Evanescent-wave fluorescence excitation in aqueous solutions using symmetric planar waveguides

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Abstract: *We describe a method of delivering light to biological samples using a planar waveguide structure with a symmetric cladding environment. The symmetry of the waveguiding structure allows the penetration depth of the evanescent field to be tuned over a wide range by varying the thickness and/or refractive index of the waveguide. Furthermore, the symmetrical structure facilitates efficient excitation of waveguide modes using, e.g., optical fibers. We have performed fluorescence excitation experiments using of dielectric as well as metallic (surface plasmon) waveguides. The method is well suited for evanescent wave microscopy of biological samples, surface sensing and other applications that require illumination spread over macroscopic areas but confined to penetration depths ranging from 100 nm up to several μm.*

Introduction

Fluorescence microscopy (FM) can be regarded as one of the most important characterization techniques within cell biology, molecular biology and related fields [1]. Although most FM studies are carried out using conventional epi-fluorescence microscopes, there exists a wide range of more specialized techniques, including confocal laser scanning microscopy, two-photon absorption, resonance energy transfer and total internal reflection fluorescence microscopy (TIR-FM). In TIR-FM, the excitation light is usually incident on the sample from a substrate with higher refractive index (e.g. a thin glass slide) under an angle large enough for it to undergo total internal reflection at the substrate-sample interface. Fluorescence in the sample is therefore excited only within the penetration depth of the exponentially decaying evanescent field associated with the total internal reflection, typically extending approximately 100 nm into the low-index medium. A review of TIR-FM can be found in Ref. [2].

Fluorescence excitation by an evanescent field can also be accomplished using a planar waveguiding structure, where the sample (typically an aqueous solution) forms one of the waveguide cladding layers. The penetration depth of the evanescent field into the sample in this case is defined by the relationship between the refractive index of the sample, substrate and waveguide materials, as well as the thickness of the waveguide layer.

In this paper, we will present a new type of planar waveguide structure developed specifically for fluorescence microscopy applications [3], where the cladding material is chosen to match the refractive index of the sample. We discuss the special features of this waveguiding geometry and present experimental results obtained on samples containing fluorescent beads. In particular, we will focus on surface-plasmon excited fluorescence which currently attracts considerable interest in biological research [4].

Waveguide-excitation fluorescence microscopy

The use of planar waveguide structures for fluorescence excitation and microscopy has been previously reported in the literature [5]. The waveguide structure consisted of a glass substrate coated with a high-index thin film supporting a guided mode with 100–200 nm penetration depth of the evanescent field. A similar waveguide configuration was used to realize two-photon fluorescence excitation over a macroscopic surface area [6]. Conversely, Horváth et al. [7] developed a so-called "reverse-symmetry" waveguide sensor using nanoporous silica (n=1.2) as bottom cladding in order to greatly increase the penetration depth of the guided mode into the sample. This type of sensor geometry has been used to monitor cell attachment and spreading [8] but, to our knowledge, it has not been used for FM applications.

In the abovementioned experiments, light was coupled into the waveguides using surface gratings illuminated by light incident under a specific resonance angle that depends on the grating period, effective waveguide index and the excitation wavelength. Due to the asymmetry in the refractive index of the top and bottom cladding layers, the waveguides have a cut-off thickness below which no guided mode is supported by the structure, limiting the range of possible penetration depths.

The symmetric waveguide structures demonstrated in the present paper provide several advantages over previous designs. The penetration depth of the symmetric mode can be tuned over a wide range without cut-off. The guided mode can be efficiently excited directly from an optical fiber, eliminating the need for patterning of gratings and resonance-angle excitation. This also implies that the sample can be excited with multiple wavelengths through the same fiber. Aside from the fiber-coupled light source, the necessary components are no more than a few millimeters in height, meaning that a standard light microscope (normal or inverted) can be configured to deliver TIR-FM performance.

