Coupling efficiency of fluorescent molecules to a sensing waveguide

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The use of planar waveguides as a platform for optical biosensors provides a highly efficient and selective excitation of molecules in close proximity to the waveguide surface. Due to the coupling of the fluorophores to the evanescent field, a substantial part of the fluorescent light is coupled back into and collected by the waveguide. The utilization of this signal for fluorescence detection and analysis can allow a significant simplification of the optical instrumentation.

1 Introduction: waveguide based biochips

Optical biosensors are often based on fluorescence analysis of chemical reactions where one reagent is immobilized on the surface of a suitable substrate. The application of a thin optical waveguide at the substrate surface enables a highly efficient and selective excitation of fluorescent molecules in close proximity to the waveguide surface via the evanescent field of the guided mode. This excitation scheme turns out to be advantageous in a number of applications [1-3].

The waveguide is usually made of a thin film of a high index metal-oxide, e.g. Ta_2O_5. If its thickness is of the order of a quarter wavelength, the waveguide supports only a single mode and features a strong evanescent field which, for example, is capable of exciting two-photon fluorescence on macroscopic areas [4]. The waveguide excitation scheme can also be used for sophisticated fluorescence analysis techniques like fluorescence correlations spectroscopy (FCS).

Usually, the fluorescence light that is emitted in the space above or below the sensor chip is collected and analyzed by suitable free space optics and a detector. However, due to the vicinity of the fluorescent molecules to the interface of the waveguide layer, a substantial part of the fluorescence light is also coupled back into and collected by the waveguide. The coupling efficiency depends on position, environment and orientation of the molecules. The utilization of this integrated optical detection channel for fluorescence analysis instead of free-space observation could enable a much more compact and cost effective instrumentation on the detection side.

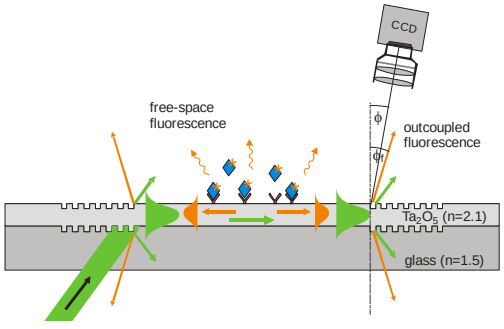

Fig. 1 *Principle of a waveguide biosensor and sketch of the experimental setup. A laser source is coupled via a grating coupler into a thin (single-mode) planar waveguide. The evanescent field of the guided mode excites fluorescent molecules in close vicinity to the surface. A substantial part of the excited fluorescence light is collected by the waveguide and guided to the grating couplers. It is deflected under the coupling angle ϕ_f determined by the dispersion properties of the grating.*

2 Detecting waveguide collected fluorescence

The collected fluorescence is guided to and ejected by the initial or a second grating coupler. It can be measured by a suitable detector, e.g. a CCD camera, which images the grating under the coupling angle ϕ_f. Due to its angular dispersion, the grating provides a spectral separation without an additional spectrometer. However, a long wave pass filter is employed in the imaging optics to completely block the excitation light.

Fig. 1 depicts the principle of the fluorescence collection and sketches the experimental setup. The used dye, rhodamine B, is immobilized on the waveguide surface by evaporation of the solvent in

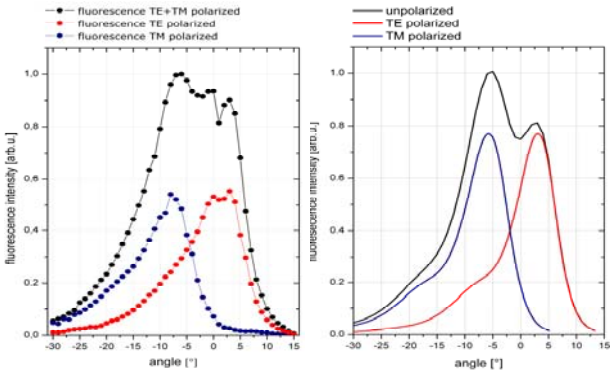

Fig. 2 *Angular spectra of collected fluorescence intensity over coupling grating. Left: Experimental data , TM-polarized excitation. Right: Angular spectra as calculated from fluorescence spectrum and grating dispersion.*

a concentration of 7×10^{-11} mol/cm². The excitation source is a Nd:YAG laser at its second harmonic with wavelength 532 nm. The detected fluorescence intensity at the coupling grating versus observation angle ϕ is shown in Fig. 2. The data are in perfect agreement with the angular spectra as calculated from the fluorescence spectrum of the dye and the dispersion properties of the grating.

