Energetic ultrafast fiber laser sources tunable in 1030-1215 nm for deep tissue multi-photon microscopy

WEI LIU,1,2,5 SHIH-HSUAN CHIA,1,2,5 HSIANG-YU CHUNG,1,2 RÜDIGER GREINERT,3 FRANZ X. KÄRTNER,1,2,4 AND GUOQING CHANG 1,4,*

1center for Free-Electron Laser Science, DESY, Notkestraße 85, 22607 Hamburg, Germany
2Physics Department, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany
3Skin Cancer Center Buxtehude, D-21614 Buxtehude, Germany
4The Hamburg Centre for Ultrafast Imaging, Luruper Chaussee 149, 22761 Hamburg, Germany
5These authors contributed equally to the paper
*guoqing.chang@desy.de

Abstract: We demonstrate an energy scalable approach to implement ultrafast fiber laser sources suitable for deep tissue multi-photon microscopy imaging. Enabled by fiber-optic nonlinearities (dominated by self-phase modulation), these unique ultrafast sources produce nearly transform-limited pulses of 50-90 fs in duration with the center wavelength tunable in the wavelength range of 1030-1215 nm. The resulting pulse energy can be scaled up to 20 nJ by optimizing fiber dispersion, shortening fiber length, and using large-mode-area fibers. We applied such an energetic source to a proof-of-principle study of \textit{ex vivo} human skin based on harmonics (i.e., second-harmonic generation and third-harmonic generation) imaging. This new type of fiber-format energetic ultrafast source provides a robust solution for multiphoton microscopy applications.

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References and links
1. Introduction

Using multiphoton signals (i.e., multiphoton fluorescence and optical harmonics) for microscopic imaging allows visualizing deep tissue image with submicron optical resolution, which offers new insights for the biomedical community [1, 2]. However, the capability of multiphoton microscopy (MPM) strongly depends on the availability of suitable pulsed laser sources to efficiently excite deep-tissue nonlinear signals before damage occurs. An ideal source should provide energetic (>10 nJ) femtosecond (~100 fs) pulses with their center wavelength located in the optical penetration window (e.g., 1100–1300 nm and near 1700 nm) of the tissue [2, 3]. Such a source benefits MPM twofold: (1) higher pulse-energy improves the generation efficiency of nonlinear signals, allowing the feasibility of higher-nonlinearity-order imaging modalities (e.g., third harmonic generation and three-photon excitation fluorescence) and (2) the optimized center wavelength of these femtosecond pulses mitigates tissue attenuation (i.e., scattering, absorption) and allows much deeper penetration.

Besides the requirement on pulse energy and center wavelength, development of robust hands-off sources is crucial for applying MPM outside research environments. For the last two decades, femtosecond solid-state bulk lasers (e.g., Ti:sapphire lasers Cr:forsterite lasers, and optical parametric oscillators) have become the standard MPM driving sources [4–6]. However, these lasers, which deliver energetic optical bursts in the optical penetration window, demand precise cavity-alignment and environmental control (i.e., water cooling, temperature and humidity...
feedback loop, vibration isolation etc.), which limits the use of MPM for deep tissue imaging to specialized laboratories.

Femtosecond fiber lasers can be constructed from all polarization-maintaining fibers without any free-space elements [7–9], undoubtedly offering robust solutions for deploying MPM systems in rugged environments especially for in vivo imaging and clinical applications. The most mature femtosecond fiber lasers developed to date are Yb-fiber lasers in 1020-1060 nm and Er-fiber lasers in 1520-1570 nm. To cover the optical penetration window in tissue, such a narrowband wavelength coverage can be substantially expanded using various fiber-optic nonlinear mechanisms. For example, output pulses from an ultrashort fiber laser can be continuously wavelength red-shifted using soliton self-frequency shift (SSFS) inside an optical fiber—a phenomenon that originates from stimulated Raman scattering [10]. SSFS requires negative group-velocity dispersion (GVD) and can be realized in standard single-mode fibers if the center wavelength of input pulses is larger than ~1300 nm—the typical zero-dispersion wavelength (ZDW) of the fundamental mode. Using SSFS inside a rod-type large-mode-area (LMA) photonic crystal fiber (PCF), femtosecond pulses generated by a high-energy Er-fiber laser at 1550 nm are red-shifted to 1675 nm with 67-nJ pulse energy [2, 3]. Despite the successful wavelength-shift to the optical penetration window of 1700 nm, ultrafast fiber-based femtosecond sources with >10-nJ pulse energy operating in 1100-1300 nm (another optical penetration window) remains particularly challenging [11]. This wavelength window plays a crucial role in MPM imaging because the generated two-/three-photon signals (from the most commonly used fluorophores or from optical harmonics) are located at the visible wavelength range corresponding to less bio-tissue absorption and optimal detection efficiency. Recently, this excitation window is of great importance for functional imaging using voltage sensitive dyes [12–14]. Furthermore, 1300-nm three-photon excitation enables functional imaging of GCaMP6s-labeled neurons beyond the depth limit of two-photon microscopy [15].

