Enhanced Two-Photon Fluorescence Excitation by Resonant Waveguide Structures

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Abstract: Two photon fluorescence excitation (2PE) minimizes photobleaching and discriminates easily fluorescence from the excitation light. Novel low loss high finesse resonant polymeric nanostructures enhance 2PE, without the need for a highly focused laser excitation light.

OCIS codes: (130.2790) Guided Waves; (300.2530) Fluorescence, laser-induced.

Two photon excitation (2PE) performed with near-IR radiation has a number of advantages when compared to one photon excitation. The larger energy gap between the excitation and emission radiation results in low scatter and the quadratic dependence of the absorption on intensity reduces damage and static photobleaching. Yet, with the conventional 2PE which requires a highly focused laser light, the time in which photodamage can occur is relatively shortened.

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 nanostructures (period 523 nm, height 450 nm). These resonant devices are basically multilayered structures consisting of a substrate, a polyimide waveguide and a grating layer on top. When such a structure is 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, the grating waveguide structure (GWS) shows a resonance when a 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 2PE. We chose the conventional tetramethylrhodamine (TMR) dye for our experiments. A drop of millimolar TMR solution in milli-Q water (ph=7.5) was deposited on top of the GWS and, for comparison, on top of a quartz prism. After evaporation of the solvent, the TMR molecules remained immobilized on the surface. A mode-locked Ti:Sapphire laser operating at 844 nm was used as excitation source.

The experimental results are presented in Figs 1 and 2. Figure 1 shows the results with the GWS. In order to ensure that the fluorescence is indeed due to the GWS enhancement we tuned the incident laser wavelength from 830 nm to 856 nm and changed polarization. At 844 nm, the resonant wavelength for TE-mode, the signal had its maximum value while for the TM mode at the same wavelength the signal was almost zero. At the maximum absorption wavelength of 850 nm, for 2PE of TMR, the fluorescent signal was reduced by a factor of two. For wavelengths at the upper and lower limit of the resonant bandgap, the measured fluorescence decreased by approximately 20 %. For other wavelengths, the fluorescence decreased by one order of magnitude (not shown). Figure 2 compares the experimental results with the GWS to those with the prism. As evident, the results with the GWS are better by at least a factor of three.

Our procedure and results indicate that the detection of 2PE can indeed be improved with the resonant GWS. We expect that the overall detection sensitivities can increase even more as the fabrication of the GWS is improved.

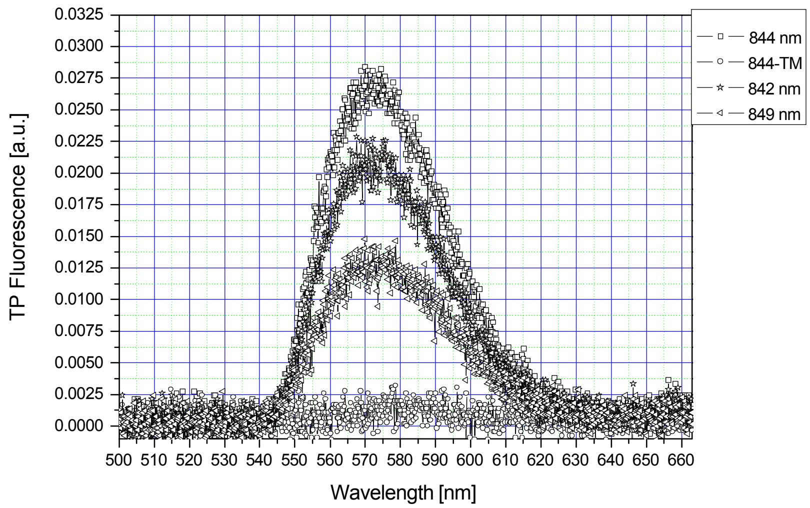

Fig. 1. Two Photon (TP) Fluorescence signal with GWS , for different excitation wavelengths. Squares denote excitation wavelength of 844 nm; down-side triangle 844 nm TM-mode; left sided triangle 842 nm (lower limit of the bandgap); stars 849 nm (maximum absorption wavelength). Energy fluence 12.7 10^5 W/cm^2.

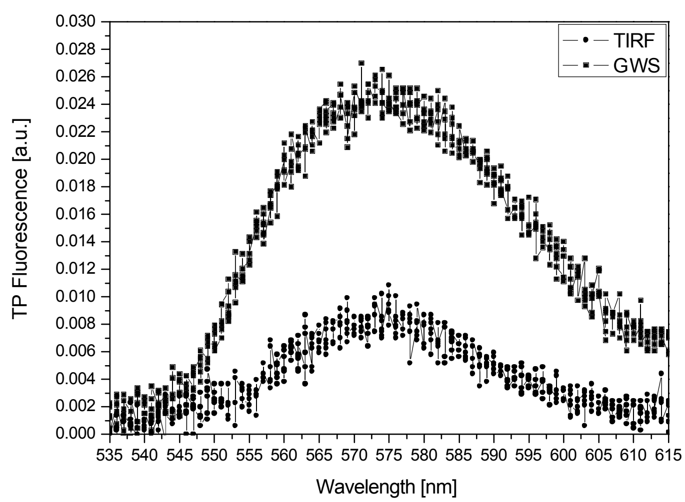

Fig. 2. Two Photon (TP) Fluorescence signal excitation wavelength of 844 nm with GWS and prism. Squares denote GWS enhanced signal; circles prism in total internal reflection.