

Two-photon cross-sections of the photosensitizers *m*-THPC and *m*-THPP in the 1.05-1.45 μm range

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Abstract

The two-photon absorption of the photosensitizers 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin and corresponding chlorin has been studied in the near infrared (IR) range using open-aperture z-scan technique. We found cross sections of 10-40 GM in the Q-band region, comparable to values in the Soret band. Both photosensitizers are promising candidates for IR two-photon photodynamic therapy given the better penetration of the IR light into tissue.

Keywords: two-photon absorption, z-scan, tumor photosensitizer, near IR, cross section.

Introduction

Two-photon absorption has attracted considerable interest in optics, biological and medical investigations where technologies, such as two-photon imaging microscopy [1], optical limiting [2], activating photosensitizers in photodynamic therapy (PDT) [3-5] and others, are used.

In particular, PDT with two-photon excitation has an advantage due to the relatively longer laser wavelength employed resulting in a deeper penetration of laser light into tissue. Compounds of the tetrapyrrole type have found considerable interest in this respect as they already play a strong role as photosensitizers in PDT [6] and due to the fact that some of them exhibit high two-photon absorption cross-sections [7]. Here we present a comparative investigation of the two-photon excitation of two structurally closely related photosensitizers of the porphyrin and the chlorin type: 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP) and 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, Temoporfin). Investigations of the two-photon excitation of the photosensitizer *m*-THPC at about 800 nm have already been reported in Ref. 5,8.

We measured the cross sections of two-photon absorption (TPA) of *m*-THPC and *m*-THPP [9-11] in the spectral range of 1.05-1.45 μm , where the penetration depth is even better than at 800 nm. To measure absolute TPA cross sections we use the established open aperture z-scan technique [12-14].

Experimental methods and samples

In our study, we used a home-built femtosecond optical parametric amplifier (OPA) as the source of ultra-short spectrally tunable pulses. The OPA is pumped by a mode-locked Ti:sapphire laser (CPA-2001, Clark-MXP, Inc.) delivering ~ 150 fs pulses of 0,5 mJ energy at a repetition rate of 1 kHz. The tuning range of the OPA is from 1.05 to > 1.45 μm , pulse energy of 5-30 μJ , full-width at half-maximum (FWHM) pulse-length of 100-125 fs assuming a gaussian pulse-shape. The pulse-length is measured with an autocorrelator of APE GmbH.

The experimental scheme is shown in Fig. 1. The pump beam is focused into a sample with a 75-cm or 40-cm focal length lens (L1). The transmitted energy through the sample is measured with the photodiode D1. The reference photodiode D2 is used to diminish the influence of laser fluctuations by taking the ratio of the signals of photodiodes D1 and D2. A pump beam waist w_0 measured with a scanning knife-edge method varied in the range of 0.3 to 0.4 mm, which corresponds to a Rayleigh parameter of $z_R = \pi w_0^2 / \lambda = 30-40$ mm at wavelengths λ of 1.05-1.45 μm . These measurements and the fitting of the z-scan curves show that the intensity-profile of the beam is rather well described as gaussian.

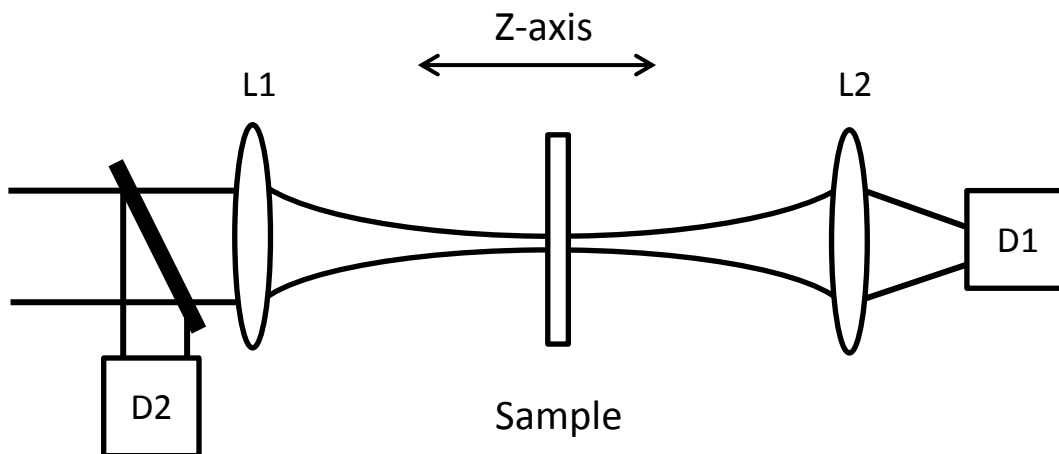


Fig.1

The sample thickness is 5 mm, which is shorter than the Rayleigh parameter. In this case, we can with good accuracy take intensity to be approximately the same along the sample cell. To achieve accuracy in fitting z-scans, the analytical expressions describing the

