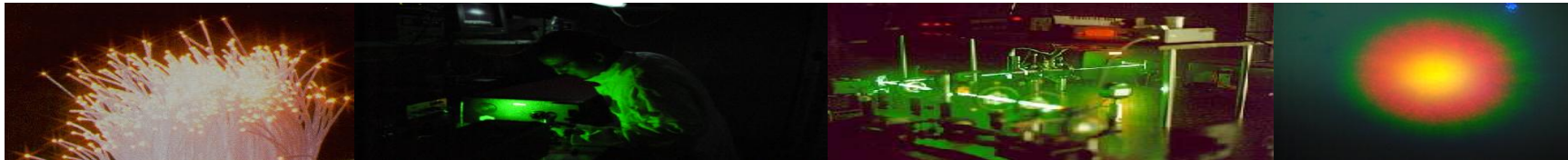


Two- and Three photon excited fluorescence in Y-shaped molecules



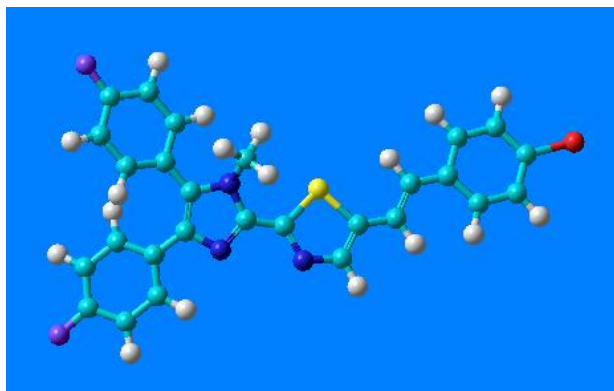
Abstract

In this work we have studied the two- and three-photon excited fluorescence on a new series of Y-shaped chromophores, using pulses at 750 nm and 1400 nm from an optical parametric amplifier pumped by 150 fs pulses from a Ti:sapphire chirped pulse amplified system. The measured two- and three-photon absorption cross-sections are in the order of $1000 \times 10^{-50} \text{ cm}^4 \text{ s}$ and $5 \times 10^{-78} \text{ cm}^6 \text{ s}^2$, respectively. Besides, the two-photon excited fluorescence signal, achieved using low energy pulses (Ti:sapphire modelocked oscillator), were used in an evolutionary strategy to optimize either the laser pulse or the two-photon excited fluorescence.



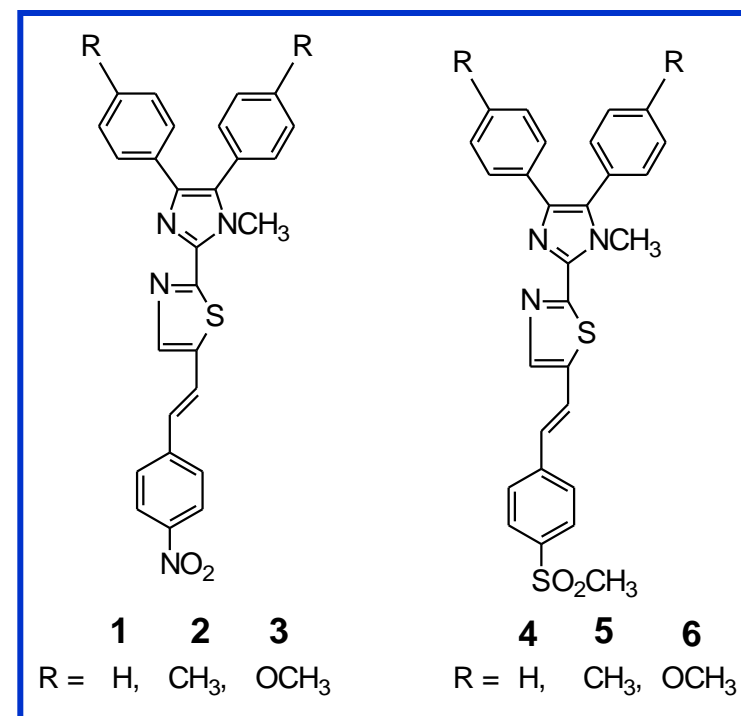
Y-shaped chromophores

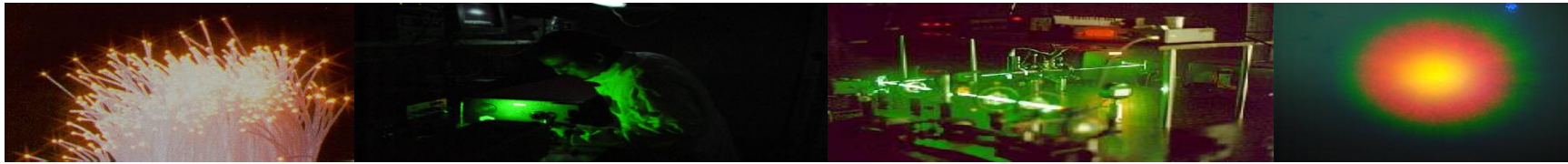
Two new series of sulfonyl and nitro-based chromophores with an imidazole-thiazole core were synthesized in order to study their nonlinear optical properties. The samples were dissolved in dimethyl sulfoxide (Aldrich) and placed in a 2-mm thick quartz cuvette to perform linear and nonlinear optical measurements. These compounds are stable and can be stored at ambient temperature for months without any degradation.



● : H, CH₃, OCH₃

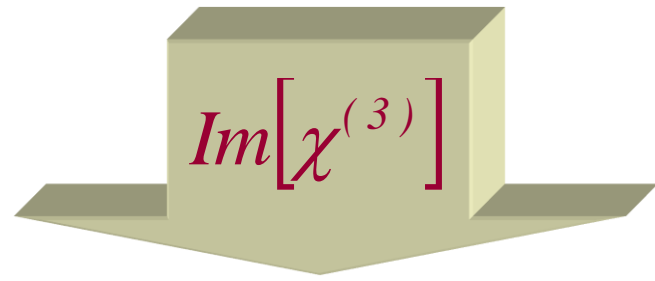
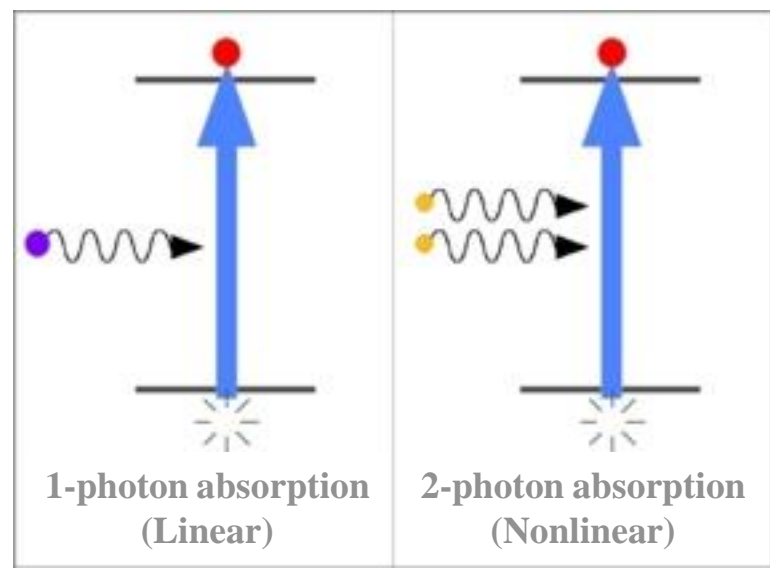
● : NO₂, SO₂CH₃





