

Resonant double grating waveguide structures as enhancement platforms for two-photon fluorescence excitation

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We report a strong enhancement of two-photon fluorescence (TPF) excitation in the evanescent field of a double grating waveguide structure (DGWS). For a suitable combination of wavelength, polarization, and angular orientation of the incident laser light DGWSs show resonant behavior resulting in a large field enhancement at the waveguide surface. We demonstrate that at resonance, TPF spectroscopy reveals a 330-fold enhancement of the fluorescence signal of a tetramethylrhodamine thin film prepared from a picomolar aqueous solution. This shows the large potential of DGWSs as TPF-based high-sensitivity sensor platforms for biotechnological and biophysical application. © 2005 American Institute of Physics. [DOI: 10.1063/1.2033130]

In the past few years fluorescence-based planar waveguide technology has been widely used for optical biochemical sensors, mainly due to their superior sensitivity.^{1,2} In particular, for immunosensor applications, the fluorescence-based sensor platforms have shown impressive detection limits.³ In most of these studies conventional one-photon fluorescence (OPF) is used as analytical tool. Recently, two-photon fluorescence (TPF) measurements were performed motivated by the advantages of TPF compared to OPF.^{4–6} Specifically, the use of near-infrared (NIR) excitation light minimizes the photodamage of biomolecules, and the large separation in wavelength between excitation and emission together with the reduction of autofluorescence greatly reduce the background noise, increasing the signal to noise ratio. However, TPF excitation usually requires high photon densities available only in highly focused ultrashort pulses. In order to overcome such a difficulty in our previous studies we resorted to resonant devices.^{6,7}

We propose here the use of a double grating waveguide structure (DGWS) as fluorescence enhancement platform. DGWSs are commercially available and their first application as a sensitive fluorescence transducer for the analysis of biomolecular affinity systems was demonstrated using OPF as an analytical tool.⁸ In this letter, we report an enhancement of the TPF signal by more than two orders of magnitude for films made of 100 pM tetramethylrhodamine (TMR) dye aqueous solutions.

DGWSs consist of a thin waveguide layer on top of a substrate with one grating at the substrate-waveguide interface and one on top of the waveguide. Under resonance conditions, i.e., for a specific combination of wavelength, polarization, and incident angle of the incident laser beam, they show vanishing transmission while most of the light is reflected. The resonance bandwidth of a DGWS is given by the sum of the resonance bandwidths of the two single gratings above and below the waveguide.⁹ Therefore, such DGWSs show a good spectral acceptance for broadband ultrashort

pulses resulting in improved coupling efficiencies while preserving their high peak intensities.

The DGWS sample we used in our experiments (Unaxis Balzers, Liechtenstein) consisted of a glass substrate (Schott AF45) of 0.7 mm thickness with a rectangular grating of 360-nm period and 40-nm depth coated by a 150-nm thin-film layer of Ta₂O₅. Consequently, the surface architecture etched into the glass is transferred to the top of the waveguide, thereby creating a second identical grating at the surface of the structure [Fig. 1(b)]. A high refractive index material (Ta₂O₅) is chosen to generate a strong localized evanescent field on top of the waveguide surface.

A nonamplified mode-locked Ti:sapphire laser (Coherent, Mira 900f) was used to perform the TPF measurements [Fig. 1(a)]. The laser system produces 150 fs pulses of 8 nm spectral bandwidth at a repetition rate of 76 MHz, with tunable wavelength over the range 690–980 nm and an average power up to 1.2 W. The polarization and the incident power of the laser were controlled by a combination of a $\lambda/2$ wave plate and a polarizer.

For the TPF measurements, we used the collimated laser beam with 2 mm in diameter without focusing. The TPF signal was analyzed and detected by a spectrometer (Jobin Yvon, Triax 180) combined with a double cooled back-thinned charge coupled device (CCD) linear array (Hamamatsu, HC230-1007). The excitation light was

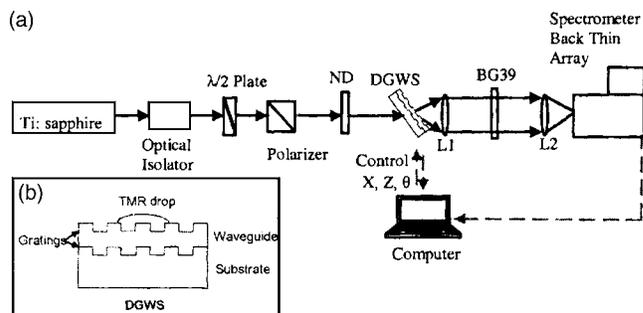


FIG. 1. (a) A schematic diagram of the experimental arrangement. (b) DGWS with a drop of TMR on it.

