Development of an applicator for multiphoton PDT

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ABSTRACT

Multiphoton excitation of photosensitizers for laser induced fluorescence diagnosis (LIFD) and photodynamic therapy (PDT) of tumors has the advantage of greater tissue penetration due to the longer wavelength of irradiation. However, multiphoton LIFD and PDT are presently not clinically applicable as there are no applicators available for the delivery of the pulsed laser radiation to the operating room. As an approach, in this contribution the beam delivery through photonic crystal fibers has been investigated. Pulses of a Ti:sapphire laser of 100 fs pulse duration and an average power of 150 mW have been transported through such a fiber of 25 m length and the resulting pulses show the absence of nonlinear contributions but still a broadening of the pulse to 2 ps due to the dispersion of the fiber. It is planned to compensate this broadening by a grating in front of the fiber. Alternatively, the transport of laser radiation of 150 fs and 100 mW through a mirror-joint-arm used for conventional CO_2 lasers has been tested showing no broadening of the laser pulses. Two-photon photodynamic activity of mTHPC-CMPEG$_4$ shall serve as a test of the laser light transport system.

Keywords: photodynamic therapy, multiphoton excitation, photonic crystal fiber

1. INTRODUCTION

In laser-induced fluorescence diagnosis (LIFD) and photodynamic therapy (PDT) of malignant tumors the activation of the photosensitizers (PS) needs not necessarily be achieved by single-photon (SP-) absorption. When irradiated with short (fs) laser pulses of approximately twice the wavelength of the single-photon absorption the PS can also be activated if the photon density is high enough to allow two-photon absorption[1]. Due to the wavelength used (700-1100 nm) and the sufficiently low applied average power the penetration depth of the light in the tissue is much higher, unwanted photobleaching and tissue damage due to unspecific absorption are reduced while the desired effect, the reduction of cell vitality, still occurs. Unfortunately multiphoton (MP-) LIFD and PDT are not clinically applicable at present, since there are no applicators available which do not broaden the pulse shape via dispersion and therefore decrease the available peak intensity.

In the recent years, laser technology has greatly advanced, significantly enhancing the availability of robust femtosecond laser sources. One of the work horses is the Ti:sapphire laser, with commercially available average power levels of more than one Watt, pulse durations of about 100 fs, pulse energies of about 10 nJ, and pulse repetition rates of 100 MHz. This translates into pulse peak powers of 100 kW.

Despite the robustness of modern mode-locked lasers, they often cannot be directly tolerated in a clinical environment. Also, these lasers are still very large and cannot be miniaturized to fit into the hand of a surgeon. All these aspects call for a beam delivery system. For continuously operated lasers, a multitude of different fiber types is available for that purpose. However, with the extremely high peak powers of femtosecond lasers, dispersive and nonlinear optical effects forbid the use of a simple fiber for beam delivery over more than a few centimeters, which practically renders this approach useless for femtosecond lasers.

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Group delay dispersion is one of the effects, which causes temporal broadening of the laser pulse in an optical fiber. The effect of dispersion can be estimated according to

$$\tau(z) \approx \tau_0 \sqrt{1+\left(\frac{\beta_2 z}{\tau_0^{\;2}}\right)^2} \; , \qquad (1)$$

where τ_0 is the initial pulse duration and $\tau(z)$ the duration after propagation through a length z of fiber[2]. Assuming β_2=40fs^2/mm (a typical value for 800 nm wavelength), we find that that a pulse with an initial width τ_0=100 fs is already stretched to double its original length after propagating through some 40 cm of fiber. After 10m of propagation, the pulse is stretched to several ps. This effect may already appear serious, but can be easily compensated to some extent by dispersion compensation, e.g. by grating or prism pairs. However, even more seriously, nonlinear optical effects, namely self-phase modulation, set in. In contrast to dispersion, these effects cannot easily be compensated for. For an estimation on the severity of this effect, we calculate the nonlinear length

$$L_{\mathrm{NL}} = \frac{A_{\mathrm{eff}}\,\lambda}{2\pi n_2 P_0} \; , \qquad (2)$$