Two-photon absorption

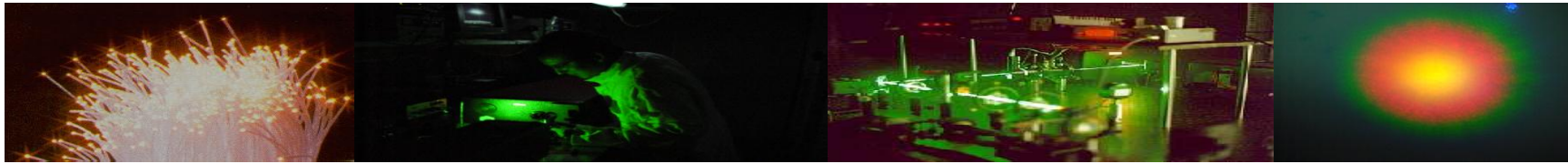
$$\vec{P} = \chi^{(1)} \cdot \vec{E} + \chi^{(2)} : \vec{E}\vec{E} + \chi^{(3)} : \vec{E}\vec{E}\vec{E} + \dots$$



$$\alpha = \alpha_0 + \beta I$$

β : two-photon absorption coefficient

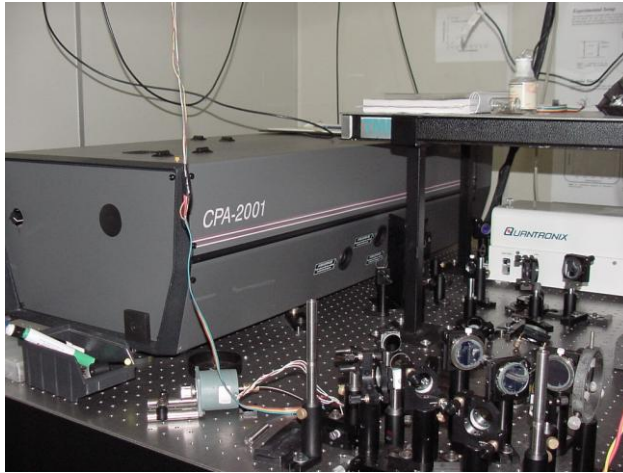
The rate of energy absorbed is proportional to the square of the excitation intensity, which allows for spatial resolution in applications such as microfabrication, two-photon fluorescence imaging, two-photon photodynamic cancer therapy and three-dimensional (3D) optical storage.



Z-scan experimental setup

* 150 fs Laser

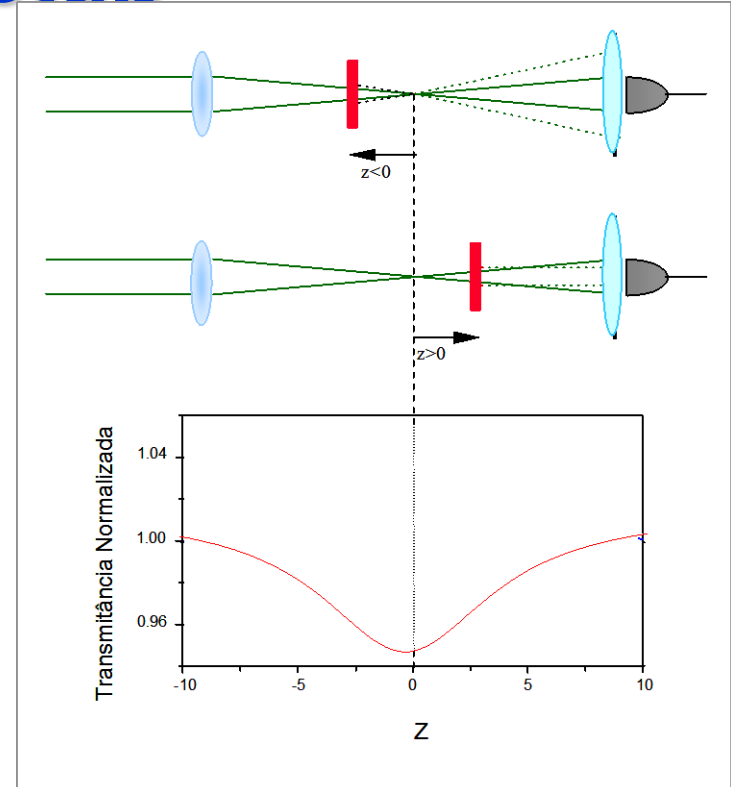
Clark – MAXR CPA - 2001
 $\lambda=775 \text{ nm}$; $f=1 \text{ KHz}$;
 $E_p=800 \mu\text{J}$