transmission of gaussian laser pulses through a two-photon absorbing medium, which are valid for arbitrary absorber thickness [14] are used.

As there are some differences in definition of the TPA cross section [15], we first present the definitions and approximations for the quantitative estimations we use. The TPA in a sample is described by the following differential equation:

$$\frac{dI}{dz} = -\beta I^2 . \quad (1)$$

Here the two-photon absorption coefficient β is related to the cross section σ_2 as $\sigma_2 = h\nu\beta/N$, where $h\nu$ is the exciting photon energy and N is the concentration of the TPA molecules in solution and I is the pump intensity[14,16,17]. For weak absorption and a sample thin in comparison to z_R , the solution of the Eq. (1) can be expanded in a series taking only a few terms. The actual quantity of interest in the open-aperture z-scan experiment is the average transmitted power as a function of the sample position. For this one can integrate over the transverse coordinates to obtain the instantaneous transmitted power and then integrate over the duration of the pulse. The transmitted power in these approximations at sample-position z is given by

$$T(z) = 1 - \beta I_0 d/2 \left(1 + (z/z_R)^2 \right) , \quad (2)$$

where I_0 is the incident laser intensity on the Gaussian beam-axis at the waist and d is the sample thickness. From the fit of the $T(z)$ curve the nonlinear absorption coefficient β and the cross section σ_2 is obtained.

The samples under investigation were DMSO (dimethyl sulfoxide) solutions of *m*-THPC and *m*-THPP at typical concentrations of 4-6 mM/l. *m*-THPC was obtained from biolitec research GmbH, and *m*-THPP was prepared according to the literature [18]. The photosensitizers *m*-THPC and *m*-THPP both are tetrapyrrole-based photosensitizers. In *m*-THPC one of the pseudoolefinic β,β' -double bonds in porphyrins has been removed compared to *m*-THPP (see in Fig.2) resulting in a chlorin-type tetrapyrrole. It is a change reducing the symmetry of the molecule and imparting distinct changes to its optical absorption properties [9,19]. *m*-THPP has recently found attention as the photoactive compound in nanoparticle-based photosensitizer formulations [20]. *m*-THPC (Temoporfin) is in established clinical use as a photosensitizer: *m*-THPC is the active pharmaceutical ingredient in the medicinal product Foscan which is authorized in the European Union for the palliative treatment of head and neck cancer [21]. The treatment of cancer by PDT, that is, the destruction of the cancer tissue, depends on the presence of molecular oxygen and is in essence a photodynamic effect. A decisive role in such photoreactions is played by the efficiency of populating the excited

triplet state of the photosensitizer. For this reason it is of interest to study the excitation of lowest singlet states of the photosensitizers, which could have a coupling to a triplet state.

