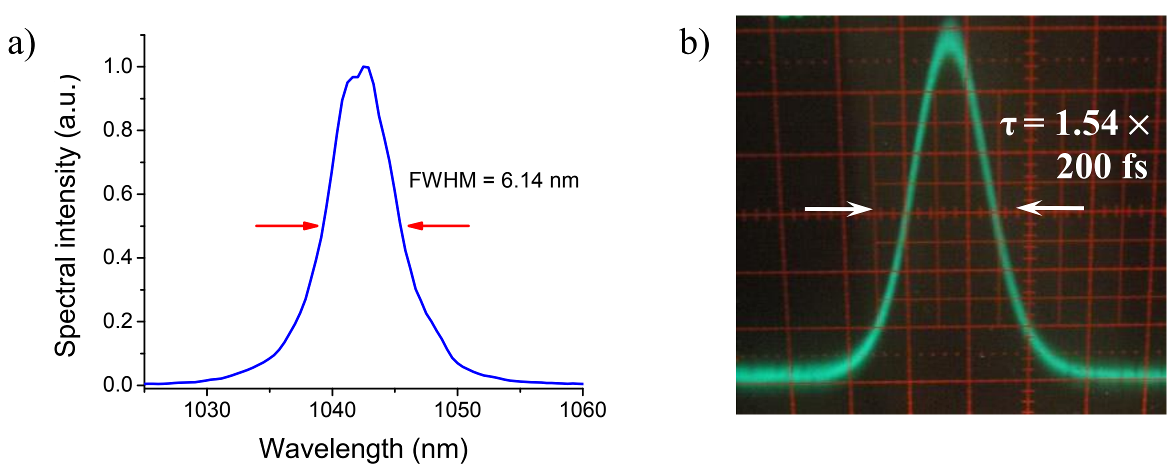

Figure 3. Spectrum (a) and autocorrelation (b) of femtosecond pulses.

Passive mode locking was completely self-starting and could be sustained for hours of operation. The maximum energy fluence on the SESAM (with the mode spot diameter of ~420 μm) was calculated to be 720 μJ/cm^2 and no damage to the device was observed in the femtosecond regime.

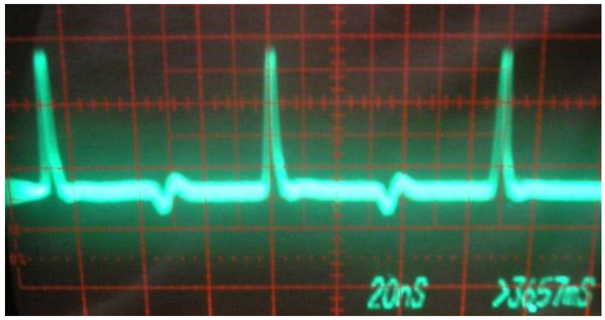

Figure 4. A trace of the pulse train at 14.6 MHz repetition rate.

Pulse repetition period was measured to be ~70 ns, as can be seen from the oscilloscope trace in Fig. 4, which displays a pulse train. A fast photodiode was used for these measurements (combined resolution of ~1 ns for the photodiode and oscilloscope).

5. NONLINEAR MULTIMODAL MICROSCOPY AT 1 μm

Using the laser and a home built nonlinear laser scanning microscope [16], capable of simultaneous detection of two-photon fluorescence (MPF), second and third harmonic signals (SHG and THG, respectively), we investigated the structure of muscle cells from *Drosophila melanogaster* larvae *in vivo* by acquiring high-resolution images. The schematic design of the nonlinear microscope is illustrated in Fig. 5.

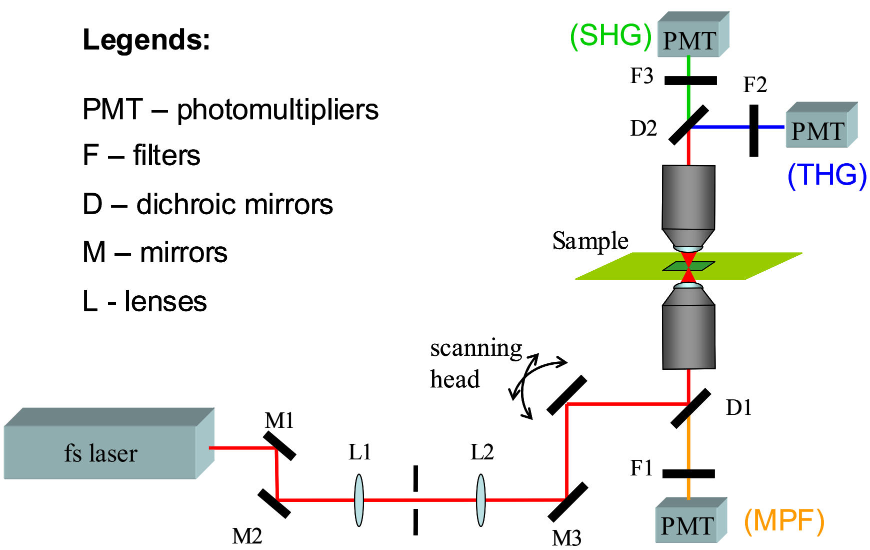

Figure 5. Schematic design of the nonlinear microscope.