* OPA

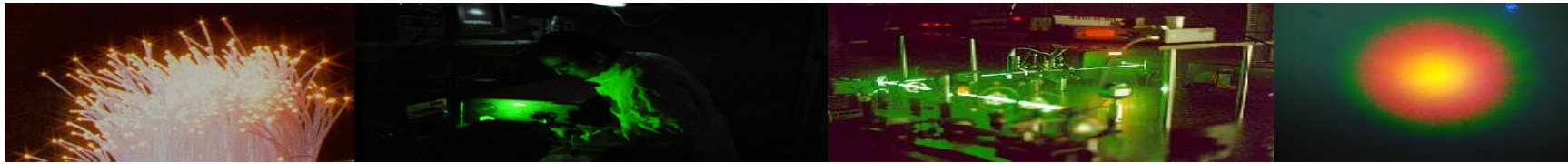


Pump - Laser Clark
 460 - 2600 nm
 $\approx 120 \text{ fs}$
 20-60 μJ



$$\alpha(I) = \alpha_0 + \beta I$$

$$\Delta T \propto \beta$$



Two-photon optical spectrum

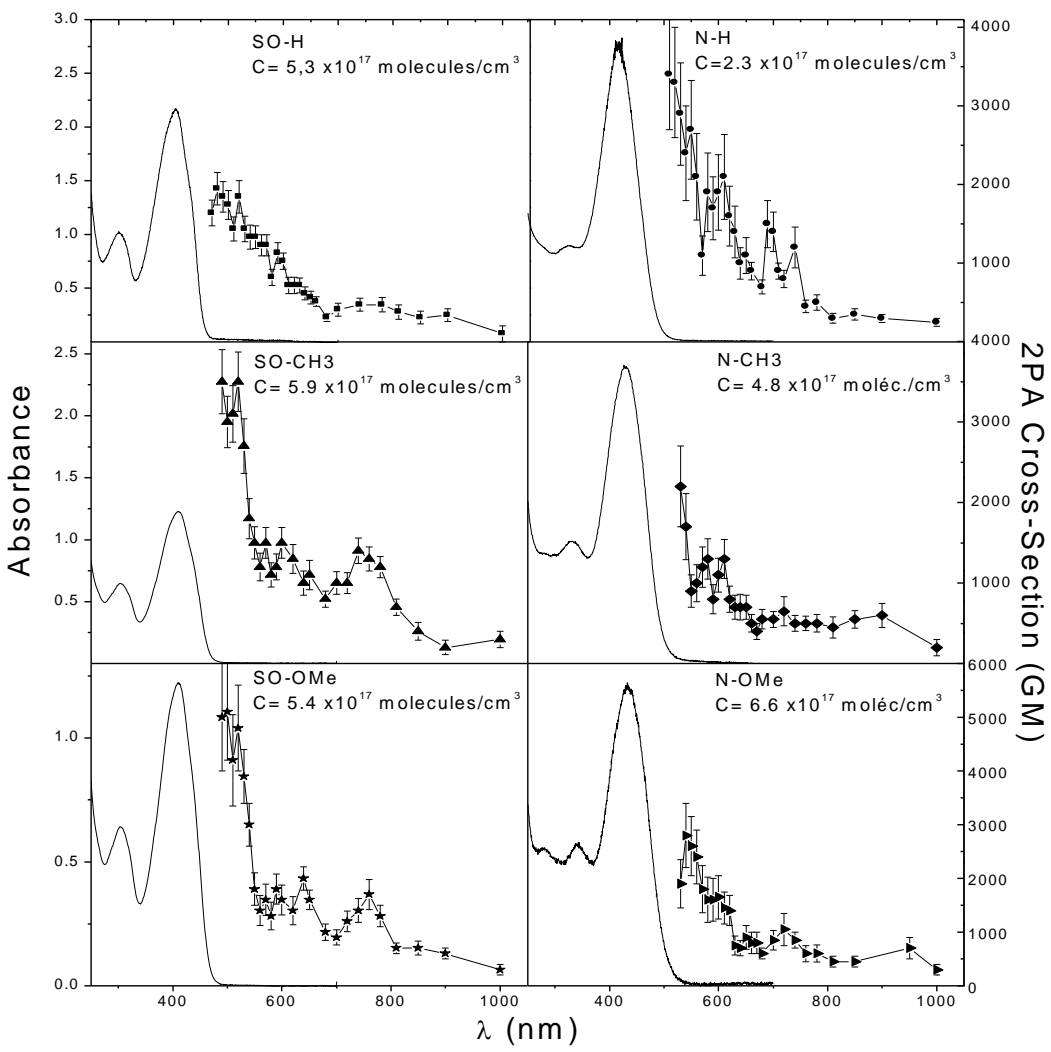
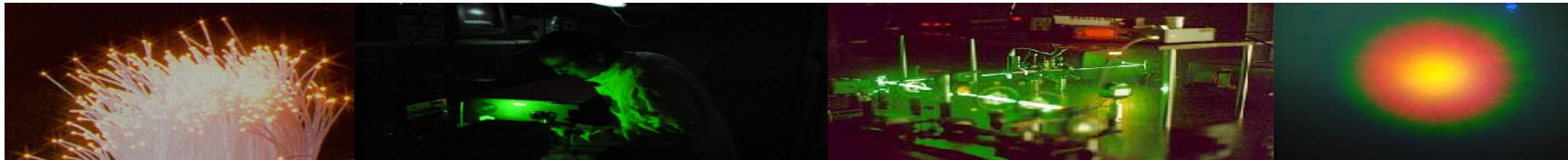


Table 1. 2PA cross-section coefficients

	[C] ^a	2PA λ_{max} (nm)	δ (GM) ^b
1	2.3	690	1500
2	4.8	720	650
3	6.6	720	1050
4	5.3	740	460
5	5.9	600, 740	1500, 1400
6	5.4	640, 760	2000, 1700

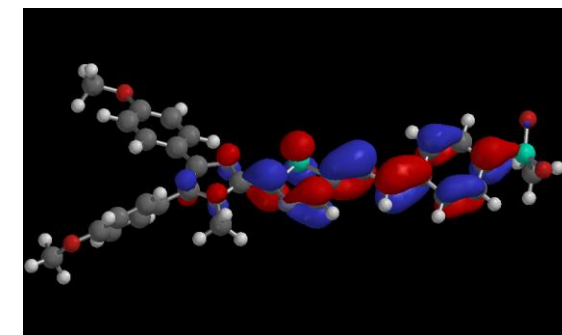
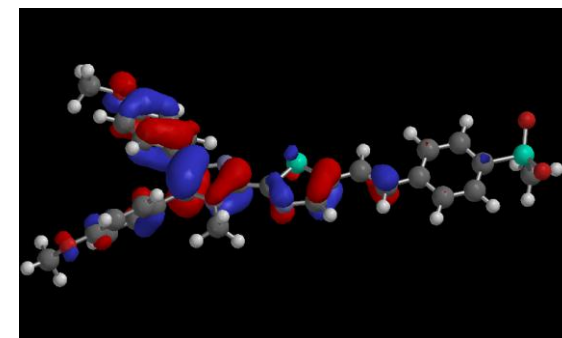
a: 10¹⁷ molecules/cm³; b: 1 GM = 10⁻⁵⁰ cm⁴ s photon⁻¹



Molecular geometry

We have examined the molecular geometry to determine the conjugation pathway of this unsymmetric system. Two phenyl rings have different twists from imidazole, and the conjugation from one phenyl ring is better than the other with imidazole.¹⁴ The optimized molecular geometry by DF-63-1G* shows that one phenyl (at the 4-position) is only twisted 26-29° from the imidazole ring while the other (at the 5-position) is twisted 53-57°, suggesting that one donating group primarily contributes to the push-pull structure through better conjugation due to less twist between two adjacent aromatic rings.