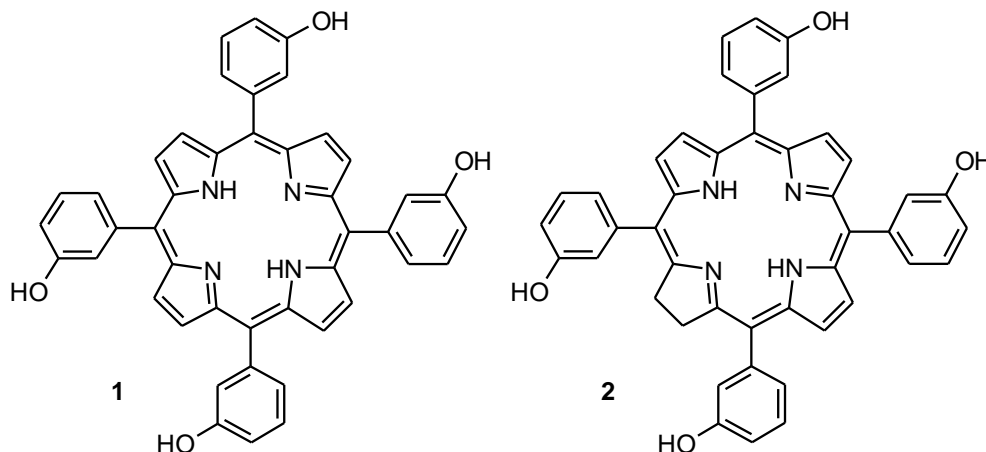


Fig.2

The photophysical and some TPA properties and the photodynamic activity of *m*-THPC are described elsewhere [5,8-9]. Studies on *m*-THPP have also been reported in Ref. 9-11.

Results and discussion

To confirm that the change in the transmission is due to the TPA, the power dependence of the change was measured regularly. There are a number of nonlinear effects, as, for example, Kerr-lensing and thermal lensing, which can affect the measurements. They influence the collection of the transmitted signal on the detector and can be interpreted as a change in the transmitted power. Typically, dependence of the absorption on the power was close to quadratic if the relative nonlinear absorption does not exceed 10-15%. For higher absorptions, the dependence is not quadratic and the beginning of saturation is seen.

The setup was tested by measuring the TPA cross section of the well studied dye Rhodamine 6G (Rh6G) dissolved in methanol at 795 nm. We determined the value of the cross section to be 26 ± 5 GM for Rh6G in a methanol solution. Here 1 GM is equal to 10^{-50} cm⁴/photon/molecule. It agrees with the value measured in [22]. A detailed comparison of Rh6G TPA cross section at 798-806 nm has been reported in Ref.15. It varies from 12.8 to 134 GM.

Our measurements of TPA cross sections at 795 nm for *m*-THPC and *m*-THPP dissolved in DMSO give the magnitudes of 28 ± 8 GM and 56 ± 15 GM, respectively. The value

reported in the literature [5] is $\sigma_2 = 18 \text{ GM}$ at 800 nm for *m*-THPC dissolved in 20% ethanol, 30% polyethylene glycol and 50% distilled water.