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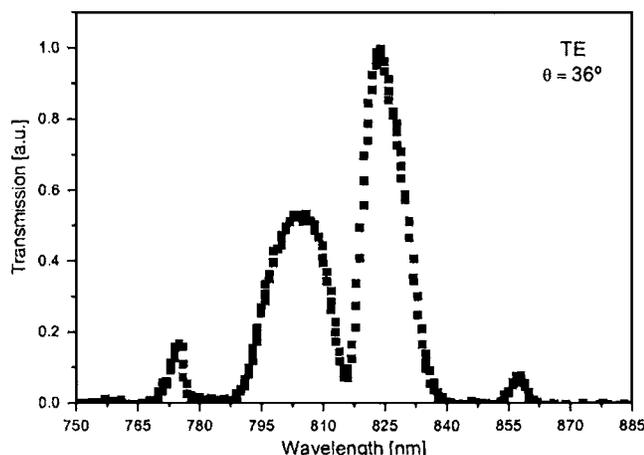


FIG. 2. Transmission spectrum of the DGWS for the TE polarization at an incident angle of 36° .

blocked by placing a NIR filter in front of the entrance slit of the spectrometer.

Our measurements show that the TE grating resonance at 815 nm occurred at an angle of incidence of 36° . Since the TM resonance at the same wavelength occurred at a significantly higher angle of incidence, it was possible to use the TM polarized pulses for the nonresonant excitation of TPF at the same incident angle of 36° . The DGWS transmission spectrum for the angle of incidence of 36° and TE polarized pulses normalized to the incident beam is shown in Fig. 2. The side peaks observed in Fig. 2 correspond to higher diffraction orders of the double grating, collected by the spectrometer at angles different from normal incidence.

We prepared uniform thin layers of TMR by depositing a $10 \mu\text{l}$ drop of the 100 pM TMR solution in milli-Q water ($\text{pH}=7.5$) on top of the DGWS. After the evaporation of the solvent, the TMR molecules remained immobilized on the surface of the DGWS, covering an area of about 18 mm^2 .

Figure 3 shows the TPF emission spectra. We first tuned the excitation wavelength from 810 to 825 nm at fixed polarization, and then we varied the polarization at fixed wavelength. The angle of incidence was kept constant at 36° . The incident laser beam (536 mW) was not focused and the fluorescence spot could be observed by the naked eye on top of the beam spot, reproducing the beam profile and size of the exciting laser pulses. As expected, near and at resonance the TPF from the DGWS could be readily observed, while far away from resonance no TPF could be observed. Near resonance, the TPF signal increases strongly, reaching its maximum at the resonance wavelength of 815 nm, indicating a strong field enhancement. Off resonance, the TPF signal was at the same level of background noise. In order to be able to compare the off-resonant TPF signal with resonant TPF, we prepared a reference sample made up of the same dye solution on top of a bare glass (BK7). In this case the immobilized TMR molecules covered an area comparable to the area on the DGWS. However, no TPF was detected under identical experimental conditions. To obtain some signal from the reference sample, we had to focus with an objective ($10\times$, 0.25 numerical aperture). The detection system was kept identical.

To compare both TPF intensities, we normalized the TPF signal from the DGWS by relating it to the TPF signal from

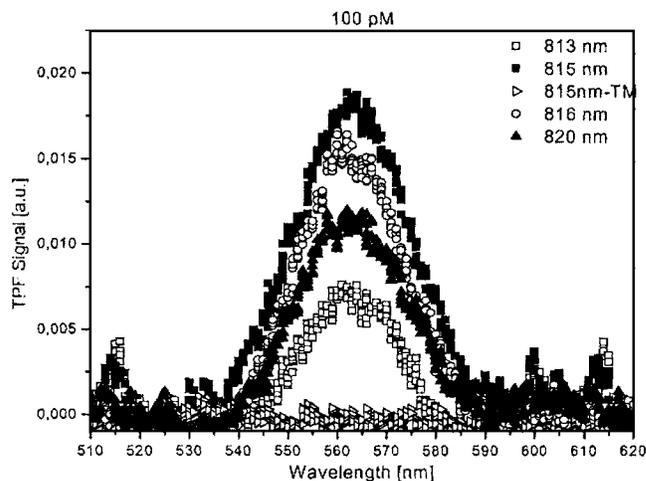


FIG. 3. TPF emission spectra of a thin layer of 100 pM TMR solution deposited onto the DGWS at different excitation wavelengths. Open squares 813 nm; filled squares resonant wavelength 815 nm; open triangles off resonance, 815 nm at TM polarization; open circles 816 nm; and filled triangles 820 nm.