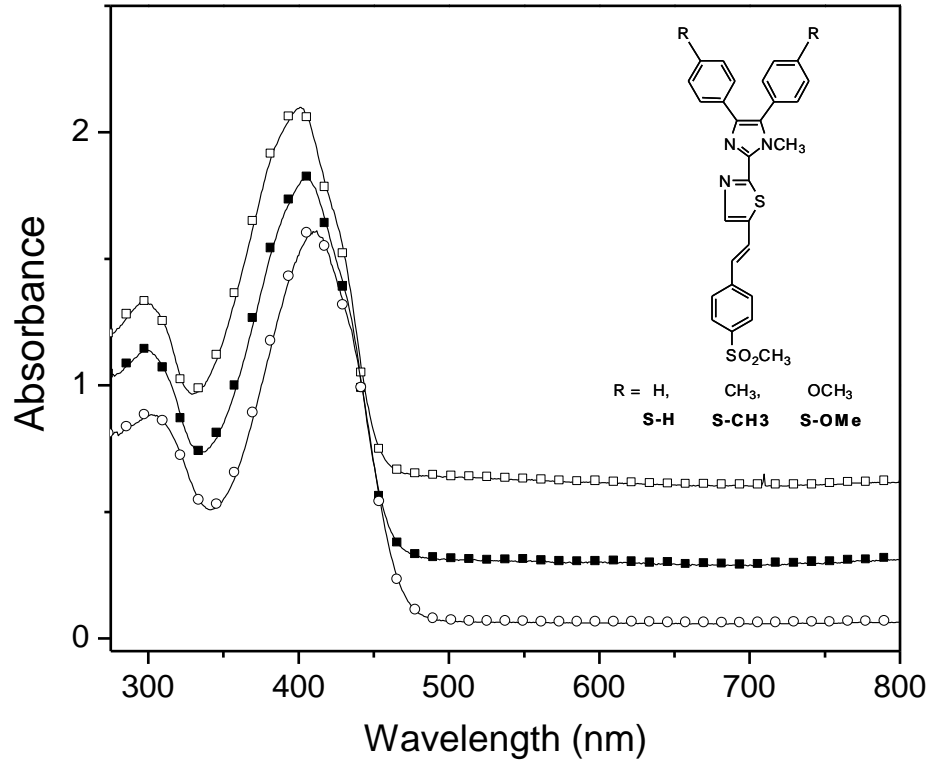
For the rest of the chromophores, namely from imidazole to thiazole and all the way to the accepting group, the geometry is relatively planar. For instance, the twists of the two phenyls from imidazole in **6** are 28.82° and 53.59°, respectively. Imidazole and thiazole are almost co-planar, with the deviation of only 1.89°. Vinyl moiety is also positioned well with two adjacent aromatic rings-thiazole and phenyl with deviations of 1.11° and 3.87°, respectively. Accordingly, the HOMO and LUMO mainly reside on the following pathway: phenyl with accepting group, vinyl, thiazole, imidazole, and the phenyl at the 4-position (Figure 3). Both series, regardless of the type of electron-accepting groups, possess the same conjugation pathway.



The HOMO (top) and LUMO (bottom) of **6**.