i.e., the propagation length after which a serious deformation of the pulse shape sets in[2]. Here A_{eff} is the effective core area of the fiber (a few 10 µm^2 for standard single mode-fibers), n_2 is the nonlinear coefficient of the glass ($n_2 \approx 3.2\times10^{-16}$ cm^2/W), λ is the wavelength, and P_0 is the peak power of the pulse. Even using a rather optimistic value of 100 µm^2 for the mode area, which can only be attained in specially designed large-mode-area fibers, we compute a nonlinear length of only 4 mm at a peak power P_0=10 kW. This estimation readily identifies self-phase modulation as the more serious problem. Its avoidance would require a power reduction by at least two orders of magnitude, corresponding to a few 100 W peak power or continuous power levels of single milliwatts. Using standard single mode fibers at 800 nm, power levels would have to be reduced by another order of magnitude to avoid catastrophic effects on the pulse shape. We have performed numerical simulations based on the split-step Fourier method, which support these conclusions, indicating a fragmented pulse shape of more than 100 ps length if the full power of a mode-locked Ti:sapphire laser is launched into standard single mode fiber.

If one wants to stay with the general concept of fiber-based laser power delivery, there are two fundamental ways to lessen or circumvent the nonlinear limitation. The most established approach is a concept similar to chirped-pulse amplification (CPA[3]). Here the pulse is stretched in time by a grating sequence, reducing the peak power accordingly. The pulse can then be launched into the fiber without irreversible effects on the pulse structure. After propagation, the pulse is recompressed to close to its original duration by a second grating pair with exactly opposite dispersion of the stretcher. From the considerations above, one immediately sees that the pulse needs to be stretched approximately by a factor of 1000, i.e. to a pulse duration of 100 ps. This is feasible, in principle, but already requires relatively large gratings. Additionally, such an approach decreases the overall throughput of the beam delivery system, as both the stretcher and the compressor cause a 50% loss. Together with typical fiber losses (mainly caused upon launching), one has to expect a maximum transmission of such a CPA-based system of 10%.

A possible approach is the use of photonic crystal fibers for waveguiding[4]. Such an applicator can be a valuable tool for LIFD and PDT, especially when combined with a suitable scanning unit. The pulsed laser for MP-excitation shall provide radiation to several workplaces in neighboring laboratories and in the operating room. As the laser system is neither transportable nor suitable for use in the operating room, a system for the transport of the radiation has to be realized which can transport the pulses over a distance of 20 m without changing the quality of the laser pulse severely. Two-photon photodynamic activity of mTHPC-CMPEG$_4$ upon excitation with 150 fs pulses at 780 nm wavelength shall serve as a test of the laser light transport system.

2. MATERIALS AND METHODS

A cell-biological workplace is realized on the basis of an inverted microscope (Axiovert 100, Carl Zeiss). With a spatially resolved spectrometer (Spectra Cube, Applied Spectral Imaging) a complete spectrum (spectral range 400-1000 nm, resolution 6 nm) can be recorded for each pixel (800x600 pixels).

As pulsed laser for MP-excitation a Ti:sapphire laser (Tsunami, Spectra Physics; pulse duration 100-200 fs, repetition rate 82 MHz, average power 0,5-0,75 W, wavelength range 780-820 nm) has been used. For the determination of the pulse duration an autocorrelator (409, Spectra Physics) has been used. The pulse spectrum has been recorded with a fiber optic spectrometer (S2000, Ocean Optics).

A laser light transport system on the basis of a photonic crystal fiber (Blaze Photonics) and motorized x,y,z-optic-coupler (Thorlabs) has been realized. Alternatively the laser pulses can be transported by a mirror-joint-arm conventionally used for CO_2 lasers. This mirror-joint-arm consists of 3 metal tubes (total length 1,0 m) with reflection prisms at the joints of the tubes deflecting the laser beam into the direction of the tube.