the glass substrate using the following equation for the unquenched TPF intensity¹⁰

$$\text{TPF} = k \frac{\phi_2}{2} n_2 \sigma_2 l I_0^2, \quad (1)$$

where ϕ_2 is the quantum yield, n_2 is the fluorophore number density, σ_2 is the two photon absorption cross section, l is the path length, I_0 is the incident intensity, and k is a dimensionless constant that depends on the optical setup (i.e., the collection efficiency and illumination area). Using Eq. (1) and assuming the same ϕ_2 , σ_2 , and l for both samples, we obtain an expression relating the TPF intensity corresponding to the DGWS to the TPF intensity corresponding to the glass substrate

$$\text{TPF}_{\text{DGWS}} = \frac{k_{\text{DGWS}} n_{2\text{DGWS}} l_{0\text{DGWS}}^2}{k_G n_{2G} l_{0G}^2} \text{TPF}_G. \quad (2)$$

Considering the facts that the density of molecules in the TMR films on both the DGWS and the reference sample are

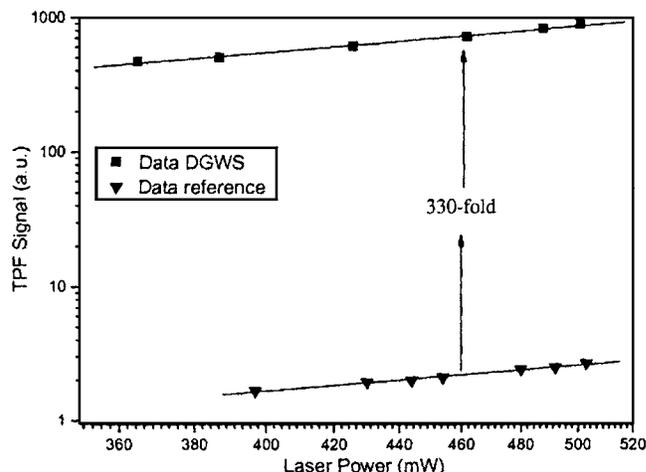


FIG. 4. Logarithmic representation of the measured TPF signal vs incident laser power obtained from the DGWS sample as well as from the reference sample. A linear slope of nearly 2 proves the quadratic dependence of TPF signal in both cases. The TPF intensity is enhanced by a factor of 330 due to the DGWS.

virtually identical (we obtained the same film area on both samples for the solution volume of 10 μl) and that the collection efficiencies are the same for both measurements (detection system was unchanged) we calculate an enhancement factor for the TPF signal of 330 from Eq. (2). In Fig. 4 we show the maximum two-photon fluorescence signal of the DGWS and the reference sample at the resonant wavelength of 815 nm of the DGWS for TE polarization as a function of the incident laser power. The experimental results are plotted in logarithmic scale and the slope of the linear fit is very close to 2, proving the quadratic dependence of TPF. The data for the reference sample are normalized by taking into account the calculated enhancement factor of 330. This allows the direct display of this factor in Fig. 4. An enhancement of this magnitude can be attributed to the evanescent field at the corrugated waveguide layer surface. The broad resonance bandwidth of 4 nm of the DGWS at full width half maximum gives a coupling efficiency of up to 50%, which leads to increased evanescent field intensities and therefore to increased TPF intensities. We can exclude other nonlinear effects like surface second harmonic generation from our measurements since no TPF was detected in the spectral region between 350 and 500 nm or in regions without the immobilized TMR.

In conclusion, we demonstrated a 330-fold enhancement of the TPF signal in TMR thin films induced by the large evanescent field on a DGWS surface. Our results indicate that DGWSs allow the detection of TPF from picomolar solutions without the need for focusing the laser beam. Therefore, DGWSs represent a powerful tool for sensitive fluores-

cence detection and enhancement, which strongly encourages their use as sensor platforms for biotechnological and spectroscopic applications.

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