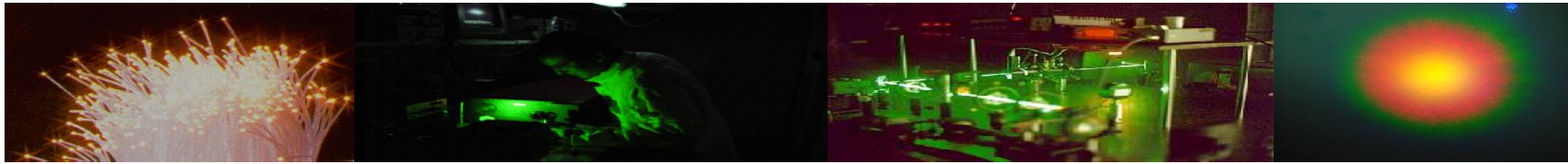


Multi-photon excited fluorescence

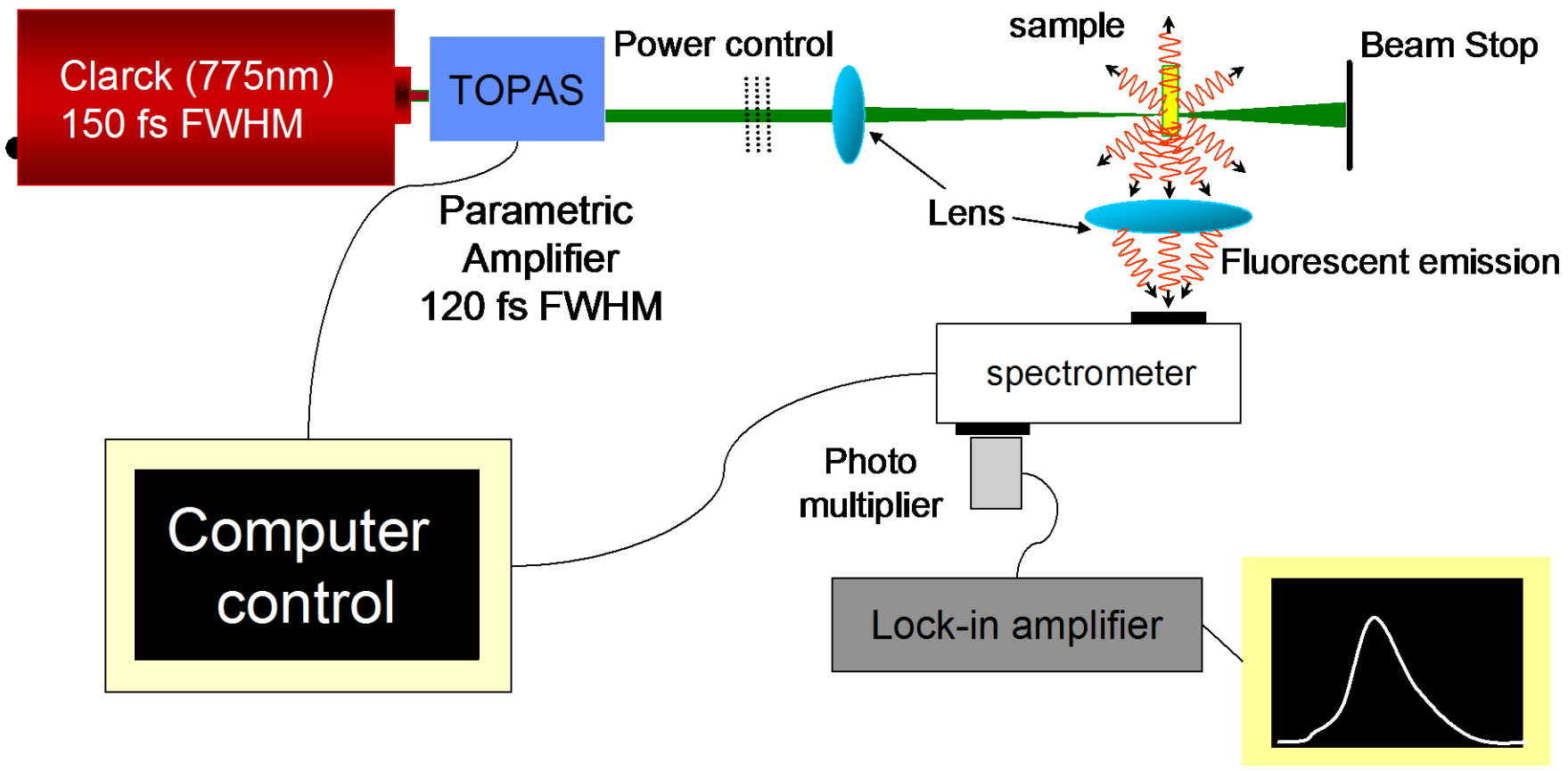


Through our 2PA results it was observed that sulfonyl-based chromophores turn out to be somewhat more efficient, in nonresonant conditions, than nitro-based ones. Thus, we study the two- and three-photon excited fluorescence of three different sulfonyl-based Y-shaped compounds, namely S-H, S-CH₃ and S-OMe.

UV-vis absorption spectra of S-OME (○), S-CH₃ (■) and S-H (□) DMSO solution. The spectra of S-CH₃ and S-H were shifted to clarify presentation. The inset shows the molecular structure of the studied samples. All compounds investigated possess absorption bands in the range between 300 and 500 nm, being transparent in the near infrared region.