3 Fluorescence waveguide coupling: theory

In the theoretical description of the fluorescence coupling efficiency to the waveguide, the dye molecules are modeled as electromagnetic dipoles close to a dielectric surface and the radiation is calculated from electromagnetic theory [5]. In case of a waveguide layer on a substrate of infinite thickness, four different contributions to the dipole radiation can be distinguished, see Fig. 3. Using the experimental dimensions and a vanishing dipole distance, a calculation of the different contributions to the radiated power for a random dipole orientation yields the results shown on the right hand side of Fig. 3. Note, that a substantial part P_{fo} of the radiation is found in the substrate at angles greater than the critical angle α_c ('forbidden zone'). With increasing thickness d, when the waveguide starts to support the first modes, an increasing part of the radiation is coupled into the waveguide. Its contribution to the total radiated power can be larger than the free-space radiation into the upper and lower halfspace (P_{up} and P_{al}).

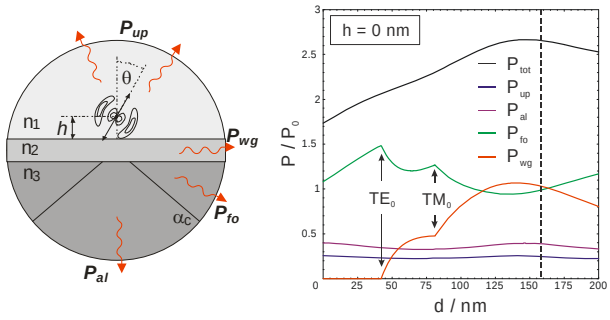

Fig. 3 *Left: Nomenclature of distinguished components of the dipole radiation in the asymptotic far-field. Right: Calculation of the different contributions to the radiated power in dependence of the waveguide thickness.*

4 Experimental verification

The dipole model also yields a prediction for the free-space radiation pattern in dependence of the observation angle, where a finite substrate thickness is now included. A comparison of corresponding results with experimental data is shown in Fig. 4 for two different assumptions. The remarkably good agreement of the experimental data with the calculations for a plane parallel dipole orientation indicates that the rhodamine B molecules immobilize on the waveguide in such a way that their dipole axis is orientated parallel to the surface.

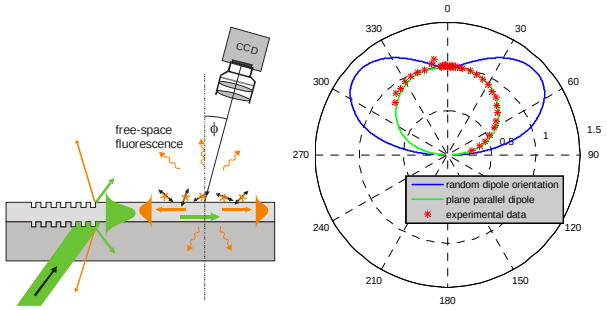

Fig. 4 *Angular dependence of the detected fluorescence intensity in free space above the waveguide. Experimental setup and data, compared to the prediction of the dipole model for the assumption of a random (blue line) and surface parallel (green line) dipole orientation.*

The radiation pattern changes if a liquid glycerol dye solution is applied to the waveguide surface instead of the immobilized dye molecules (Fig. 5). Experimental data from the free-space below the substrate suggest that the dye molecules follow a random orientation distribution in this case. Detailed calculations for the upper halfspace with this geometry will require the inclusion of additional dielectric layers above the waveguide surface.

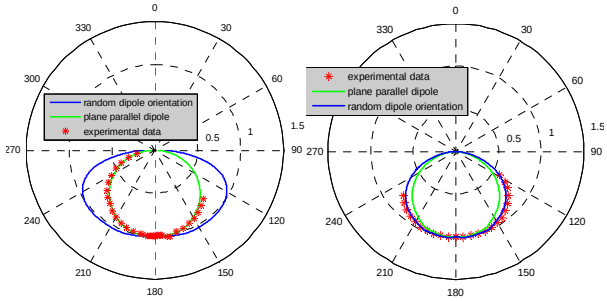

Fig. 5 *Same as Fig. 4 for the free space below the substrate. Left: immobilized dye; right: dye in glycerol solution.*

Acknowledgements

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