mTHPC-CMPEG$_4$ has been obtained from the German Cancer Research Center, Heidelberg (Dr. H.-J. Sinn). Phosphor-buffered Saline PBS, without Ca and Mg (Gibco BRL), nutrition solution HAM F12 (20 mM HEPES, pH 7.4) with (during growth) and without (for sensitizer uptake) foetal bovine serum (Gibco BRL) have been used. For the determination of the photoactivity of the PS the light induced ability of mTHPC-CMPEG$_4$ to restrict cell vitality has been tested in human colon carcinoma cells (HCT-116 multi-drug-resistant tumor cell line). After a cell monolayer had formed in the cell culture flasks the cells were incubated with 2 µg/ml of mTHPC in HAM F12 without serum for 24 hours. The cells were washed once with 50 ml of PBS and the cell culture flasks were filled with 50 ml of PBS in order to prevent dehydration of the cells during laser irradiation. The cells were irradiated with the pulsed Ti:sapphire laser (150 fs pulses, 780 nm, 600 mW average power focused with a lens with 60 mm focal length, for 300 s). Subsequently the cell culture flasks were emptied, refilled with nutrition medium and kept at 37 °C for 24 h. The effect of the photodynamic treatment on the cell vitality was documented by pictures of the cells before and after (24 h) the irradiation, taken with the Spectra Cube system.

3. RESULTS AND DISCUSSION

3.1 High power beam delivery system for femtosecond laser pulses

We investigated a scheme based on a newly available photonic crystal fiber[5]. This scheme is depicted in Fig. 1. Other than conventional fibers that guide light due to total internal reflection, photonic crystal fibers guide light within an air core, which by itself does neither exhibit a substantial amount of dispersion nor any nonlinearity. Guiding is achieved due to Bragg reflection in a cellular glass structure surrounding the hollow core. Compared to total internal reflection, this guiding mechanism is not totally lossless, but damping coefficients on the order of 0.15 dB/m have been reported, which add up to a total loss of 50% over a propagation length of 20m. Additionally, the bandwidth of photonic crystal fibers is smaller than in conventional fiber geometries. Typical values of 10% of the carrier wavelength have

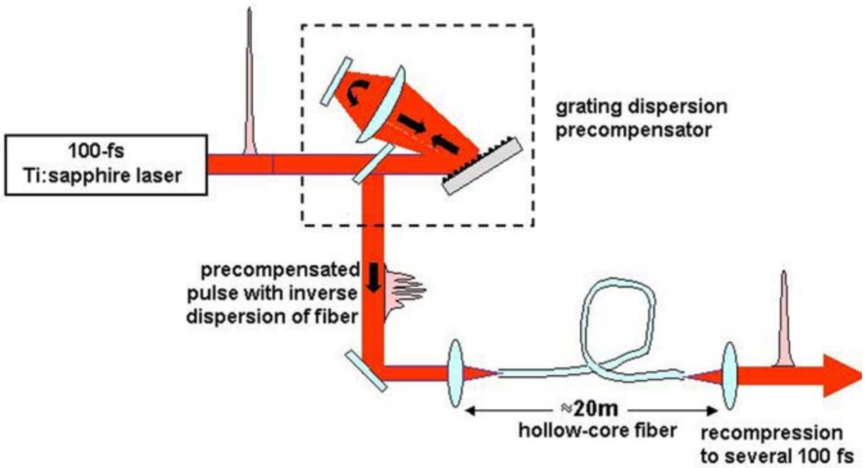

Figure 1: Schematic set-up of the proposed femtosecond laser delivery system. The system consists of a grating stretcher, which precompensates the dispersion of the fiber. Nonlinear effects are avoided by using a photonic crystal fiber, which guides the light in a hollow core.

been achieved, which translates into a useful bandwidth of about 80 nm in the Ti:sapphire wavelength range. As the mode field extends into the outer cellular structure, these fibers exhibit only waveguide contributions to their dispersion.

This dispersion is mainly third-order, with the zero-dispersion wavelength located close to the short-wavelength cutoff of the transmission band (see Figure 2).