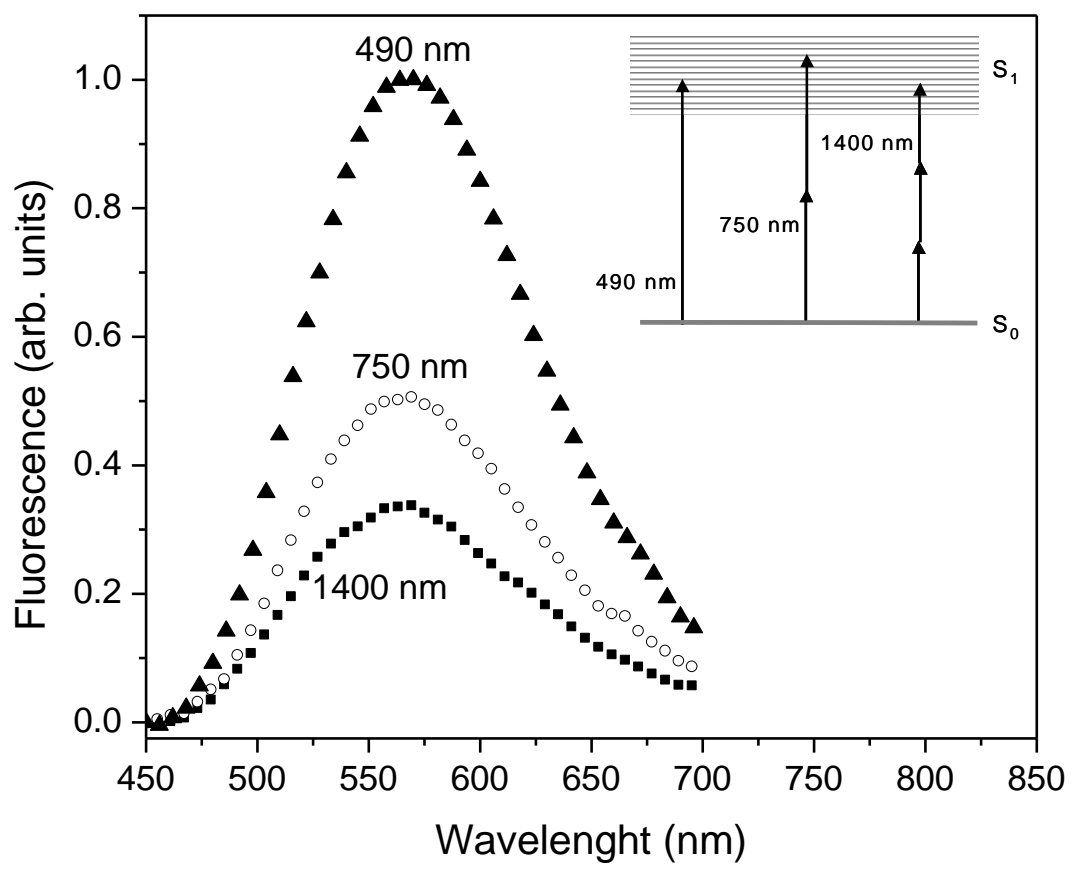


Multi-photon excited fluorescence

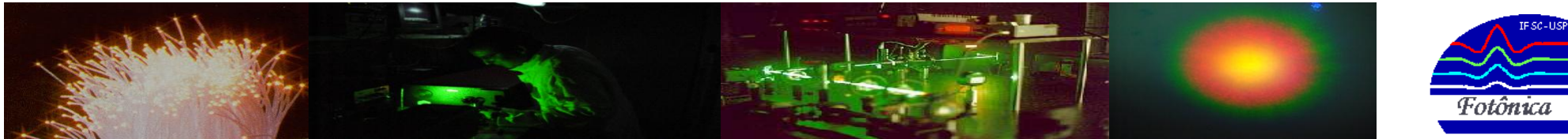




Multi-photon excited fluorescence



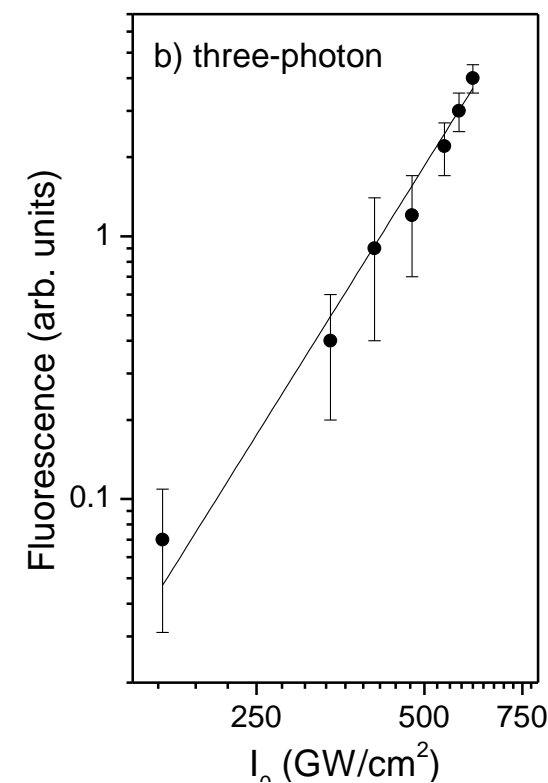
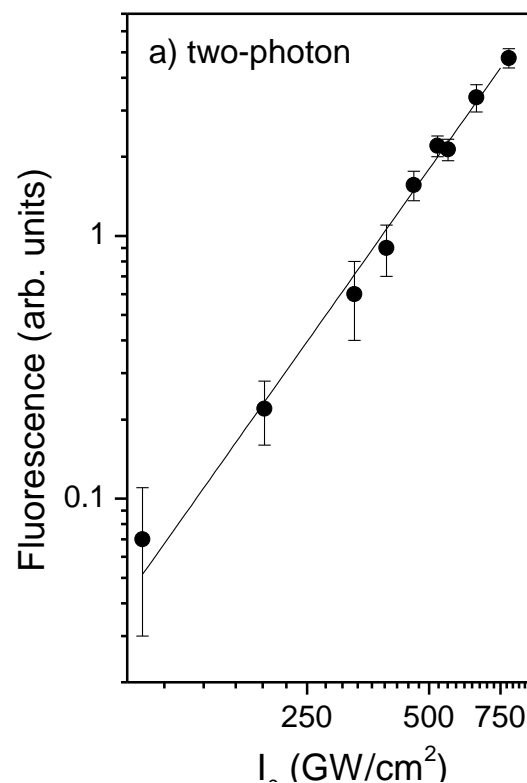
These results shows the fluorescence spectrum for S-OMe obtained for excitation at 490 nm (one-photon), 750 nm (two-photon) and 1400 nm (three-photon). The y-scale was adjusted to clarify presentation. As expected these spectra are identical, indicating that the emission corresponds to the relaxation from the first excited state for all excitation wavelengths employed.

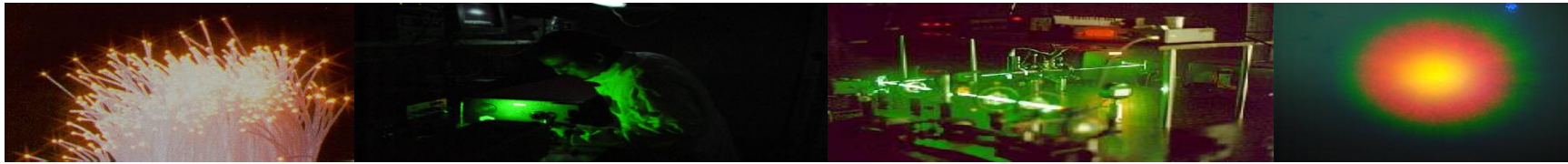


Multi-photon excited fluorescence

In order to demonstrate the two- and three-photon nature of these excited fluorescences we have measured the emission intensity as a function of the pump beam irradiance, as shown.

Similar results were obtained for the other samples. The solid lines in this figure represents the best linear fits obtained with slopes of 2.1 and 3.4, clearly demonstrating the two- and three-photon nature of the observed nonlinearities at 750 and 1400 nm, respectively.





Multi-photon absorption cross-section

The two- and three-photon absorption cross-sections of these compounds were determined using the Z-scan technique at 750 and 1400 nm.

For an absorptive nonlinearity,

$$\alpha = \alpha_0 + \alpha_2 I + \alpha_3 I^2 + \dots$$

where I is the laser beam irradiance and α_0 , α_2 and α_3 are the linear, two- and three-photon absorption coefficients, respectively.

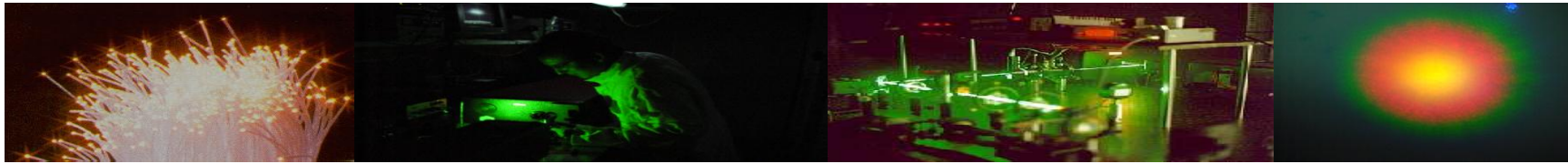
The two- and three-photon absorption cross-section can be obtained, respectively, through the expressions

$$\sigma_2 = \frac{(h\nu)\alpha_2}{N} \qquad \sigma_3 = \frac{(h\nu)^2\alpha_3}{N}$$

The two and three photon absorption cross-sections determined are listed in Table 2.

