Absolute measurement of molecular two-photon absorption cross-sections using a fluorescence saturation technique

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Introduction

We demonstrate direct measurement of the absolute molecular two-photon absorption (TPA) cross-section using a fluorescence technique that measures the onset of excited-state saturation. The saturation onset depends on the TPA cross-section and the local irradiance but is independent of the detection efficiency and the quantum yield, thus eliminating the need of detection calibration. Our technique only requires precise knowledge of the space-time profile and the pulse energy of the exciting femtosecond laser beam and the spatial profile of the observation beam. The exciting beam is focussed using a microscope objective after passing through a hollow core photonic crystal fiber that acts as a spatial filter. The observation is done confocally using a conventional single-mode fiber. An in-house built profiling tool is used for the diagnosis of the tightly focussed, highly divergent beams. A theoretical model to describe the fluorescence photon counting signal generated by a femtosecond laser pulse was developed to fit the measured data. Measurements of the TPA cross-sections of Rhodamine 6g and Rhodamine B in methanol at 806 nm give $\sigma_{2R6g} = 23.0 \pm 3.0$ **GM** and $\sigma_{2RB} = 50.1 \pm 3.0$ **GM** (linearly polarized excitation), respectively .

Theory

Fluorescence photon counting signal N_F per laser pulse:

Laser pulse duration · Dye concentration

$$N_F = \eta\, q\, \sigma_2\, \bar{I}^2\, \tau\, \bar{c}\, V_0\, \Theta \left\{ 1 + c_1\, \sigma_2\, \bar{I}^2\, \tau + c_2 \left(\sigma_2\, \bar{I}^2\, \tau\right)^2 + \mathcal{O}\left[\left(\sigma_2\, \bar{I}^2\, \tau\right)^3\right] \right\}$$

TPA cross-section · Mean square intensity · Overlap volume · Solid angle of observation · Overlap coefficients $c_n = V_n / V_0$

$$\bar{I}^2 = \frac{1}{\sqrt{2\pi}} \frac{Q^2}{\pi^2\, a_{E0}^4\, \tau^2}$$

Q: Photons / laser pulse

$$V_n = \frac{(-1)^n}{(n+1)!} \int Y_E^{(2\,n+2)}(\vec{r})\, Y_O(\vec{r})\, d^3r$$

Spatial profile for excitation and observation beam

Our model assumes:

- Spatial **Gaussian profile** for excitation beam Y_E and observation beam Y_O
- Temporal **Gaussian profile** for intensity I(t)

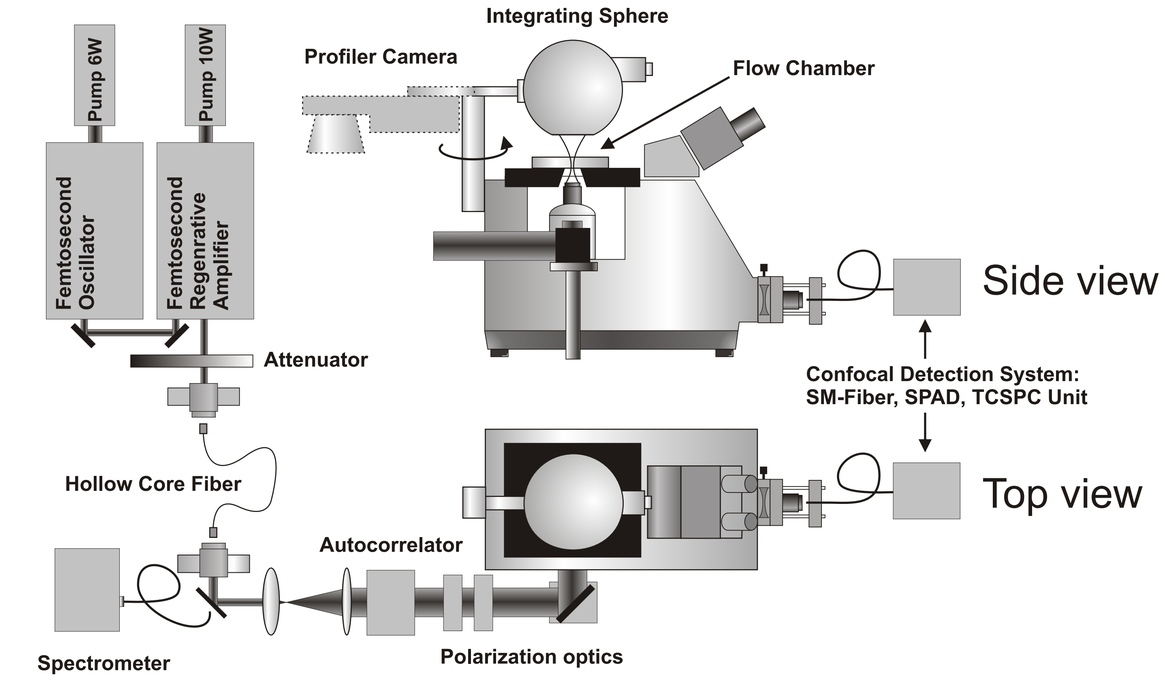
What we need to know:

- σ_2, τ and properties of Y_E and Y_O

- Detection efficiency η and quantum yield q do not influence **saturation onset**

Material and Methods

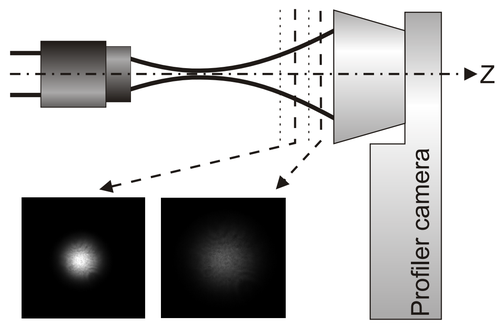
Experimental Setup:



Beam-Diagnostic Tool:

Spatial:

The beam radii of the excitation and observation beam (a_{E0} and a_{O0}) are determined by off-focus profiling, based on the propagation theory of a nonparaxial Gaussian beam[1]. Several far-field images of the laser beam at different z-positions after the microscope objective are taken. After concatenating all of these images, a 3-dimensional far-field map of the Gaussian beam can be assembled and processed using a nonlinear 3-dimensional least-square fit. Images were taken with an adapted CMOS-camera (PL-A741-R, Pixelink), equipped with a fiber optic taper, directly bonded onto the CMOS chip (Schott, Proxitronic). The taper allows us to take images in planes sufficiently close to the focus, so that the whole profile of the divergent beam can be captured. Moreover, the taper prevents the formation of disturbing interference fringes. The single-mode beam radii are determined to be a_{E0}**=342 ± 10 nm** and a_{O0}**=296 ± 10 nm**.



[1]W.H. Carter, Electromagnetic Field of a Gaussian Beam with an Elliptical Cross Section, J. Opt. Soc. Am. 62 pp. 1195-1201 (1972)

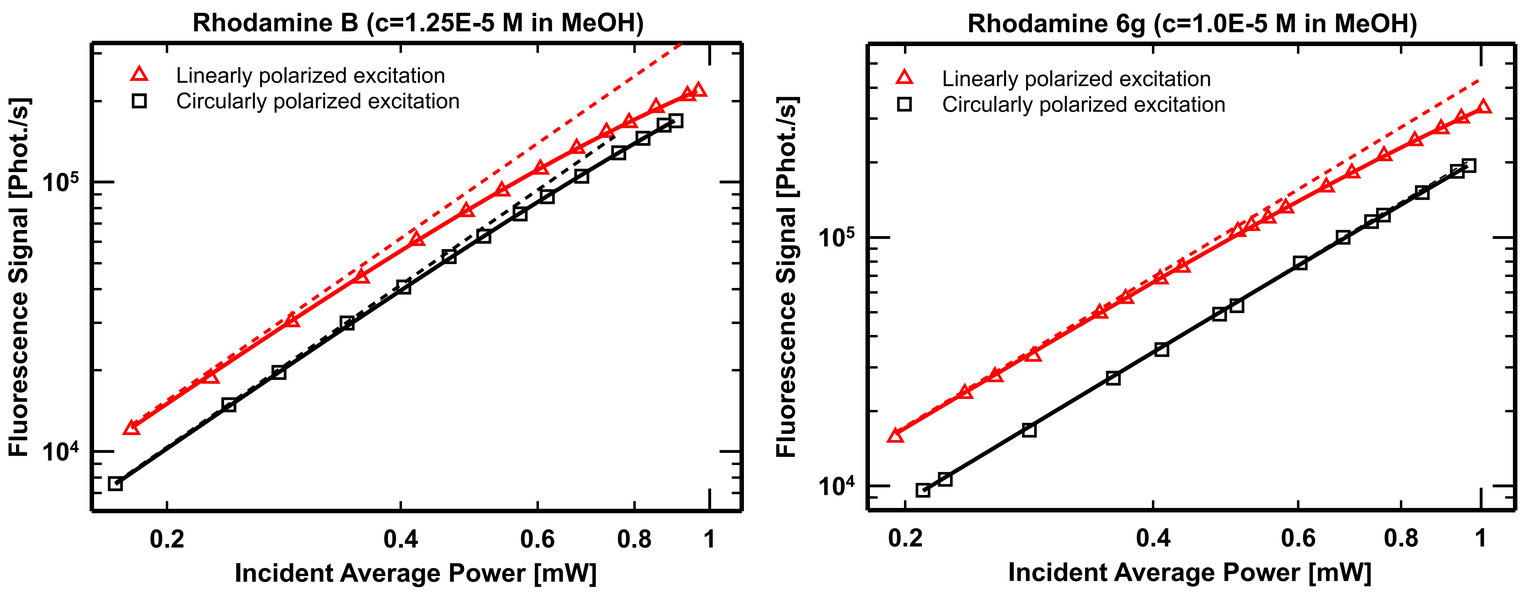
Temporal:

The 1/e-pulse width τ **= 193 fs** of the temporal Gaussian laser pulse is measured directly in the focus on the microscope stage using an adapted intensity autocorrelator (Carpe, APE). The two-photon excited fluorescence from the sample is used as the quadratic medium for the pulse width measurement.

Average excitation power:

The number of photons per pulse $Q=P_L/(h\nu f)$ was determined by measuring the average laser power P_L and the laser repetition rate f. As a power meter we employed a 4" integrating sphere (LPM-040-SF, Labsphere) containing an input port of 1" diameter and a silicon detector. Placed above the sample in the microscope, this detector allows precise diagnostics of laser beams with divergences of up to NA = 0.65 and power in the range of 15 μW to 15 W.

Results



Log-log plot of the dead-time corrected fluorescence signal at different laser average power

- Good agreement with σ_2-values measured with different methods, but we achieve much **higher sensitivity**.
- High sensitivity allows in-situ measurement, e.g. within a biological cell
- Dead-time correction of the measured signal $\langle M_F \rangle$: $\langle N_F \rangle = \ln\left(1 - \langle M_F \rangle\right)$
- Fluorescence lifetime was found to be independent of the excitation intensity

Values for the two-photon cross-section for Rhodamine 6g and Rhodamine B in MeOH as they given in the literature. Note that some values are converted to our definition of σ_2 (TPA cross-section per single molecule)

Dye	Conc.	TPA cross-section	Polarization	Wavelength	Ref.
Rh. 6g	9.7 μM	$\sigma_2 = 23.0 \pm 3.0$ GM[+]	linear	806 nm	Our work
Rh. 6g	10.23 mM	$\sigma_2 = 16.2 \pm 2.4$ GM	unknown	806 nm	4
Rh. 6g	0.11 mM	$\sigma_2 \approx 20/q$ GM	linear	800 nm	2
Rh. 6g	30 mM	$\sigma_2 = 15.3 \pm 2.0$ GM	unknown	800 nm	3
Rh. 6g	30.9 mM	$\sigma_2 = 18/q \pm 6$ GM	unknown	800 nm	5
Rh. B	9.6 μM	$\sigma_2 = 50.1 \pm 3.0$ GM	linear	806 nm	Our work
Rh. B	9.6 μM	$\sigma_2 = 21.5 \pm 3.0$ GM	circular	806 nm	Our work

[+]1 GM = 10^{-50} $m^4 s$ / $Phot^2$ / Molecule

[2]M.A. Albota et al., Appl.Opt. 37, pp. 7352-7356, (1998); [3]P. Tian et al., Opt. Lett. 27, pp. 1634-1636 (2002); [4]P. Sengupta et al., J. Chem. Phys. 112, pp. 9201–9205 (2000); [5]R. Kapoor et al., J. Opt. Soc. Am. B 20, pp. 1550-1554 (2003)

Conclusion

Considering the wide spread of the concentrations used in previous studies, the agreement between the results is surprisingly good. Our technique allows us to measure the TPA cross-section with high sensitivity, resulting in a concentration 10x lower than possible in previous measurements. This will allow us to determine the TPA cross-sections *in-situ*, for example within a labelled biological cell. We also note that once the detection efficiency η is calibrated using a well- characterized dye, such as Rhodamine 6g, the setup can be used for the determination of the two-photon cross-section at low average laser power, well below the onset of saturation. Moreover, once we have used our technique to calibrate the detection efficiency η, we can simultaneously determine the quantum yield q and the TPA cross-section of a dye.

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