As a first test, we launched ≈150 mW of a 100-fs Ti:sapphire laser into 25m of such a fiber[6] and found the absence of nonlinear contributions confirmed. However, caused by the relatively strong third-order dispersion of these fibers, the pulses still get broadened to a duration of about 2 picoseconds, as shown in Fig. 3. This agrees roughly with the net effect the dispersion of a conventional fiber would have on the pulse. For the future, we plan to compensate these effects by a suitably designed grating sequence to be placed between laser source and fiber input coupling. As dispersion of the fiber is mostly negative, this grating sequence would formally equal a standard stretcher, prestretching

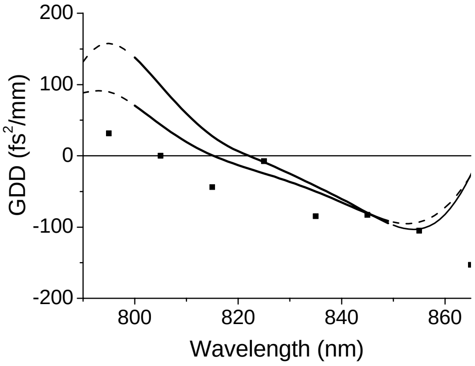

Figure 2: Measured dispersion of the hollow core fiber[6]. Shown is data over the high-transmission band (loss<0.3 dB/m). Lines indicate own measurements, dots manufacturer data. Solid lines indicate regions, where a reliable measurement of dispersion was possible. Note the slight difference between the two measurements on the same fiber sample. Minimum transmission losses are achieved in the vicinity of 840 nm.

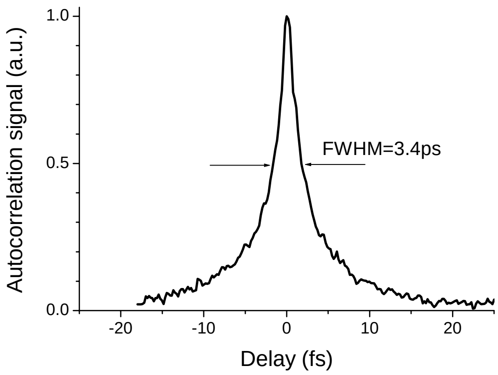

Figure 3: Measured autocorrelation of a 100-fs Ti:sapphire laser pulse after propagation through 25m of HC-800-1 hollow core fiber. The laser was tuned to a center wavelength of 817 nm. The measured autocorrelation width of 3.4 ps corresponds to a pulse duration of about 2.2 ps.

the pulse before fiber propagation, cf. Fig. 1. The fiber itself would then recompress the pulse close to its original pulse duration. Recompression to less than half a picosecond requires a careful balance of second and third order dispersion. It also requires adjustment of the grating sequence if major tuning of the wavelength is required. In contrast to all other solutions discussed so far, however, use of a photonic crystal fiber is still the most promising way of combining a relatively high throughput and negligible pulse distortions.

As an alternative we tested a light transport system on the basis of the mirror-joint-arm. The main requirement of the laser light transport system is the retention of the pulse structure and the transport of the laser radiation as free of dispersion as possible. Therefore it has been tested if the pulsed laser radiation exhibits a change of the spectrum after passing through the mirror-joint-arm. With the autocorrelator it has been established that the Ti:apphire laser is in pulse operation and the intensity and pulse spectrum before and after passing through the mirror-joint-arm have been measured. Figure 4 shows the pulse spectrum before and after passing through the mirror-joint-arm. The FWHM's determined from the recorded spectra are FWHM (before) = 6,6±0,4 nm and FWHM (after) = 7,3±0,4 nm.