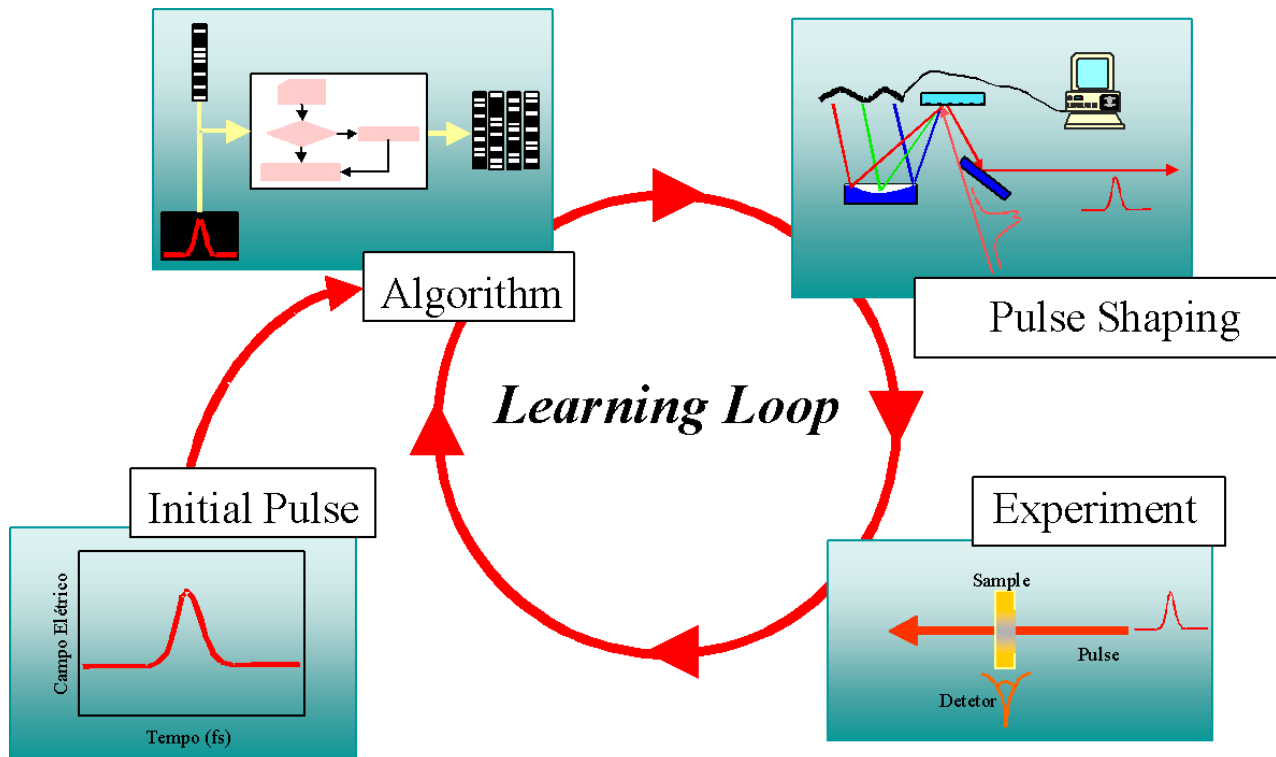
Table 2: Concentration, two- and three photon absorption cross-sections for the compounds studied.

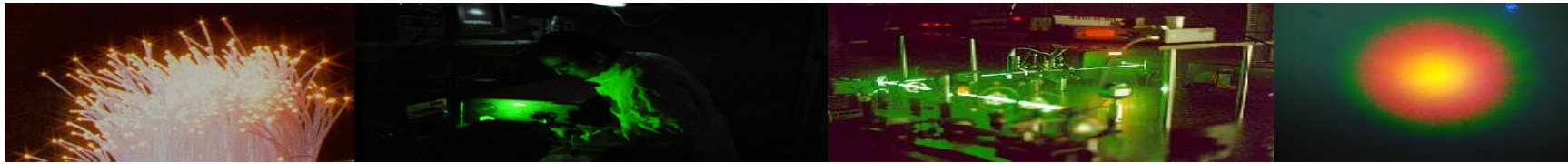
Sample	N (10^{18} molecules/cm ³)	σ_2 (10^{-50} cm ⁴ s) @ 750 nm	σ_3 (10^{-78} cm ⁶ s ²) @ 1400 nm
S-H	3.2	500	4.5
S-CH3	2.6	1450	5.6
S-Ome	2.4	1550	7.3



Pulse shaping using Y-shaped compounds

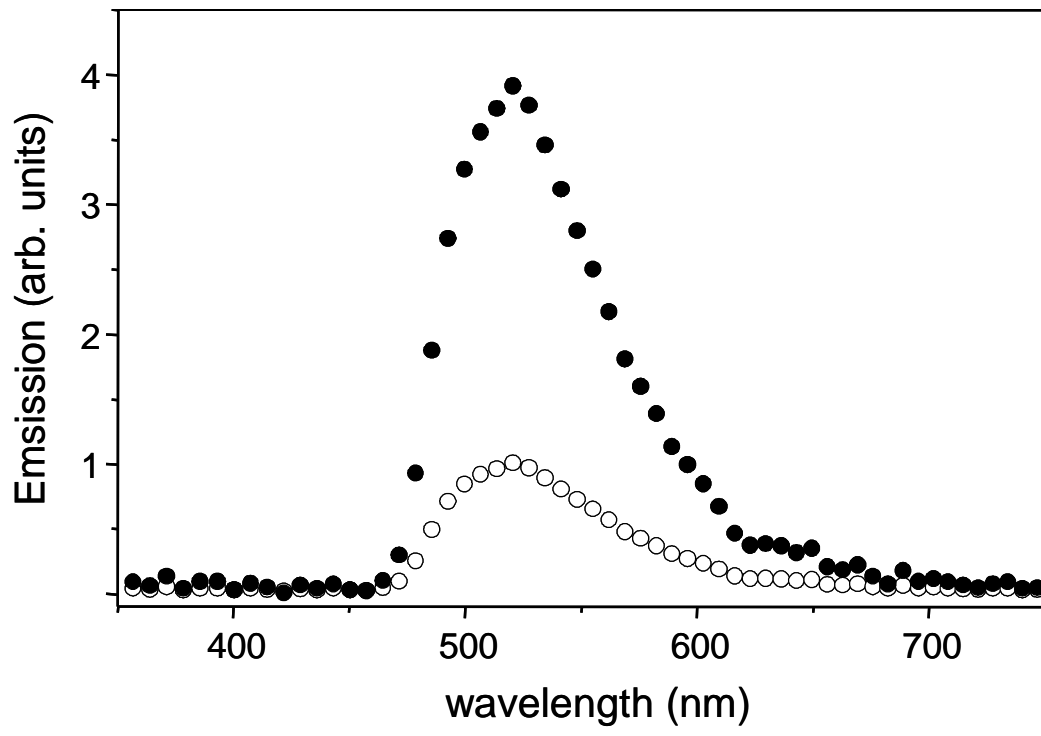
A micro-machined deformable mirror (DFM) was used to shape the spectral phase in a zero-dispersion stretcher configuration. Optimization of the laser pulse is obtained using an evolutionary strategy that begins with a set of random pulse shapes whose associated 2PEF signals are measured. Those pulses that produce the most intense fluorescence are retained, duplicated, perturbed, and reproduced, as the Genetic Algorithm requires. This process is repeated until a desired number of interactions.