In order to achieve SSFS for the excitation wavelengths below 1300 nm, PCFs with a small (<3 μm) mode-field diameter (MFD) are usually employed to shift the ZDW below 1030 nm—the typical Yb-fiber laser wavelength [16, 17]. The associated strong nonlinearity in these PCFs limits the resulting wavelength-converted pulses to <1 nJ in pulse energy [18–20]. Higher-order-modes (HOMs) permitted by an optical fiber may exhibit both negative GVD in the wavelength range of 1000-1300 nm and much large MFD than the fundamental mode. These two unique features benefit SSFS pumped by Yb-fiber lasers and allow generation of 200-fs pulses tunable between 1064 and 1200 nm with 1-2 nJ pulse energy [21]. A recent experiment employed LP09 mode to perform SSFS and achieved ~50-fs pulses at 1317 nm with 30 nJ pulse energy [22]. However, the system requires a spatial light modulator to excite the LP0,9 mode and an axicon at the fiber output to convert the LP0,9 mode to a Gaussian-like beam profile. It is difficult to adapt such a system to an all fiber-format. Furthermore, the demonstrated source has a repetition rate of only 120 kHz, too low for practical MPM applications. Scaling to high repetition rate such as >10 MHz—which is necessary for video rate imaging—is challenging due to the limited average-power handling capability of spatial light modulators [23].

Recently we demonstrated a new method of producing high repetition-rate, widely wavelength-tunable femtosecond pulses [24]. The essence of this method is to minimize the dispersion effect such that the fiber-optic broadening of an input optical spectrum is dominated by self-phase modulation (SPM) plus self-steepening. Due to the minimal effect of dispersion, the resulting broadened spectrum features well isolated spectral lobes. Each of these spectral lobes can be selected by an optical bandpass filter, producing nearly transform-limited pulses. In Ref. 20, we employ PCFs with a MFD of 2.2 μm for spectral broadening and then use optical bandpass filters to select the leftmost or rightmost spectral lobes. Without external compression, the filtered spectral lobes correspond to ~100-fs (nearly transform-limited) pulses, tunable from 825 nm to 1210 nm with >1 nJ pulse energy [24]. More specific, we obtain ~3 nJ pulse energy
in the wavelength range of 1100-1215 nm at 55-MHz repetition rate. Although these results already represent an order of magnitude improvement in pulse energy for high repetition rate (>10 MHz) sources in comparison with SSFS-based soliton sources achieved using single mode fibers, higher pulse energies (>10 nJ) are demanded for practical MPM imaging systems. In this paper, we present another energy scaling method using optical fibers with larger MFD. Using large-mode-area (LMA) fibers (e.g., MFD of 7.5 μm) with proper dispersion for SPM-dominated spectral broadening allows us to scale up the pulse energy up to 20 nJ in the wavelength range of 1030-1215 nm at 55-MHz repetition rate. Another drawback of broadening optical spectrum using fibers with smaller MFD (MFD of 2.2 μm in our previous work) is that the power coupled into the fiber is environmentally sensitive, making the resulting tunable source unpractical for MPM applications. In this paper, we employ LMA fibers with about 10 times mode-area compared with the fibers in previous work; consequently the resulting widely tunable source exhibits excellent wavelength and power stability. To demonstrate its capability to drive MPM, we apply such a fiber-based ultrafast source to harmonic virtual biopsy of ex vivo human skin.