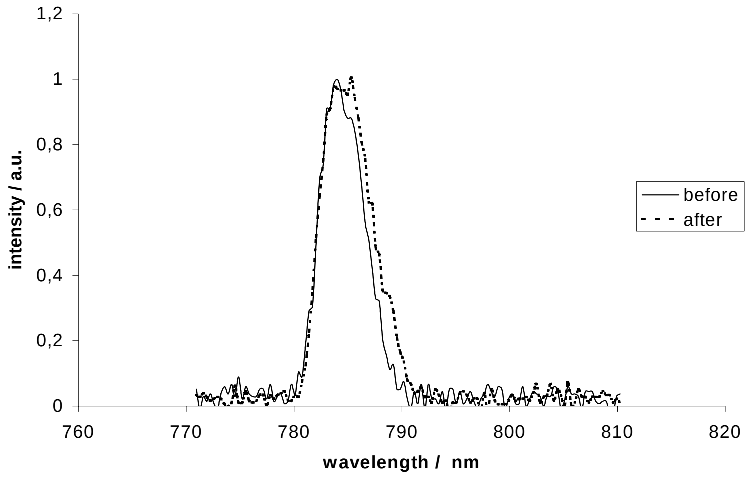

Figure 4: Pulse spectrum before and after passing through the mirror-joint-arm

By adjusting the prism assembly of the Ti:sapphire laser the FWHM of the spectrum of the laser pulses has been varied and the resulting pulse width determined from the autocorrelation signal. The dependence of the pulse width on the FWHM of the spectrum has been depicted in Figure 5. It can be concluded that the pulse duration corresponding to the FWHM of 7 nm before and after passing through the mirror-joint-arm amounts approximately to 150 fs. The pulse duration is not changed measurably by the mirror-joint-arm.

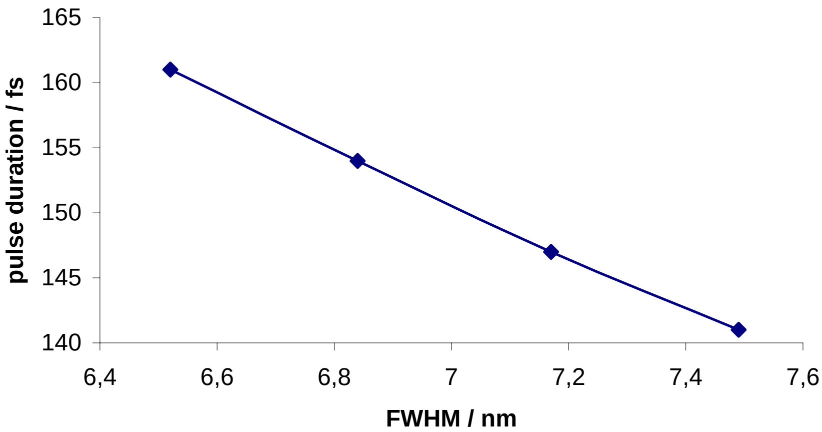

Figure 5: Pulse duration in dependence of the FWHM of the pulse spectrum of Ti:sapphire laser

3.2 Two-photon induced photodynamic activity

To verify that a laser illumination with focused fs pulses does not induce any damage to the cells by itself untreated cells were irradiated according to the same data as used for the photodynamic therapy. Fig. 6 shows that the laser irradiation itself does not reduce the cell vitality.

Cells were treated with the sensitizer as described above. Subsequent laser irradiation that induces two-photon excitation (150 fs pulses, 780 nm, 300 s, average power 600 mW focused with a lens with 60 mm focal length) leads to a drastic reduction of the cell vitality, as shown in Fig. 7.

