

# Enhanced two-photon fluorescence excitation by resonant grating waveguide structures

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Enhanced two-photon fluorescence (TPF) spectroscopy with novel high-finesse resonant polymeric grating waveguide structures (GWSs) is presented. Under resonant conditions the field enhancement at the surface of a GWS can be exploited for TPF spectroscopy without the need for highly focused laser excitation light. We compare the TPF obtained by placing a drop of tetramethylrhodamine (TMR) on top of a GWS with that obtained with TMR on top of a glass substrate. Our procedure and results indicate that the detection of TPF can be improved by a factor of 10 with resonant GWSs. © 2004 Optical Society of America

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Two-photon excitation performed with near-infrared radiation has a number of advantages compared with one-photon excitation. These advantages include (1) low scattering because of the large energy gap between excitation and emission radiation, (2) reduction of static photobleaching of the dyes that are used because there is a quadratic dependence of the absorption on intensity,<sup>1,2</sup> (3) greater tolerance of cells and tissue to near-infrared radiation, and (4) less autofluorescence.

Conventional two-photon excitation, however, requires highly intense, focused laser light, so the photodamage threshold is lowered.<sup>3</sup> To achieve the required high instantaneous photon flux densities ( $\sim 10^{31}$  photons/cm<sup>2</sup> s<sup>2</sup>) and yet avoid tight focusing, we resort to low-loss high-finesse polymeric resonant grating waveguide structures (GWSs).<sup>4</sup> The basic configuration of such a GWS consists of a substrate, a waveguide layer, and a grating layer on top. When the GWS is illuminated with an ultrashort light pulse, most of the light is directly transmitted through the structure and some is diffracted by the grating, trapped in the waveguide layer, and subsequently partially rediffracted outward. At a specific wavelength and angular orientation of the incident beam, the GWS resonates, where the rediffracted beam destructively interferes with the transmitted beam and most of the incident light is reflected. Specifically, a sharp dip occurs in the spectrum of the illuminating pulse.<sup>5</sup>

For our purposes the most important feature of a GWS is the large optical field enhancement that can be achieved at the grating's surface, despite the low coupling efficiency. Thus the GWS can be exploited for two-photon fluorescence (TPF) excitation of a layer that is deposited on top of the grating layer. GWSs have a number of additional attractive features for TPF applications. In particular, they are compact and ro-

bust to simplify experimental setups, and they are relatively easy to fabricate so they can readily be incorporated into widespread biological and chemical applications.

In recent years there have been some attempts to enhance TPF by use of resonant phenomena such as surface plasmon resonance on smooth silver films<sup>6</sup> and on silver nanoparticle fractal clusters.<sup>7</sup> However, although in both cases the performance is excellent, the technical requirements and costs are high, and, for clusters, special dyes must be synthesized.

To evaluate the efficacy of our GWS we performed several experiments. First we determined the resonant behavior of the GWS with and without a drop of tetramethylrhodamine (TMR) on top. Then, for the GWS with the TMR drop, we compared the TPF intensity emitted on and off resonance; the TPF intensity was measured as a function of wavelength at a fixed polarization and as a function of polarization at a fixed wavelength. Finally, we compared the TPF intensity when a GWS was used with that obtained when a 90° quartz prism in total internal reflection or glass substrate or a waveguide layer was used.

Several GWSs of 1-cm diameter were fabricated. We used optical quality glass substrates onto which the waveguide and the photoresist layers were spin coated. The waveguide consisted of a polyimide layer of approximately 430-nm thickness (refractive index,  $n = 1.7$ ). The grating was holographically recorded into a Shipley S1805 photoresist layer. Typically the grating period was  $\sim 523$  nm and the grating thickness was 450 nm. In principle, a decrease in the grating thickness narrows the resonance bandwidth, whereas a change in the grating period shifts the resonance wavelength. The grating structure was optimized to minimize surface roughness, with different exposures, development procedures, and heat treatments. The

waveguide layer also served as a stopping layer for the wet-etching development of the grating layer and thus assisted in achieving a high uniformity. Because of the nonlinearity of the etching process the resultant grating had a square profile with an approximately 40% duty cycle.<sup>4</sup> Afterward a drop of 15- $\mu$ M 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 immobile on the surface of the GWS. The presence of this TMR layer should lead, according to rigorous coupled-wave analysis, to a shift of approximately 10 nm in the resonance wavelength as a result of the change of index of refraction.