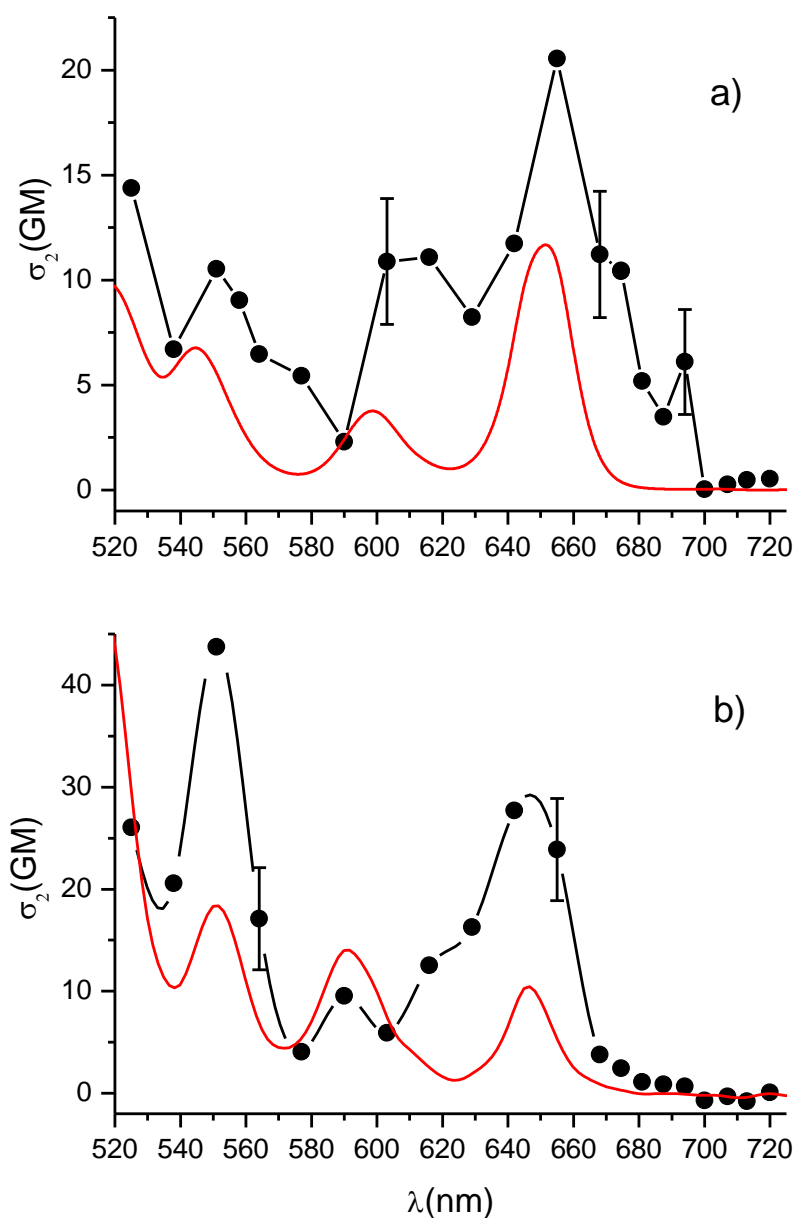


Fig.3

The two- and one-photon absorption spectra of *m*-THPC and *m*-THPP are presented in Fig.3. The TPA spectra are plotted against half the excitation wavelength value to allow comparison to the one-photon absorption spectra. It can be seen that the transition structure for the one- and two-photon absorptions are very similar, and if we could more precisely determine the maxima of the TPA spectra most probably the same. It is possible that one- and two-photon excitations may reach the same excited state if a molecule is not centrosymmetric

or through relaxation of symmetry rules by vibronic coupling [15,23-25]. As a result, the same band should appear in both, one- and two-photon absorption spectra, although with different relative intensities. Of the molecules in this study *m*-THPP is centrosymmetric, but the *m*-THPC is not. In the *m*-THPC the symmetry is broken by the reduced double bond. The decrease in symmetry results in higher intensity of the long wavelength Q-band in the absorption spectrum of chlorins. The reduced symmetry increases the TPA probability for the symmetry-forbidden transitions. The observation of the relatively high TPA in the Q-band region [11] between 500 and 700 nm for both symmetric and not symmetric molecules shows that the vibronic coupling plays here an important role [25].

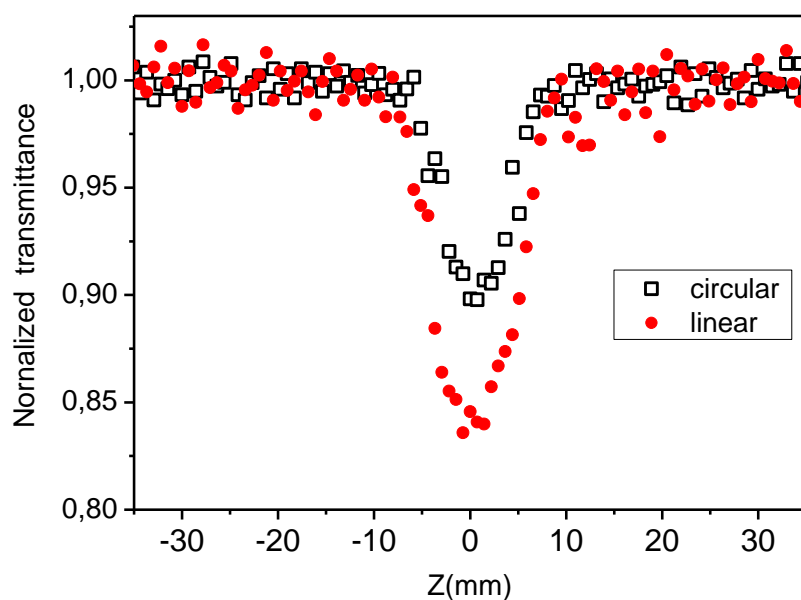


Fig.4

We have also measured the ratio of TPA cross sections for circular and linear polarized laser beams in the vicinity of the absorption peaks. An example of different transmittance curves for linear and circular polarizations is presented in Fig. 4. The ratio of both transmittance signals provides information on the symmetry of excited states for randomly oriented molecules [26,27]. It is stated that this ratio is <1 for any transition involving two states of the same symmetry on any point group with some rare exceptions [26]. For all peaks in the measured TPA spectra the ratio of the cross section for circularly polarized light over that for linearly polarized light does not exceed 1. The averaged value for a number of measured peaks in our experiment is $73 \pm 16\%$. It means that the ground and excited Q-band states are of the same symmetry.