2. SPM-enabled, widely tunable ultrafast fiber laser source

Figure 1 depicts the schematic of our experimental setup. A home-built Yb-fiber oscillator produces 55-MHz ultrashort pulses centered at 1033 nm with 70-mW average power. These pulses were first stretched to ∼20 ps in a fiber-based stretcher [25], and then amplified to 10-W average power by two amplifiers. After an optical isolator, a grating-pair compressor de-chirped the amplified pulses to 190 fs in duration with 8-W average power. The fiber-based stretcher provides positive second-order dispersion and negative third-order dispersion, which are carefully managed such that the de-chirped pulses are nearly transform-limited. The compressed pulses were coupled into an optical fiber for SPM-enabled spectral broadening followed by optical bandpass filters to select the rightmost spectral lobe. A half-wave plate and a polarization beam splitter were used to adjust the power coupled into the nonlinear fiber. Finally, as a proof-of-principle, we employed the filtered femtosecond source to drive a video-rate laser scanning MPM microscope.

We tested three commercially available fibers: LMA-8, HI-1060, and LMA-5. As listed in Table 1, their MFDs are 7.5 μm (LMA-8), 5.8 μm (HI-1060), and 4.7 μm (LMA-5); the corresponding GVDs at 1030 nm are -21, -42 and -5 ps/nm/km, respectively. The strength of the fiber-optic nonlinearity can be quantified by the well-known nonlinear parameter γ, which
is defined as \( \gamma = \frac{2\pi n_2}{\lambda A_{eff}} \), where \( n_2 \) is the nonlinear-index coefficient of fused silica with a typical value of \( 2.3 \times 10^{-20} m^2/W \). \( \lambda \) denotes the wavelength and \( A_{eff} \) the mode-field area. The last column of Table 1 lists the calculated nonlinear parameter for these three optical fibers. Apparently fiber LMA-8 has the smallest nonlinear parameter (3.2 \( W^{-1} km^{-1} \)) due to the largest MFD and fiber LMA-5 has the largest nonlinear parameter (8.1 \( W^{-1} km^{-1} \)) due to the smallest MFD.

Table 1. Properties of optical fibers used for spectra broadening

<table>
<thead>
<tr>
<th>Type</th>
<th>( \lambda_{ZDW} ) nm</th>
<th>D@1030 nm ps/km nm</th>
<th>MFD@1030 nm ( \mu )m</th>
<th>( \gamma @ 1030 ) nm ( W^{-1} km^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMA-8</td>
<td>1160</td>
<td>-21</td>
<td>7.5</td>
<td>3.2</td>
</tr>
<tr>
<td>HI-1060</td>
<td>1310</td>
<td>-42</td>
<td>5.8</td>
<td>5.3</td>
</tr>
<tr>
<td>LMA-5</td>
<td>1050</td>
<td>-5</td>
<td>4.7</td>
<td>8.1</td>
</tr>
</tbody>
</table>

For a fair comparison of their energy scalability, all three fibers under test are 70 mm long. We varied the coupled power into each fiber such that the rightmost spectral lobe peaks at 1100 nm. Figures 2(a-c) show the entire broadened spectra from these three fibers. Also labeled in these figures are the coupled average power: 1.46 W for LMA-8, 1.36 W for HI-1060, and 0.4 W for LMA-5. Apparently, fibers with smaller nonlinear parameter require more coupled power to generate the rightmost spectral lobe peaking at 1100 nm. It is noteworthy that fiber dispersion plays an important role as well during the nonlinear spectral broadening. For instance, larger dispersion in fiber HI-1060 tends to stretch the input pulse more rapidly, leading to a decreased pulse peak power; consequently the overall accumulated nonlinearity is compromised. This explains why the required power for fiber LMA-8 is only slightly more than that for fiber HI-1060 (1.46 W versus 1.36 W) despite a large difference between their nonlinear parameters (3.2 \( W^{-1} km^{-1} \) versus 5.3 \( W^{-1} km^{-1} \)).

We then used a 50-nm optical bandpass filter centered at 1100 nm to filter the rightmost...
spectral lobes of these spectra and recorded them in Fig. 2(d). The filtered spectrum generated from fiber LMA-8 corresponds to the highest average power of 340 mW (6.2-nJ pulse energy), much higher than the average power resulting from the other two fibers: 285 mW for HI-1060 and 113 mW for LMA-5. The power conversion efficiency is 23.3%, 21.0%, and 28.3% for fiber LMA-8, HI-1060, and LMA-5, respectively. Furthermore, a larger MFD allows fiber LMA-8 to handle higher coupled power than the other two fibers before surface damage takes place. Therefore fiber LMA-8 showed an overall better scaling performance.