The experimental arrangement for evaluating TPF together with a GWS configuration is shown schematically in Fig. 1. In our experiments the excitation light was derived from a mode-locked Ti:sapphire laser (Coherent) operated at a frequency of 76 MHz. The pulse width was 150 fs and the spectral bandwidth was  $\sim$ 8 nm. The wavelength of the excitation light could be tuned from 690 to 980 nm but was generally centered at the resonant wavelength of the GWS. The GWS was mounted upon a positioning stage (Physik Instrumente), which allowed for transverse translation and rotation to ensure the normal incidence for which the GWS was designed. At normal incidence, the angular alignment tolerances of the incident beam are relaxed.<sup>8</sup> The incident laser beam of 21-kW peak power was slightly focused to a 500- $\mu$ m beam waist. TPF occurs at the location of the excitation beam. The TPF was collected by a spherical lens ( $f = 50$  mm, N.A. = 0.8), focused onto the entrance slit of a spectrometer (Jobin Yvon), and detected by a backthinned CCD linear array (Hamamatsu). The excitation light of the Ti:sapphire laser was blocked by a near-infrared filter (BG39, Schott) placed in front of the entrance slit of the spectrometer.

Some representative experimental results are shown in Figs. 2 and 3. Figure 2 shows the transmitted spectral response of the bare (without TMR) GWS, the GWS with TMR, and the incident pulse (reference). The GWS was illuminated at normal incidence with transverse electric (TE) polarization, in which the electric field of the incident light is parallel to the grating grooves. As is evident from these experimental results, the transmitted intensities do not decrease to zero, indicating that our GWS has some scatter loss. Also, the resonance peak of the GWS with TMR was shifted by 9 nm from that of the bare GWS. With transverse magnetic (TM) polarization illumination the resonance peak was shifted by 10 nm to a shorter wavelength. As is evident, the transmission's spectral profile has a dip at the resonant wavelength of  $\lambda_{\text{res}} = 844$  nm with a FWHM of 2 nm for TE polarization excitation. For the TM polarization at the same wavelength, i.e., out of resonance, the spectrum of the transmitted light corresponded to the full spectrum of the incident pulse.

Figure 3 shows TPF as a function of wavelength for several excitation wavelengths obtained with a GWS onto which TMR has been deposited. Five TPF measurements were made at each wavelength. To ensure that the fluorescence is indeed due to the GWS

enhancement we first varied the incident laser wavelength from 830 to 856 nm at fixed polarization and then varied the polarization at fixed wavelengths. As is evident from Fig. 3, there is very low background TPF away from the resonance. Near resonance the TPF intensity increases strongly, reaching its maximum at 844 nm, indicating strong field enhancement. The peak intensity is approximately ten times

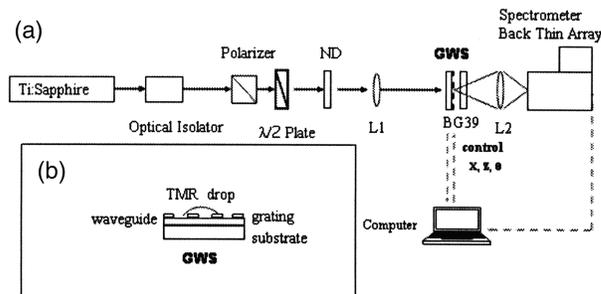


Fig. 1. Experimental arrangement for evaluation of TPF: (a) Experimental arrangement, (b) GWS configuration with a TMR drop. ND, neutral-density filter; L1, L2, lenses.