The laser beam was first spatially filtered and directed by mirror M3 through a laser scanning head and dichroic mirror D1 into the focusing objective. Raster scanning of the beam allowed for the acquisition of the 2D images in a x-y plane of the sample. To allow for a 3D imaging, the sample was mounted on a piezoelectric precision stage, which could translate the sample along the optical axis with 50 nm resolution.

As can be seen from Fig. 5, the multi-photon fluorescence signal was detected in the backward direction, and SHG and THG signals in the forward direction, using the appropriate dichroic mirrors D and filters F. All signals were detected by photomultiplier tubes operating in the single photon counting regime, and directed to a data acquisition card within a computer. A Labview interface program was used to control the microscope.

As an example, a white light image of the investigated *Drosophila melanogaster* larvae muscles is displayed in Fig. 6. White light imaging is limited by poor contrast and high scattering, consequently, the muscle structure can not be clearly identified.

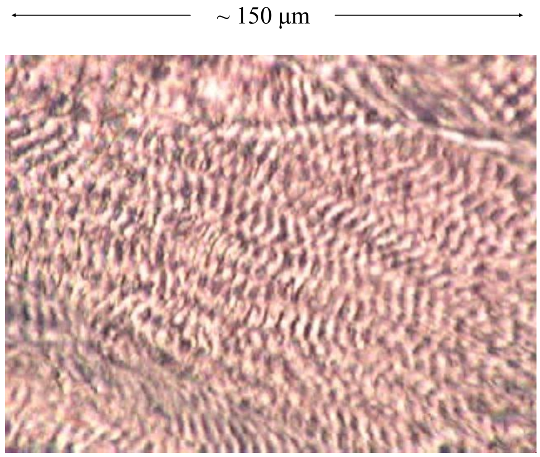

Figure 6. White light image of the larval muscle cells.

The muscle structure, visualized with the SHG signal is presented in Fig. 7. The image provides excellent contrast and individual sarcomeres (unit of a muscle cell) can be clearly identified.

It was recently shown that strong second harmonic signal originates from the actin-myosin microcrystalline structure and can be used for visualization of the anisotropic bands of the sarcomeres [16, 17]. Owing to laser excitation at ~1 µm wavelength, no structural or functional alterations were observed during imaging, confirming that the developed laser system is suitable for investigations of biological samples.

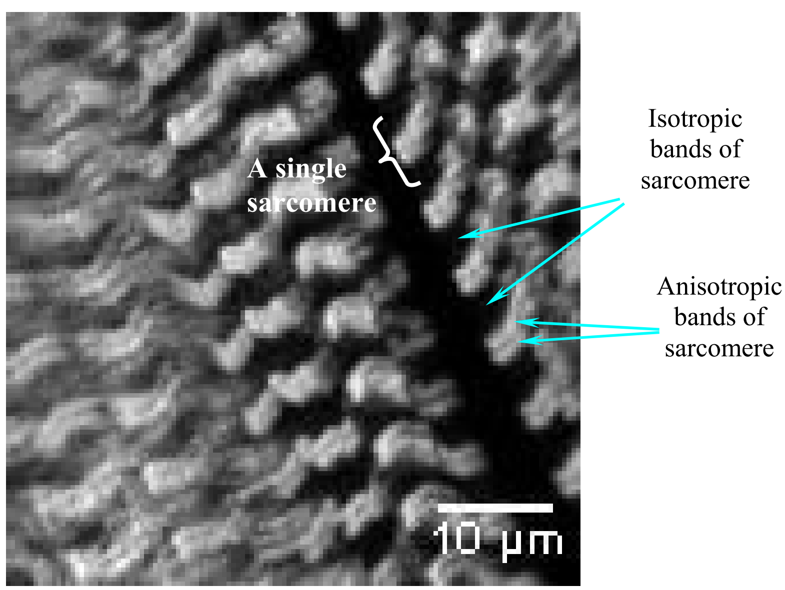

Figure 7. SHG image of larval muscle structure.

For more details on applications of the developed laser system please refer to the following two manuscripts which were submitted to the SPIE Proceedings (to be presented in the Biomedical Photonics session of the Photonics North 2006 conference) [18, 19].

6. CONCLUSIONS

In conclusion, we have developed a high pulse energy diode-pumped femtosecond Yb:KGW laser that produced pulse energies up to 60 nJ in 200 fs pulses centered at 1042 nm wavelength at a repetition rate of 14.6 MHz. The laser uses a simple, inexpensive design and can be scaled up to higher pulse energies.

The developed laser was used as a femtosecond excitation source for nonlinear multimodal microscopy. Laser operation at ~1 μm wavelength is particularly beneficial for high-resolution nonlinear imaging of biological samples. An example of the *in vivo* imaging of the structure of muscle cells from *Drosophila melanogaster* larvae with second harmonic generation microscopy was presented.

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