Conclusions

We have measured the TPA cross sections of two promising photodynamic therapy photosensitizers *m*-THPC and *m*-THPP in the excitation range of 1.05-1.45 μm . The TPA in this Q-band region is comparable with the TPA at 800 nm, but the infrared light has better penetration into tissue. Thus, both photosensitizers are promising candidates for two-photon photodynamic therapy applications. The question under study now is how effectively the triplet state is populated by the excitation of the Q-band and its vibronic components.

References

1. W. Denk, J.H. Strickler, W.W. Webb. Two-photon laser scanning fluorescence microscopy. *Science* 248 (1990) 73-75.
2. T.C. Lin, G.S. He, Q. Zheng, P.N. Prasad. Degenerate two-/three-photon absorption and optical power-limiting properties in femtosecond regime of a multi-branched chromophore. *J. Mater. Chem.* 16 (2006) 2490-2498.
3. J.D. Bhawalkar, J.N.D. Kumar, C.F. Zhao, P.N. Prasad. Two-photon photodynamic therapy. *J. Clin. Laser Med. Surg.* 15 (1997) 201-204.
4. W.G. Fischer, W.P. Patridge, C. Dees, E.A. Wachter. Simultaneous two-photon activation of Type-I photodynamic agents. *Photochem. Photobiol.* 66 (1997) 141-155.
5. M. Atif, P.E. Dyer, T.A. Paget, H.V. Snelling, M.R. Stringer. Two-photon excitation studies of *m*-THPC photosensitizer and photodynamic activity in an epithelial cell line. *Photodiagn. Photodyn. Ther.*, 4 (2007) 106-111.
6. a) L. B. Josefsen, R.W. Boyle. Unique Diagnostic and Therapeutic Roles of Porphyrins and Phthalocyanines in Photodynamic Therapy, Imaging and Theranostics. *Theranostics* 2 (2012) 916-966; b) R.R. Allison, C.H. Sibata. Oncologic photodynamic therapy photosensitizers: A clinical review. *Photodiagn. Photodyn. Ther.* 7 (2010) 61-75.
7. M. Pawlicki, H.A. Collins, R.G. Denning, H.L. Anderson. Two-Photon Absorption and the Design of Two-Photon Dyes. *Angew. Chem. Int. Ed.* 48 (2009) 3244 - 3266; *Angew. Chem.* 121 (2009) 3292 -3316.
8. M. Schneider, G. Grasczew, T.A. Roelofs, E. Balanos, S. Rakowsky, H.J. Sinn, P. Schlag. Multiphoton excitation and photodynamic activity of macromolecular derivatized *m*THPC. *Proc. SPIE* 3909 (2000) 60-65.
9. R. Bonnett, P. Charlesworth, B.D. Djelal, S. Foley, D.J. McCarvey, T.G. Truscott. Photophysical properties of *m*-THPP, *m*-THPC and *m*-THPBC: a comparative study. *J. Chem. Soc., Perkin Trans. 2* (1999) 325-328.