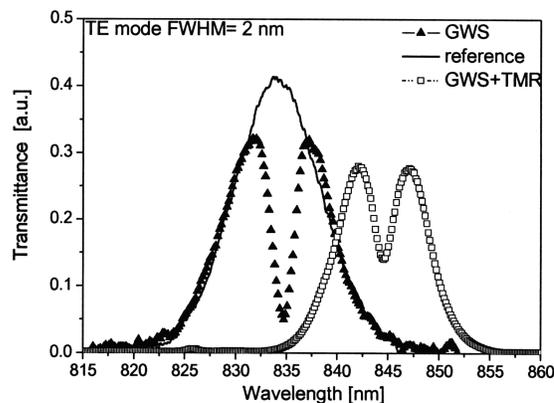


Fig. 2. Experimental measurements of the spectral response of a polymeric GWS for TE-polarization excitation: triangles, without TMR; squares, with TMR; solid curve, reference pulse. For a bare GWS the transmission intensity drops by 90%.

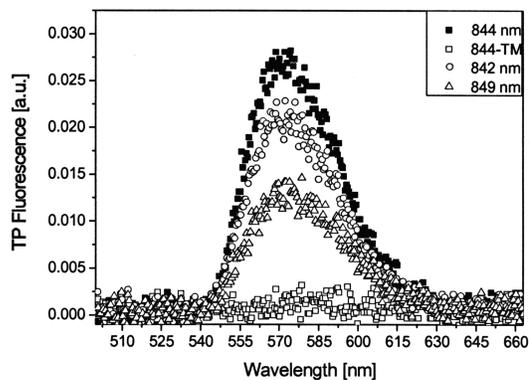


Fig. 3. TPF signal with GWS for several excitation wavelengths. Filled squares denote the excitation wavelength at resonance (TE mode); open squares, the excitation wavelength out of resonance (TM mode); triangles, maximum two-photon absorption (TE mode); circles, lower limit of the FWHM (TE mode). Average intensity, 127 W/cm<sup>2</sup>.

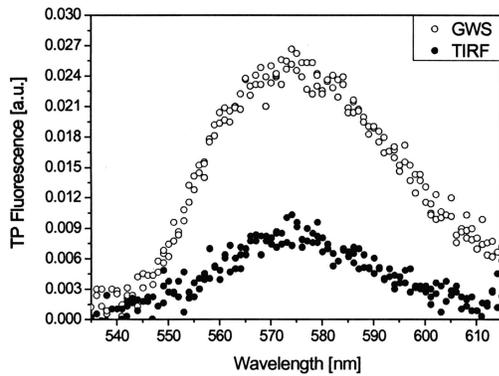


Fig. 4. TPF signal for  $\lambda = 844$  nm. TIRF, total internal reflectance fluorescence.

higher than the background. Close to the maximum two-photon absorption of TMR,  $\lambda_{\max} = 849$  nm, the fluorescence signal was reduced by a factor of 2. For wavelengths at the FWHM of the resonance bandwidth, the measured fluorescence decreased by approximately 20%. These results indicate that the maximum TPF intensity is observed only under resonant conditions. We also compared the experimental TPF results with the GWS at resonance to those with a prism onto which TMR was deposited. The results are presented in Fig. 4. As is evident, the results with the GWS are better by at least a factor of 3.

Finally, we performed measurements of TPF after the GWS was replaced with a substrate and a waveguide layer. The measured TPF values were comparable to the low-background values obtained away from resonance condition. This result indicates that the TPF enhancement with the GWS is an order of magnitude higher. No TPF was detected in the spectral region from 350 to 500 nm or in regions of the GWS

without an immobilized TMR. Thus one can exclude other nonlinear effects such as surface second-harmonic generation.<sup>9</sup>

To conclude, our procedure and results indicate that the detection of TPF can indeed be more sensitive with a resonant GWS. We expect that the overall detection sensitivities will increase even further as the methods for fabrication of GWS improve.

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