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Short communication

Quantitative analysis of enhanced light irradiance in waveguide-based fluorescent microarrays

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ABSTRACT

Probing microarray assays in the presence of a hybridization mix retrieves precious information on hybridization kinetics. However, in common detection schemes, useful surface signals compete with the high supernatant background from labelled targets in the mix. A known solution consists in exciting specifically the microarray surface with evanescent fields. Configurations using planar optical waveguides to produce such fields are shown here to present also a dramatic excitation irradiance enhancement at the guide/surrounding matter interface. We compare theoretically and experimentally a guided excitation with a classical external excitation. A full electromagnetic analysis predicts an irradiance increase higher than 10^4 for adequately tailored waveguides. We deposited high-index TiO_2 sol–gel waveguides on glass substrates according to best simulations. Quantitative enhancement analysis exploiting actual biological fluorescent spots perfectly confirms the irradiance amplification effect of a thin waveguide. The impact of amplification on the design of biochip readers is discussed since it leaves ample margin for simple and low-cost light couplers, advantageous in affordable readers and sensor systems.

1. Introduction

Combinatorial assays are potent tools for biological analysis, providing avenues to explore gene and protein functions in living organisms (Van Hal et al., 2000; Lueking et al., 1999). Several detection techniques are currently exploited. Label-free detection methods (e.g. Surface Plasmon Resonance (SPR), ellipsometry, ...) enable spot detection in the presence of a mix during hybridization, and thus the ability to follow hybridization kinetics or to optimize assay parameters (temperature, composition, ...). Labelled molecules are suspected to influence the binding process altering the information (Schmitt et al., 2007).

However, these methods, generally mass-sensitive, offer a poor contrast if the target size or amount becomes low. In contrast, for fluorescent or radioactive detection, the hybridization event signals only arise from labels linked to the biomolecules, without background. Among these selective techniques, fluorescence is the most used because of its simplicity (Schäferling and Nagl, 2006; Schena, 2003) and innocuity.

The sensitivity of standard top-reading fluorescence schemes is quite degraded by the hybridization mix: the reading step is thus usually performed after hybridization, washing and drying. But for fine monitoring of biological assays or shorter time to results, it is very desirable to detect spots signals in the presence of a hybridization mix. With an external excitation source, the whole mix above the spots strongly fluoresces, generating a large background and jeopardizing spot detection. These systems can regain their superior sensitivity if the microarray is excited by evanescent waves (Rowe Taitt et al., 2005) which explore only 100 nm above the surface. The spots are then well excited, unlike most of the labelled species floating above the evanescent field. Hybridized species at the surface appear with high contrast. Preferred techniques are Total Internal Reflection Fluorescence (TIRF), and optical waveguides (Lehr et al., 2003; Duveneck et al., 1997). Their advantages in terms of sensitivity are well documented (Klotz et al., 1998; Rowe et al., 1999; Kunz and Cottier, 2006).

In the present letter we quantify another aspect of thin waveguide-based excitation: a strong enhancement of the electromagnetic field at the guide/surrounding matter interface. Calculations and measurements on biological objects show that this enhancement can reach several orders of magnitude ($\geq 10^4$). We eventually discuss the impact of this enhancement on the design of

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compact and cheap high-sensitivity readers (light source, coupling in the chip, fluidics).

2. Materials and methods

2.1. Planar optical waveguides

Waveguide physics is key to our study. The predicted enhancement of irradiance at the guide interface is experimentally assessed, and confirmed by measurements on a TiO_2 sol–gel guiding layer deposited on a glass substrate (refractive index $n_{sub}(\lambda) = 1.52$) using spin-coating techniques (fabrication details will be given elsewhere). This easy in-house elaboration technique gives access to various high refractive indices and guide thicknesses. It also lends itself to low-cost fabrication.

The layer thickness and refractive index are measured by spectroscopic ellipsometry. The process is optimized in order to obtain a guiding layer thickness between 100 and 130 nm and refractive indices between 1.8 and 2. Such indices are rather low for this oxide but the fabrication process was also optimized to get a material microstructure limiting propagation losses.

2.2. Microarray preparation

In order to demonstrate the guided excitation advantage in a biological assay, we fabricated a microarray on our TiO_2 waveguides. Our aim is to assess the irradiance at the superstrate/guide interface by comparing the fluorescence of biological spots between an external and a guided excitation. From a photonic viewpoint, it is safer to use control spots instead of reading the microarray after hybridization. This method preserves the reproducibility, and ensures large signals and high experimental contrast. We thus directly deposited Alexa 647^{TM} labelled actin oligomers on the waveguide surface (few-nanometers-thick spots, see details in Supplementary material).

We underline that we did not realize a hybridization step but the fluorescent spots we deposited are good models of those that are generally detected in conventional assays.