10. N. Lourette, B. Maunit, L. Bezdetnaya, H.-P. Lassalle, F. Guillemin, J.-F. Muller. Characterization of photoproducts of mTHPP in aqueous solution. *Photochem. Photobiol.* 81 (2005) 691-696.
11. A.B. Ormond, H.S. Freeman. Effects of substituents on the photophysical properties of symmetrical porphyrins. *Dyes Pigm.* 96 (2013) 440-448.
12. M. Sheik-Bahae, A. A. Said, T. H. Wei, D. J. Hagan, E. W. Van Stryland. Sensitive measurement of optical nonlinearities using a single beam. *IEEE J. Quant. El. QE-26* (1990) 760-769.
13. J. Castillo, V.P. Kozich, O.A. Marciano. Thermal lensing resulting from one- and two-photon absorption studied with a two-color time-resolved Z-scan. *Opt. Lett.* 19 (1994) 171-173.
14. P. Sengupta, J. Balaji, S. Banerjee, R. Philip, R.G. Kumar, S. Maiti. Sensitive measurement of absolute two-photon absorption cross sections. *J. Chem. Phys.* 112 (2000) 9201-9205.
15. N.S. Makarov, M. Drobizhev, A. Rebane. Two-photon absorption standards in the 550-1600 nm excitation wavelength range. *Opt. Express* 16 (2008) 4029-4047.
16. C.H. Wang, O.Y.-H. Tai, Y. Wang, T.-H. Tsai, N.-Ch. Chang. Non-quadratic-intensity dependence of two-photon absorption induced fluorescence of organic chromophores in solution. *J. Chem. Phys.* 122 (2005) 084509; DOI: 10.1063/1.1850466.
17. A. Nag, S. Singh, D. Goswami. Structure property correlations in alcohols through two-photon absorption cross-section measurements. *Chem. Phys. Lett.* 430 (2006) 420-423.
18. A. Wiehe, Y.M. Shaker, J.C. Brandt, S. Mebs M.O. Senge. Lead structures for applications in photodynamic therapy. Part 1: Synthesis and variation of *m*-THPC (Temoporfin) related amphiphilic A₂BC-type porphyrins. *Tetrahedron* 61 (2005) 5535-5564.
19. a) R. Bonnett, B.D. Djelal, A. Nguyen. Physical and chemical studies related to the development of *m*-THPC (FOSCAN) for the photodynamic therapy (PDT) of tumours. *J. Porphyrins Phthalocyanines* 5 (2001) 652-661; b) R. Bonnett, R.D. White, U.-J. Winfield, M.C. Berenbaum. Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumor photosensitizers. *Biochem. J.* 261 (1989) 277-280.
20. a) J. Schwiertz, A. Wiehe, S. Gräfe, B. Gitter, M. Epple. Calcium phosphate nanoparticles as efficient carriers for photodynamic therapy against cells and bacteria.

- Biomaterials 30 (2009) 3324-3331; b) M. Wacker, K. Chen, A. Preuss, K. Possemeyer, B Röder, K. Langer. Photosensitizer loaded HSA nanoparticles. I: Preparation and photophysical properties. Int. J. Pharm. 393 (2010) 253-262; c) A. Preuß, K. Chen, S. Hackbarth, M. Wacker, K. Langer, B. Röder. Photosensitizer loaded HSA nanoparticles II: In vitro investigations. Int. J. Pharm. 404 (2011) 308-316.
21. a) <http://www.ema.europa.eu/ema/> b) I.B. Tan, G. Dolivet, P. Ceruse, V.V. Poorten, G. Roest, W. Rauschnig. Temoporfin-mediated photodynamic therapy in patients with advanced, incurable head and neck cancer: A multicenter study. Head Neck 32 (2010) 1597-1604.
22. R.Sailaja, P.B. Bisht, C.P. Singh, K.S. Bindra, S.M. Oak. Influence of multiphoton events in measurement of two-photon absorption cross-sections and optical nonlinear parameters under femtosecond pumping. Opt. Commun. 277 (2007) 433-439.
23. M. Drobizhev, N.S. Makarov, S.E. Tillo, T.E. Hughes, A. Rebane. Two-photon absorption properties of fluorescent proteins. Nature Meth. 8 (2011) 393-399.
24. A. Nag, D. Goswami. Solvent effect on two-photon absorption and fluorescence of rhodamine dyes. J. Photochem. Photobiol. A: Chem. 206 (2009) 188-197.
25. P. Macak, Y. Luo, H. Ågren. Simulations of vibronic profiles in two-photon absorption. Chem. Phys. Lett. 330 (2000) 447-456.
26. M.A.C. Nascimento. The polarization dependence of two-photon absorption rates for randomly oriented molecules. Chem. Phys. 74 (1983) 51-66.
27. A. Nag, D. Goswami. Polarization induced control of single and two-photon fluorescence. J. Chem. Phys. 132 (2010) 154508-(1-5).

Figure captions.

Fig.1. Scheme of open-aperture z-scan measurements. D1 and D2 are photodiodes, L1 and L2 – lenses.

Fig.2. Structures of investigated photosensitizers: 5,10,15,20-Tetrakis(3-hydroxyphenyl)porphyrin (*m*-THPP) **1** and 5,10,15,20-Tetrakis(3-hydroxyphenyl)chlorin (*m*-THPC) **2**.

Fig.3. Two-photon (filled circles and line) and one-photon (solid line) absorption spectra for *m*-THPC (a) and *m*-THPP (b) dissolved in DMSO. The TPA spectra are plotted against half the excitation wavelength.

Fig. 4. The measured transmittance curves for the *m*-THPP dissolved in DMSO at 1258nm for linear (filled circles) and circular (open squares) polarized exciting beam.