To demonstrate the wavelength tunability, we varied the coupled average power into 70-mm fiber LMA-8; the resulting spectral evolution versus coupled average power is shown in Fig. 3(a). The optical spectrum becomes broader with more coupled power into the fiber. At 4-W coupled power, the broadened spectrum spans from 850 nm to 1250 nm, and the resulting rightmost spectral lobe red-shifts from 1050 nm to 1215 nm. Another intriguing feature is that the broadened spectra exhibit clear distinct spectral lobes at the long wavelength side. These side lobes become well separated when they are in the 1100-1200 nm wavelength range. This phenomenon is caused by the fiber dispersion. Fiber LMA-8 has the ZDW at 1160 nm and

Fig. 3. SPM-enabled femtosecond sources using 70-mm LMA-8. (a) Spectral evolution versus coupled power. (b) Filtered rightmost spectral lobes for different coupled power. (c-e) Measured autocorrelation traces (red curves) of the filtered lobes at 1080 nm (c), 1140 nm (e), and 1215 nm (d). Calculated autocorrelation traces of transform-limited pulses allowed by the filtered spectra are shown as black dotted curves.
exhibits relatively small GVD in the wavelength range of 1100-1200 nm. Therefore the spectral broadening in this range is dominated by SPM, leading to the characteristic spectral lobes. We then used a set of optical bandpass filters to filter the rightmost spectral lobes and recorded seven typical filtered spectra in Fig. 3(b), their center wavelength varying from 1080-1215 nm. Figures 3(c-e) show the measured autocorrelation traces (red curves) for the filtered spectra at 1080 nm, 1140 nm, and 1215 nm. We also plot in the same figure the calculated autocorrelation traces (black dotted curves) of the transform-limited pulses corresponding to the filtered spectra. Clearly these pulses are nearly transform-limited; the full-width-half-maximum duration of their autocorrelation traces is in the range of 70-125 fs. The corresponding pulse duration is estimated to be 50-90 fs, assuming a deconvolution factor of 1.4.

In addition to managing nonlinearity using fibers with different MFDs, fiber length is another degree of freedom. We coupled 3-W average power into fiber LMA-8 at different length: 20 mm, 30 mm, 50 mm, and 70 mm. As Fig. 4(a) shows, the rightmost spectral lobe red-shifts with the increased fiber length and peaks at 1080 nm (1170 nm) for 20-mm (70-mm) LMA-8. Apparently much more power needs to be coupled into the 20-mm fiber LMA-8 such that the resulting rightmost spectral lobe will be shifted to 1170 nm; consequently the filtered spectrum at 1170 nm from 20-mm LMA-8 should result in much higher pulse energy. This constitutes another powerful energy-scaling means–using shorter fibers for SPM-enabled spectral broadening. We varied the coupled power into these fibers to generate the filtered spectra at different wavelengths. Figure 4(b) summarizes the pulse energies corresponding to the filtered spectra peaking at different wavelength for the different fiber length. As expected, the filtered spectra from shorter fibers exhibit higher pulse energies for the same wavelength shift. For example, the filtered spectrum at 1100 nm has pulse energy of 6.5 nJ, 8.5 nJ, 14.5 nJ, and 22 nJ for fiber length of 70 mm, 50 mm, 30 mm, and 20 mm, respectively. However, due to the available power from our fiber laser system, using shorter fibers reduces the wavelength-shift range; that is, the farthest reaching wavelengths are 1215 nm, 1200 nm, 1140 nm, and 1110 nm for the fiber length at 70 mm, 50 mm, 30 mm, and 20 mm, respectively. Using a more powerful fiber laser source can increase the farthest reaching wavelength. Numerical simulation shows that for 11-W average power (200-nJ pulse energy) coupled into 20-mm LMA-8, the rightmost spectral lobe is shifted to 1200 nm and the filtered spectrum exhibits >40-nJ pulse energy with >2.2-W average output power.

It is noteworthy that the ZDW of an optical fiber plays an important role to determine the
amount of wavelength shift. For fiber LMA-8, as the rightmost spectral lobe shifts beyond 1160 nm, it experiences negative GVD. The resulting soliton formation and fission starts to distort the spectral lobe, and the corresponding pulse after filtering this spectral lobe may be much longer than the transform-limited pulse. As a result, the rightmost spectral lobe generated by fiber LMA-8 is limited to 1215 nm. Since fiber HI-1060 has its ZDW at 1310 nm, it should be able to generate the rightmost spectral lobe beyond 1300 nm with enough input pulse energy. This speculation is confirmed by simulating a 250-nJ, 190-fs pulse propagating inside 2-cm long HI-1060. Figure 5(a) plots the spectrum evolution versus the fiber length. Clearly the rightmost spectral lobe is well isolated and continuously red-shifts along the fiber. At the output of the 2-cm fiber, the filtered rightmost spectral lobe–shown as the inset of Fig. 5(b)–peaks at 1310 nm with 37 nJ energy. The blue curve in Fig. 5(b) shows the corresponding pulse, which has 60-fs duration with >500 kW peak power. Also plotted in the same figure as the red curve is the transform-limited pulse given by the filtered spectrum, showing that the filtered pulse is indeed nearly transform-limited.