2.3. Experimental setup

As shown in Fig. 1(a), we couple light from a collimated He–Ne laser (excitation wavelength $\lambda = 633$ nm) into the waveguide through a conventional 90° prism coupler whose index is $n_{prism} = 1.91$ (Tien and Ulrich, 1970; Ulrich, 1970). At the residual air gap, a fraction η_C of the power is coupled into the waveguide, and is optimized by scanning the incidence angle θ.

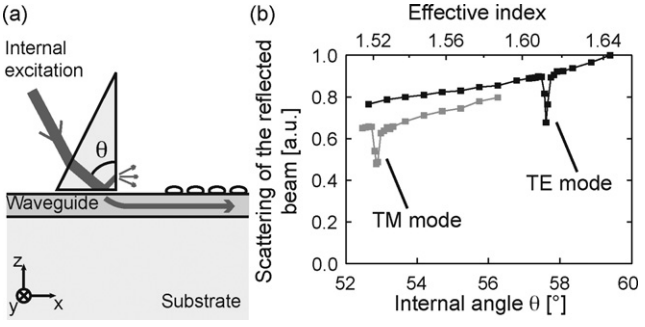

Fig. 1. (a) Coupling setup used for the internal excitation of labelled oligomers at the microarray surface. Light not coupled into the guide scatters at the unpolished prism face. (b) Typical measurement of scattered uncoupled light as a function of internal angle θ. Dips correspond to guided modes (effective indices $n_{eff}^{TE} = 1.61$ and $n_{eff}^{TM} = 1.52$).

Because measuring directly the coupling efficiency η_C commonly entails inaccuracies (Caballero-Calero et al., 2007) we rather record here the intensity variation of the prism/guide interface reflection, proportional to $(1-\eta_C)$. We do this by collecting the scattered light generated when this reflected beam impinges on the vertical unpolished prism face. The area and collection of this scattered light are such that speckle is no trouble.

Other measurements are more conventional. The guiding losses caused by waveguide defects (index fluctuations, interface roughness) are quantified with the "scattering detection method" (Nishihara et al., 1989), imaging the spatial decay of scattered light onto a charge-coupled-device (CCD). Finally, we couple this imaging system with a bandpass emission filter, yielding fluorescence images in the label emission band.

3. Results

3.1. Electromagnetic analysis

We first introduce a figure-of-merit (*FOM*) that quantitatively accounts for the gain in excitation efficiency. For a given total excitation power, if an area of 1 cm^2 is excited (i) with an external beam or (ii) with a guided wave, the *FOM* is merely the ratio of the corresponding irradiances I at the spots level (I being the square of electric field $|E|^2$):

$$FOM = \frac{I_{\mathrm{SPOT,GUIDED}}}{I_{\mathrm{SPOT,EXTERNAL}}} \quad (1)$$

Our study is restricted to applications where fluorophores lie well within the evanescence depth (~100 nm). This encompasses a large majority of techniques that use a similar localized excitation like TIRF or SPR. Within this assumption, at first order, the *FOM* reads:

$$FOM = \frac{I_{\mathrm{AIR/GUIDE\ INTERFACE,GUIDED}}}{I_{\mathrm{AIR/GUIDE\ INTERFACE,EXTERNAL}}} \quad (2)$$

For a typical ~100 nm evanescence depth into the aqueous solution, the irradiance for a fluorophore floating at only 1 μm height plummets by a factor 10^{-9} from the surface value.

Elementary geometric considerations dictate a rough *FOM* estimate. For free-space external excitation, the total exciting power is spread over 1 cm^2, against only ~1 cm × 1 μm for guided schemes (1 μm is an upper limit of a guided wave effective width), thus providing the essential source of the gain in irradiance of several orders of magnitude (~10^4). The 1 cm^2 choice is a typical biosensor area for real-time hybridization study of large spot collections (Van Hal et al., 2000; Lehr et al., 2003).

We now perform an exact calculation based on standard optical guided wave theory (Yeh, 1988). The waveguide consists of a glass substrate ($n_{sub}(\lambda) = 1.52$) coated by a high-index layer (optical index $n_g(\lambda)$, thickness t). The superstrate is provisionally air ($n_{sup} = 1$) and we present results for a core index $n_g(\lambda) = 1.95$. Fig. 2 shows the irradiance (in mW/cm^2) at the air/guide interface versus the waveguide thickness t for a total power of 1 mW carried by each of the waveguide modes. For free-space excitation, the reference surface irradiance reads 1 mW/cm^2, hence Fig. 2 directly plots the waveguide FOM.

We first note the appearance of a well-defined maximum for each mode, at a thickness just above the mode cut-off. The largest maximum, $FOM = 2.2 \times 10^4$, arises for thicknesses t comprised between 105 nm and 115 nm, which corresponds to a monomode guide for a given polarization (the cut-off is around $t = 60$ nm). The higher modes for thicker layers beat the fundamental mode at a given width, but their peak values are weaker than the fundamental mode maximum. The overall decrease