# Two-Photon Fluorescence Spectroscopy by Resonant Grating Waveguide Structures

**A. Thayil K.N., S. Soria, andGonçal Badenes**
*ICFO-Insitut de Ciències Fotòniques, Jordi Girona 29, Nexus II, 1-D 08034 Barcelona, Spain*
*Silvia.Soria@icfo..es*

**T. Katchalski and A. A. Friesem**
*Department of Physics of Complex Systems, Weizmann Institute of Science, 76100 Rehovot, Israel*

Compared to conventional fluorescence methods, two-photon fluorescence (TPF) increases the signal-to-noise ratio due to a complete rejection of background noise, and reduces dynamic photobleaching and photo-induced processes like auto-fluorescence in most biological systems. However, TPF excitation requires high photon densities. In order to achieve the required high instantaneous photon flux densities and to avoid tight focusing, we resort to low loss high finesse resonant polymeric grating-waveguide-structures (GWS) (period 523 nm, height 450 nm). The resonant GWS are basically multilayered structures consisting of a substrate, a polymeric waveguide and a grating layer on top. When such devices are illuminated with an incident light beam, part of the beam is directly transmitted through the structure and part is diffracted by the grating and is trapped in the waveguide layer. At a specific wavelength and angular orientation of the incident beam, GWS show resonant behavior where complete destructive interference occurs such that no light is transmitted, but rather is fully reflected from the GWS.

Our presentation is focused on demonstrating how such polymeric GWS can be exploited for the enhancement of TPF. We chose the conventional tetramethylrhodamine (TMR) dye for our experiments. A drop of nanomolar TMR solution in milli-Q water (ph=7.5) was deposited on top of the GWS. After evaporation of the solvent, the TMR molecules remained immobilized on the GWS surface. A mode-locked Ti:Sapphire laser ( 76 MHz, 150 fs pulse duration, 690-980 nm wavelength range) operating at the resonant wavelength was used as excitation source. In order to ensure that the fluorescence is indeed due to the GWS enhancement we tuned the incident laser wavelength for a fixed polarisation and changed polarization for a fixed wavelength. Figure 1 shows the results with the GWS. As evident, near and at resonance the TPF from the GWS could be readily observed. On the other hand, far away from resonance no TPF signal could be observed. Near resonance, the TPF intensity increases strongly, reaching its maximum at the resonance wavelength of 826 nm, indicating a strong field enhancement. Also shown are TPF signals obtained at 831, and at 828nm which are two and one FWHM away from the resonance excitation wavelength. The TPF signal at the excitation wavelength of 826nm (suitable for resonance with TE polarization) was detected as background noise signal for TM polarization. Similarly, no TPF was detected when using a reference glass with a deposited TMR thin layer under identical experimental conditions, indicating clearly the enhancement of the TPF detection with GWS [1]. In this case the TPF in resonance is enhanced by a factor of 160 with respect to the non-resonant TPF.

Since the TPF signal is proportional to the convolution of the GWS transmission spectrum and the pulse intensity envelope, considerable TPF signal is also present at wavelengths close to the resonance wavelength. Since the resonance bandwidth is considerably narrower than the pulse envelope the TPF signal is expected to resemble a slightly broadened pulse envelope (see Fig. 2.).[2]

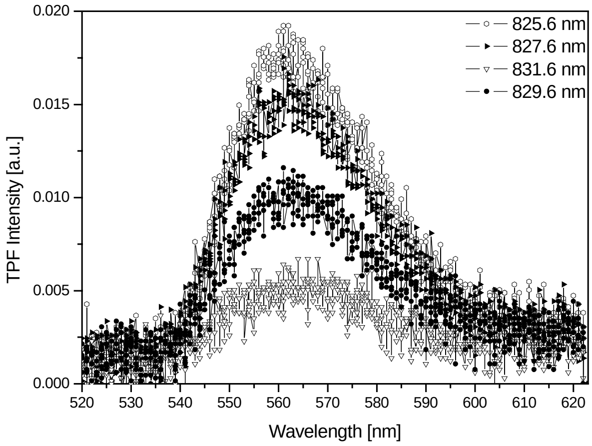


Fig. 1. Two-photon fluorescence signal with GWS, for different excitation wavelengths. Hexagons denote excitation wavelength of 826 nm; down-side triangle 832 nm ; left sided triangle 827.6 nm ; crosses 829.6 nm (maximum absorption wavelength).

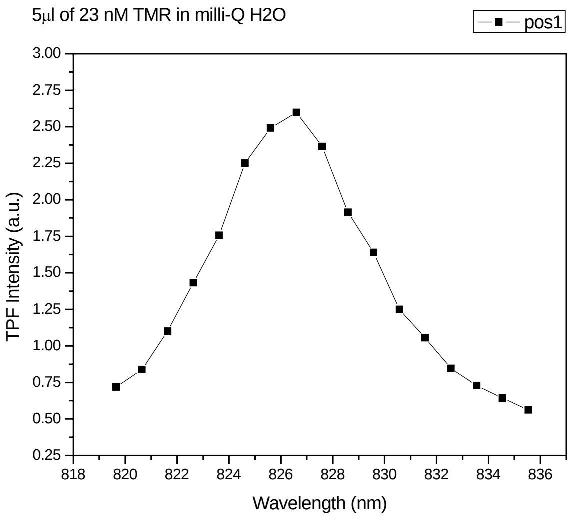


Fig. 2. Integral TPF signal as a function of central excitation wavelength of a DGWS**.**


[1]. S.Soria, T. Katchalski, E. Teitelbaum, A.A. Friesem, G. Marowsky, "Enhanced Two Photon Fluorescence excitation by resonant grating waveguide structures" Opt. Let. **29**, 1989-1991 (2004).

[2] T. Katchalski, S.Soria, E. Teitelbaum, A.A. Friesem, G. Marowsky, "Two Phton Fluorescence Sensors based on Resonant Grating Waveguide Structures" Sensors and Actuators, in press.