Two-Photon Fluorescence Spectroscopy by Resonant Single and Double Grating Waveguide Structures

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Abstract:. We show how resonant single and double grating-waveguide-structures enhance two-photon fluorescence more than two orders of magnitude without the need for a highly focused laser excitation light. Such resonant devices have an enormous potential for biosensing.

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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 present in most of biological systems. However, TPF excitation requires high photon densities.In order to achieve the required high instantaneous photon flux densities (~ 10^{31} photons/cm^{2}s) and avoid tight focusing, we resort to low loss high finesse resonant polymeric single grating-waveguide-structures (GWS, with grating period of 523 nm and grating height of 450 nm) and dielectric double grating-waveguide-structures (DGWS, with grating period of 360 nm and grating height of 40 nm). The resonant single GWS are basically multilayered structures consisting of a substrate, a polymeric waveguide and a grating layer on top, whereas the DGWS consist of a substrate and a thin waveguide layer with one grating under and one on top of the waveguide layer. 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, both GWS and DGWS show a resonance when a complete destructive interference occurs such that no light is transmitted, but rather is fully reflected from the GWS and DGWS.

Our presentation is focused on demonstrating how such polymeric GWS and dielectric DGWS can be exploited for 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 and DGWS. After evaporation of the solvent, the TMR molecules remained immobilized on the GWS and DGWS surface. A mode-locked Ti:Sapphire laser (76 MHz, 150 fs pulse duration, 690-980 nm wavelength range) operating at the resonant wavelengths of the devices was used as excitation source.

The experimental results are presented in Figs 1 and 2. In order to ensure that the fluorescence is indeed due to the GWS and DGWS enhancement, we tuned the incident laser wavelength at a fixed polarisation and varied polarization at a fixed wavelength. Figure 1 shows the results with the GWS. The normally incident laser beam (400 mW) was slightly focused to a 100 μm beam waist. 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 825.6 nm, indicating a strong field enhancement. Also shown are TPF signals obtained at 831.6 nm and at 829.6 nm, both away from the resonance excitation wavelength. The TPF signal at the excitation wavelength of 825.6 nm (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. These results clearly indicate the enhancement of the TPF detection with GWS. Specifically, the TPF at resonance shows an enhancement by a factor of 160 with respect to the non-resonant TPF [1].

Figure 2 shows the results with a DGWS which are similar to those obtained with a GWS. In this case, the maximum TPF intensity is observed at the resonance wavelength of λ_{res}=815 nm at an incident angle of 35º, for a non focussed incident laser beam (536 mW and 2 mm beam waist). Recent published experimental results reported a 350-fold enhancement using a DGWS [2].

Since the TPF signal is proportional to the convolution of the GWS and DGWS 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, that is shown in Fig.3.

No TPF was detected in the spectral region between 350 and 500 nm or in regions without the immobilized TMR. Thus, one can exclude other nonlinear effects like surface second harmonic generation.

Our procedure and results indicate that the detection of TPF can indeed be improved with the resonant GWS and DGWS. We expect that the overall detection sensitivities can increase even more as the fabrication of such devices is improved.

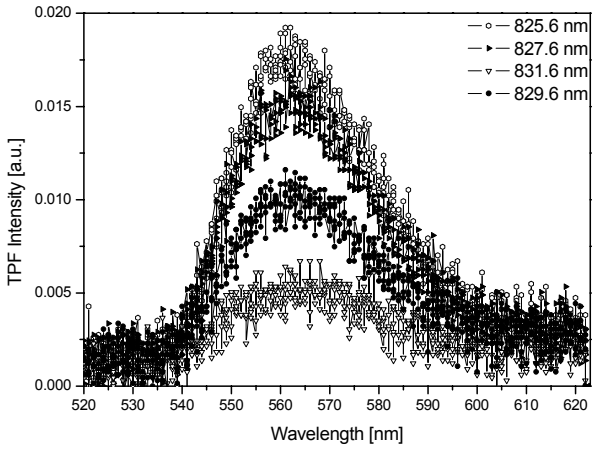

Fig. 1. Two Photon Fluorescence signal with GWS , for different excitation wavelengths. Open circles denote excitation wavelength of 826 nm; down-side triangle 832 nm ; left sided triangle 827.6 nm ; solid circles 829.6 nm .

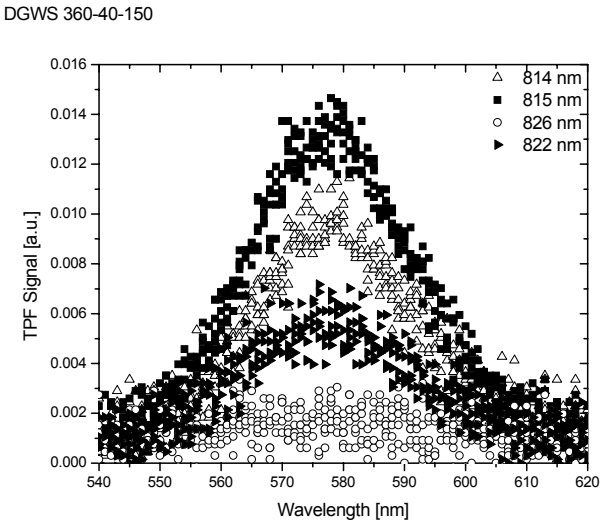

Fig. 2. Two Photon Fluorescence signal with DGWS , for different excitation wavelengths. Squares denote excitation wavelength of 815 nm; up-side triangle 814 nm; right sided triangle 822 nm; circles 826nm.

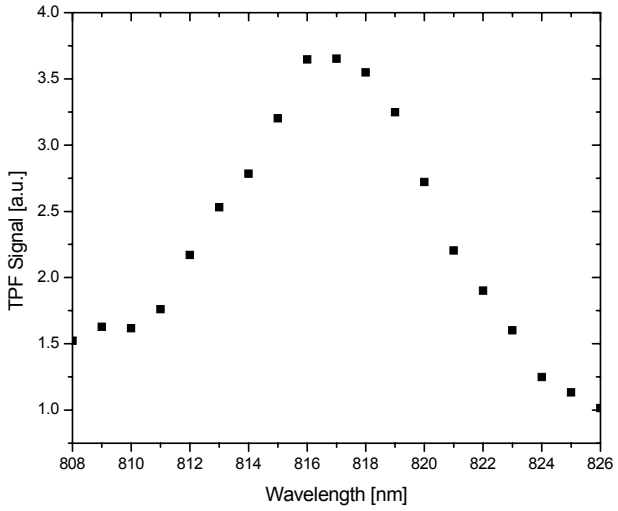

Figure 3.Integral TPF signal as a function of central excitation wavelength of a DGWS.

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[2] C. Kappel, A. Selle, T. Fricke-Begeman, M.A. Bader, G. Marowsky, „Giant Enhancement of two-photon fluorescence induced by resonant double grating structures" Appl. Phys. B **79**, 531-534 (2004).