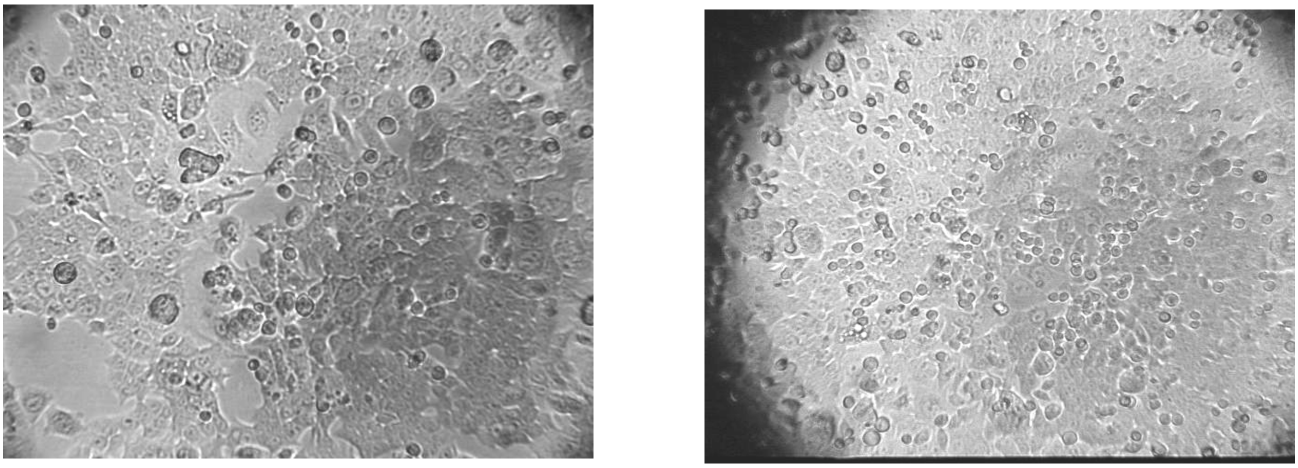

Figure 6: Untreated HCT-116 cells before (left) and 24 h after (right) laser irradiation using same data as for the sensitizer-treated cells (150 fs pulses, 780 nm, 300 s, average power 600 mW focused with a lens with 60 mm focal length). The laser treatment does not induce any noticeable restriction of the cell vitality as the cell monolayer stays intact.

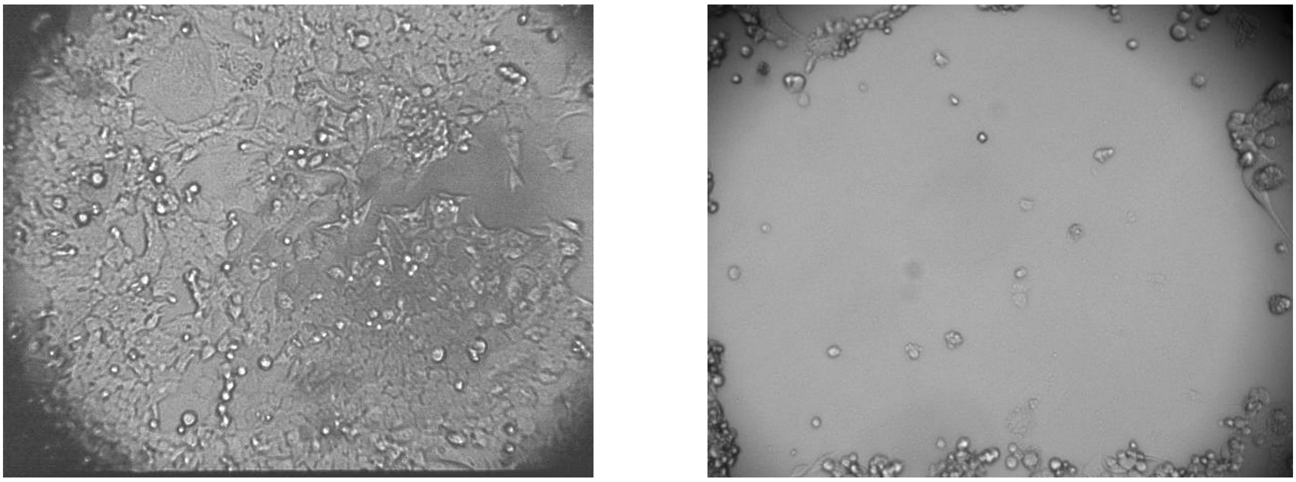

Figure 7: mTHPC-CMPEG$_4$ treated HCT-116 cells before (left) and after (right) laser irradiation (150 fs pulses, 780 nm, 300 s, average power 600 mW focused with a lens with 60 mm focal length). The two-photon excitation of the sensitizer obviously induces a strong photodynamic effect, as the cell monolayer is destructed.

4. CONCLUSIONS

The transport of fs laser pulses (100 fs) through a photonic crystal fiber (25 m length) shows a broadening of the pulse to 2 ps and the absence of nonlinear contributions. As a next step prestretching of the pulse before fiber propagation shall be tested. Alternatively, laser pulses can be propagated through a mirror-joint-arm of 1m length without measurable broadening. However, we are in favor of a light transport system based on a photonic crystal fiber as the propagation of pulses through a mirror-joint-arm with a length of 20 m seems unrealistic due to the lack of an arm of such a length and possible high manufacturing costs. Such a system could be used for a short propagation length e. g. for the delivery of pulses in the operating room. Two-photon induced photodynamic activity of mTHPC-CMPEG$_4$ shall serve as a test of the pulses delivered by the applicators.

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