Fig. 5. Propagation of a 250-nJ, 190-fs pulse inside fiber HI-1060. (a) Spectrum evolution versus fiber length. The rightmost spectral lobe of the optical spectrum after propagating 2 cm is filtered. The corresponding optical pulse (blue curve) and the calculated transform-limited pulse (red curve) from the filtered spectra are shown in (b). Inset of (b): the filtered rightmost spectral lobe.

3. Harmonic generation tomography of human skin

To show the capability of our SPM-enabled source for driving MPM, we employed it to obtain harmonic generation tomography of human skin. Complementary to multi-photon fluorescence, the coherent nature of optical harmonics offers additional insights to visualize the tissue morphology as bio-photonic crystals [26]. Free from photodamage, phototoxicity, or photobleaching, second-harmonic generation (SHG) and third-harmonic generation (THG) images reveal different optical structures and provide complementary sub-μm information of human skin [27]. With THG microscopy, the cellular morphology of human skin can be distinguished in epidermis, while the THG contrasts can also be found in dermis to show fibroblasts, erythrocytes, collagen fiber bundles, and elastin fibers [28]. On the other hand, collagen-sensitive SHG reveals the diverse collagenous structures in dermis. Figure 6 shows the combined SHG/THG images (SHG in green and THG in magenta) of ex vivo human skin from stratum corneum to the reticular dermis. We employed fiber LMA-8 to generate the filtered spectral lobe at 1100 nm. At the 55-MHz pulse repetition rate, the illumination pulse energy after the objective lens (N40XLWD-NIR, Nikon) is controlled to be 1.5 nJ to
Fig. 6. Horizontal-sectioned epi-SHG/THG images of ex vivo human skin driven by the filtered source at 1100 nm. (a-d) Cell morphology from the stratum corneum to the stratum basale in epidermis from THG contrast. Combined with the epi-SHG modality, the collagen fibers in the dermal papilla [arrowhead in (d)] is revealed. (e-h) Depth-dependent collagenous distribution in the dermis observed through epi-SHG. Magenta: THG, Green: SHG. Scale bar: 50 μm.

avoid sample damage. The excited SHG and THG signals were epi-collected by the same objective lens, separated by a dichroic mirror (DMLP505R, Thorlabs), and then detected by two photomultiplier tubes. The cell morphology in epidermis (Figure 6(a-d)) and the collagen distribution in dermis (Figure 6(e-h)) can be clearly distinguished in the 10-frame-averaged video-rate (30 Hz) images. >220 μm of imaging penetration depth from the top of the stratum corneum can be obtained. Due to the energy scalability and wavelength tunability of the demonstrated fiber-source, deeper penetration depth is possible by increasing the pulse energy and further optimizing the excitation wavelength.

4. Conclusion

To conclude, we demonstrated an energetic ultrafast fiber laser source tunable between 1030-1215 nm, capable of driving SHG/THG imaging of human skin. More pulse energy can be achieved by further shortening the fiber and increasing the coupled power. Broader wavelength coverage (e.g., 1030-1300 nm) is also possible using a fiber (e.g., fiber HI-1060) with its ZDW around 1300 nm, which is of great importance for not only harmonic generation tomography but also deep tissue functional imaging [15]. We applied such a high-energy source to a proof-of-principle study of ex vivo human skin THG/SHG images. The horizontal histology section from stratum corneum to the reticular dermis can be obtained with >220 μm penetration depth. Furthermore, applying our method to Er-fiber laser technology will result in wavelength-tunable femtosecond sources accessing both the 1300 nm and 1700 nm window. Since all the free-space components in our source can be replaced by fiber or fiber-pigtailed devices, sub-100-nJ-level femtosecond sources in an all-fiber format are possible. We believe that such SPM-enabled femtosecond fiber-laser sources will provide a robust solution for MPM applications in rugged environments (e.g., clinical applications), as well as various other applications such as sensitive mid-infrared spectroscopy via difference frequency generation.
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