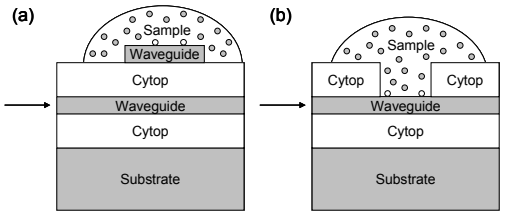

Fig. 1: Waveguide configurations used in the present study. (a) Coupled-waveguide structure using a buried "feeding core" waveguide and a surface "sensing" waveguide. (b) Single-waveguide structure with a sample well exposing part of the waveguide. Samples consisted of fluorescent beads in solution. Fluorescence is excited at the waveguide surface as indicated by the white beads.

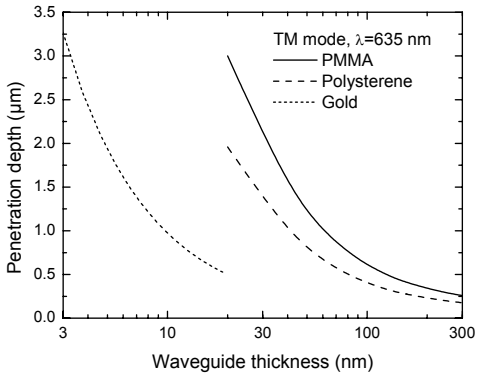

Fig. 2: Calculated $1/e^2$ penetration depth of the excitation field intensity into a sample with refractive index n=1.34.

Waveguide fabrication and system set-up

Planar waveguide structures were fabricated by spin-coating on silicon or glass substrates. Two excitation geometries were used, as shown in Fig. 1. For the bottom cladding layer we used Cytop (Asahi Glass Co.), an amorphous fluoropolymer closely index-matched to water. Waveguide layers with a wide range of thicknesses were fabricated using PMMA (n=1.49) or polystyrene (n=1.59). The waveguide film was covered with a second layer of Cytop. The area of sample excitation was defined by patterning a second waveguide layer (dielectric or metallic) on top of the cladding layer (Fig. 1a) or by making a hole through the top cladding (Fig. 1b) using reactive-ion etching. The substrates were cut into individual chips using a dicing saw.

Light at different wavelengths was coupled directly into the waveguide through the end facet using a fiber-coupled diode laser or diode-pumped solid state lasers (635 nm, 532 nm or 473 nm). Fiber-to-chip alignment was carried out by using translation stages or a specially constructed chip holder [9] while monitoring the light transmitted through the planar waveguide. A solution containing fluorescent probes (e.g. TransFluoSpheres® from Molecular Probes) was dispensed onto the waveguide chip. The fluorescence signal was collected with an Olympus BX51 research microscope and recorded with a Hamamatsu C8484 digital camera.

Mode analysis

In order to estimate the penetration depth of the excitation light into the sample, we calculated the vertical mode profile for planar dielectric waveguides of different thicknesses. Furthermore, we made similar calculations for surface plasmon polariton (SPP) waves propagating along thin gold films sandwiched between a Cytop substrate and an aqueous sample. Thin metal films in a substantially symmetric cladding environment support the propagation of so-called long-range surface plasmon polariton (LRSPP) waves, corresponding to a coupled mode of two SPP waves propagating along the top and bottom surfaces of the metal film [10]. Fig. 2 shows the penetration depth of the evanescent field into the sample for conventional dielectric waveguides as well as metallic LRSPP waveguides. The penetration depth in this case is defined as the distance where the field intensity has reached $1/e^2$ of the intensity at the surface. The LRSPP waveguide only supports a propagating TM mode whereas the dielectric waveguides also support a TE mode with a slightly shorter penetration depth (not shown).

The geometry shown in Fig. 1a supports two coupled (symmetric and antisymmetric) modes in the region of the sensing waveguide. Both modes are excited by the incoming light and interference between them results in a transfer of optical power between the "feeding core" and the surface waveguide. Similarly, efficient coupling can be achieved between a dielectric waveguide and a thin metal waveguide by matching their propagation constants. One such condition is shown in Fig. 3, where the embedded waveguide consists of a 49 nm layer of PMMA and the surface waveguide is an 8 nm gold film. The strength of the coupling between the two planar waveguides can be adjusted by tuning the thickness of the intermediate cladding layer. For the structure shown in Fig. 3, the coupling length (full power transfer from one waveguide to the other) is approximately 350 µm.

