

Evanescent-wave fluorescence microscopy of biological samples in aqueous solution using a symmetric planar waveguide.

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Fluorescence microscopy (FM) can be regarded as one of the most important characterization tools in many fields of biology today [1]. Total internal reflection fluorescence microscopy (TIR-FM) [2] is a relatively recent addition to the growing number of FM methods available to biologists. In TIR-FM, an evanescent wave is created on the interface between a biological specimen and the substrate surface on which it is placed. This wave dies out exponentially normal to the surface, exciting only fluorophores within this exponentially decaying field. This results in a superior signal-to-noise ratio compared to standard FM techniques due to the absence of fluorescence excitation outside the focal plane. Even though relatively new, TIR-FM has already proven its worth for studying events at or within the cell membrane, in fluorescence correlation spectroscopy (FCS), in studying cell morphology, cell motility, focal adhesions, etc. TIR-FM is mostly limited, however, to inverted microscopes with specially designed objectives and its setup tends to be delicate in operation. The penetration depth of the evanescent excitation field is also limited to around 100 nm.

An evanescent field at a surface-sample boundary can also be realized using planar waveguides. Fluorescence microscopy carried out with such waveguides, utilizing a high-index waveguide layer on top of a glass substrate, has been previously reported in the literature but suffers from complications in excitation light in-coupling [3]. By using a *symmetric* planar waveguide, where the waveguide cladding material is index-matched to the aqueous solution containing the biological specimen, these problems can be overcome. In addition, the symmetric waveguide configuration provides a greater selection of penetration depths than other evanescent-wave methods. We have fabricated and tested such waveguides for fluorescence microscopy, as illustrated in Figure 1. Our devices consist of a planar core-layer made of PMMA ($n \approx 1.49$), embedded in a cladding material made out of the fluorinated polymer Cytop ($n \approx 1.34$), which refractive index is close to that of water ($n \approx 1.33$). Cytop is also chemically robust and is optically transparent over wide range of wavelengths (200-1600 nm) [4].

Excitation light is efficiently coupled into the structure using a standard optical fiber, eliminating the need for patterning of gratings and use of angle-dependent coupling. The excitation wave travels through the structure, generating an evanescent wave at the core-cladding boundary. Fluorophores are excited within the reach of the evanescent wave, characterized by a penetration depth given by the exponential decay length. The resulting fluorescence signal is then picked up by a standard upright optical microscope as shown in Figure 1. The penetration depth of the symmetric mode can be tuned over a wide range without cut-off and is therefore more flexible than both TIR-FM and previous waveguide-excitation designs. The guided mode can be efficiently excited directly from an optical fiber, using end-fire excitation. This also implies that the sample can be excited with multiple wavelengths through the same fiber. The waveguide chips, together with a fiber-coupled light source, can therefore deliver TIR-FM performance on a standard (normal or inverted) light microscope. Furthermore, the chips can deliver continuous illumination of high intensity confined to a sub-micrometer penetration depth over macroscopic areas for low light level and/or high frame rate imaging that would not be possible to carry out with, e.g., a confocal laser scanning system. The waveguide device is fabricated in standard clean-room facilities using standard equipment such as spinner, oven, reactive-ion etcher and metal deposition chamber.

As a proof-of-principle, a cluster of cancer cells from a standard cell line (MCF7), labeled with antibody against the trans-membrane adhesion protein E-Cadherin, were imaged using the described waveguide device and compared to standard epi-fluorescence microscopy. Figure 2a shows that the waveguide generates a clear image with obvious cell outlines and cell-cell contact regions, as is to be expected from E-Cadherin labeling. Figure 2b shows that the fluorescence signal achieved with at standard epi-fluorescence microscope is dominated by a signal from the bulk of the cell cluster, making detection of cell outlines and weak signals from the near-surface region difficult.

Even though the chip is especially well suited for cell studies (live or fixed), its application is not limited to that alone. In principle, all fluorescence studies where excitations should be limited to a small volume close to the surface are especially well suited for the described sensor chip.

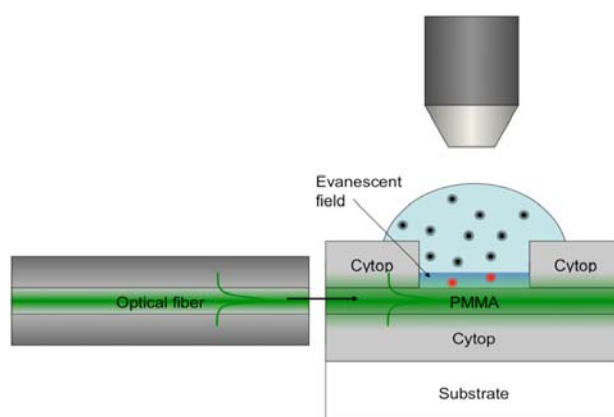


Figure 1 Schematic representation of the biosensor. Excitation light is coupled into the waveguide structure using a standard optical fiber. The excitation wave travels through the structure generating an evanescent wave at the core-cladding boundary. This wave penetrates a specific length (penetration depth) into the solution, exciting fluorophores on its way. The fluorescence signal is picked up by a standard upright optical microscope.

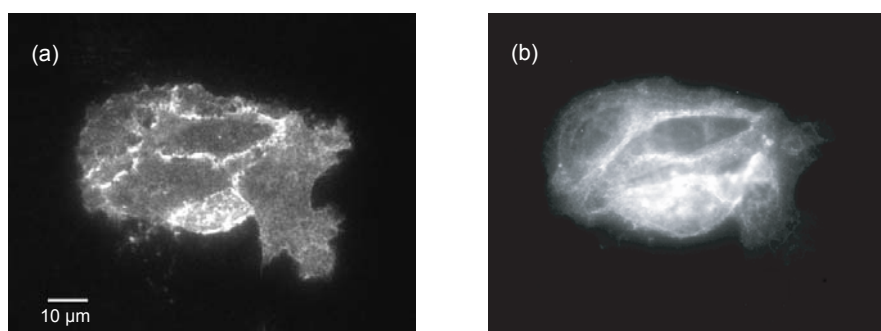


Figure 2 a) Cluster of MFC7 breast cancer cells labeled with antibody against the transmembrane protein E-cadherin imaged using symmetric waveguide structure. The penetration depth of the excitation light is about 160 nm into the cells. The image is obtained using a water immersing lens with NA=1.2 and 63 \times . b) Same cluster imaged using a standard epi-fluorescence microscope. The image is obtained using an oil immersing lens with NA=1.3 and 63 \times .

References

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