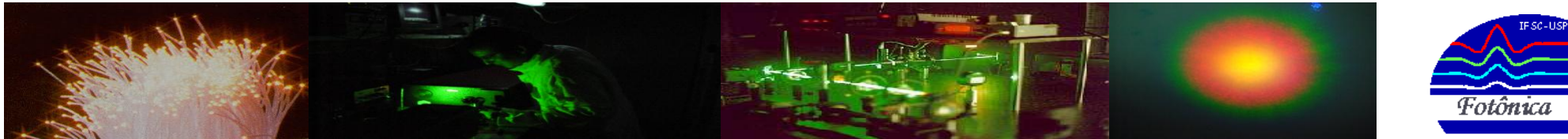


Two-photon excited fluorescence with oscillator

The relatively high two-photon absorption cross-section of Y-shaped compounds allows its application for two-photon excited fluorescence (2PEF) even without an amplified laser system (oscillator).

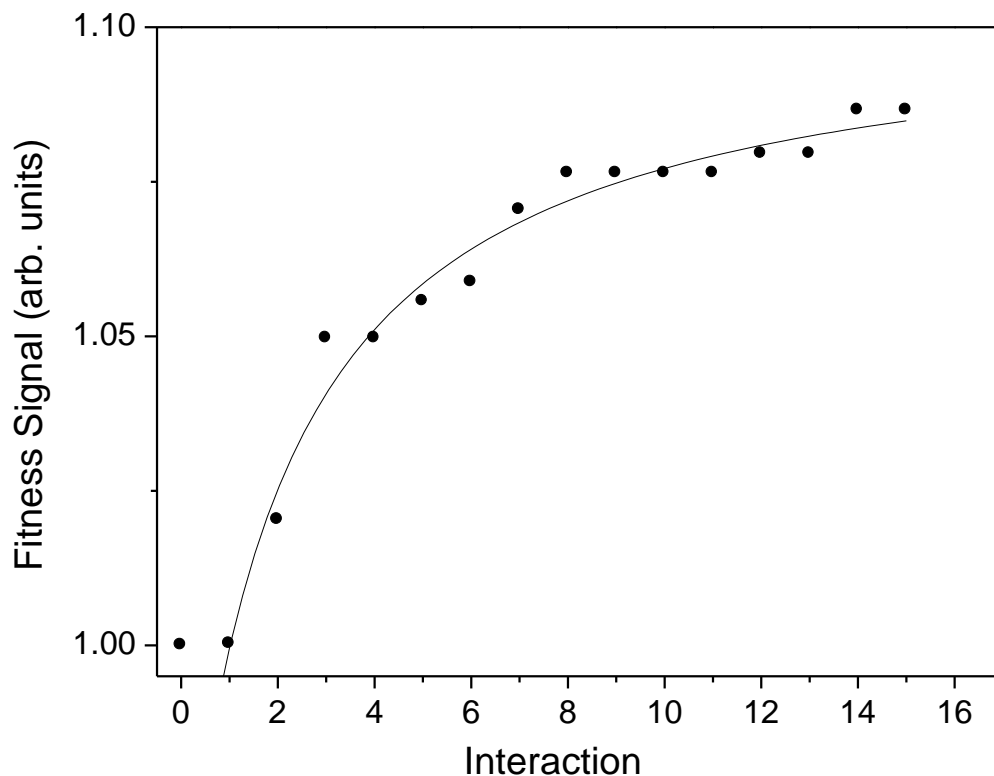


This figure shows the two-photon excited fluorescence spectrum for the S-OMe sample obtained with a KLM Ti:sapphire oscillator, with pulses at two distinct energies (2 and 1 nJ). As can be seen, the 2PEF signal increases about four times when the pump irradiance increases twice, exhibiting the quadratic dependence expected for two-photon processes.

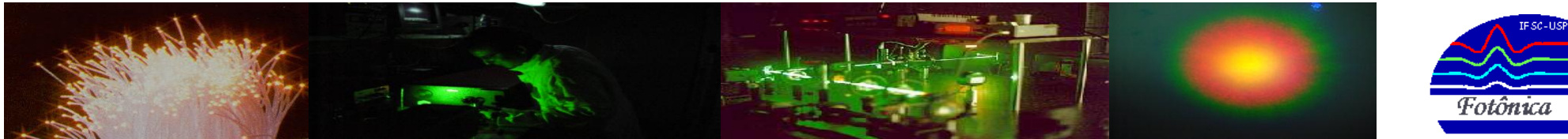


Fitness signal evolution

The fitness signal evolution as a function of the number of interaction for S-OMe is the figure below, where a clear increase in the fitness can be observed. An increase by 10 % was observed in the 2PEF for this solution, comparing with the initial condition (flat DFM).

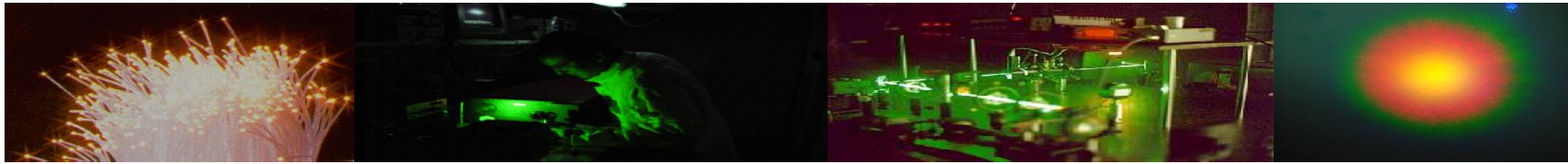


Approximately the same evolution was observed for the other compounds. Such an optimization corresponds to a decrease in the pulse duration, measured using autocorrelation, from 28 fs to 22 fs. In this way, we were able to use the multiphoton fluorescence process to improve the pulse quality, thus enhancing the fluorescence which can be used to further improve the performance of multiphoton imaging systems.



Conclusions

In summary, we have demonstrated two- and three-photon absorption through fluorescence emission measurements as function of the excitation irradiance in a new series of Y-shaped chromophores at 750 and 1400 nm, respectively. The nonlinear absorption (2PA and 3PA) cross-section values, measured using the Z-scan technique, are comparable to the best ones presented in the literature for organic compounds. These findings open the door to access a new class of materials with potential applications as fluorescent probes for two- and three-photon microscopy. Moreover, we were able to enhance the 2PEF signal via the laser pulse optimization using an evolutionary strategy, which could be employed to further improve the performance of multiphoton imaging systems.



Acknowledgements

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