Experimental results

Microscope images of fluorescence from a solution containing 100-nm TransFluoSpheres® (T8878, 633nm/720nm) are shown in Fig. 4. The waveguide structure consists of an embedded PMMA waveguide and a gold surface waveguide with a closely matched propagation constant. Excitation light (635 nm) is incident from the left side through the embedded waveguide. Very little fluorescence is recorded in the left half of the image, where no gold film is present. The power transfer between the two waveguides is clearly seen in the fluorescence intensity. The confinement of the excitation light was confirmed by collecting images with and without filtering of the excitation wavelength (Fig. 4, left and right, respec-

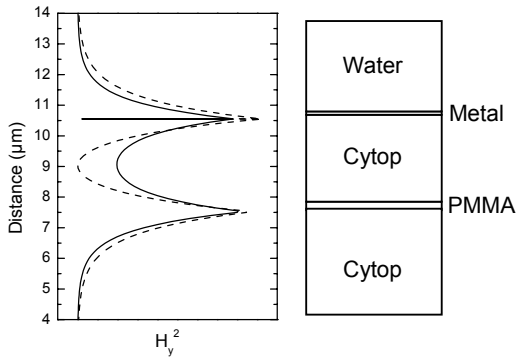

Fig. 3: Vertical mode profiles (solid line: symmetric, dashed line: antisymmetric) of a structure shown on the right, consisting of a 49 nm PMMA "feeding core," a 3.0 μm intermediate cladding layer and an 8 nm gold film surface waveguide.

tively), keeping the same camera settings. In the right image, some scattering from imperfections in the waveguides is observed but otherwise the images are very similar, confirming that fluorescence is excited primarily by light propagating in the plane of the surface.

The surface-bound nature of the excitation was also checked by observing scattering of the excitation light from gold nanoparticles. Particles in suspension appeared and disappeared as they moved randomly in and out of the excitation field. If the diffusion constant of the particles is known, the signal from a collection of particles can be used to estimate the penetration depth of the evanescent field [11].

The observed fluorescence signal in Fig. 4 decreases exponentially over a lateral distance of about 1 mm, due to absorption of the excitation light by the metal waveguide. In general, the propagation loss associated with LRSPP waveguides limits their applicability in transmitting light signals over longer distances, especially for shorter wavelengths. In order to extend the illumination range, we detuned the propagation constant by changing slightly the thickness of the embedded waveguide layer. The resulting structure supported two coupled modes, one confined mostly to the metal film and another confined mainly to the buried waveguide. Fig. 5 demonstrates how fairly uniform excitation can be obtained across a 5 mm gold film in such a structure. The signal intensity is reduced, compared to Fig. 4, because a smaller portion of the total incident power is contained within the evanescent tail.

Conclusions

We have demonstrated the applicability of symmetric planar waveguide structures to fluorescence microscopy of aqueous samples. We have also successfully carried out fluorescence imaging of live cells using the same method which will be the subject of a future publication. We believe that microscope slides using integrated optics for illumination have the potential

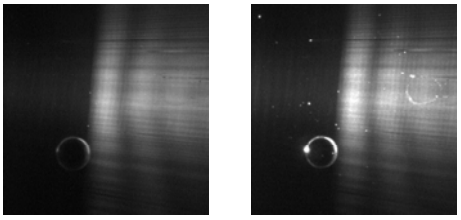

Fig. 4: Fluorescence induced on a waveguide chip with a metallic surface waveguide covering the chip in the right half of the images, with (left image) and without (right image) filtering of the excitation wavelength. The images are 1 mm across and light is incident from the left side.

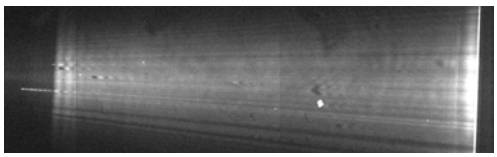

Fig. 5: Fluorescence excitation over a 5-mm wide gold film. The propagating mode is confined mainly to the dielectric feeding core. Initial beating with the rapidly decaying mode confined mainly to the metal film can be observed at the left side of the film.

to complement advanced characterization techniques in fluorescence microscopy in the future.

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