

# Bio-imaging using high vertical resolution fluorescence spectroscopy

**A.K. Swan and M. S. Ünlü**

*Electrical and Computer Engineering, Boston University, 8 St Mary's St., Boston, MA 02215*  
Telephone 617 353 1712, [swan@bu.edu](mailto:swan@bu.edu), [selim@bu.edu](mailto:selim@bu.edu)

**Y. Tong and B.B. Goldberg,**

*Department of Physics, Boston University, 590 Commonwealth Ave., Boston MA 02215*

**L. Moiseev and C. Cantor,**

*Department of Biomedical Engineering, 36 Cummington Ave., Boston MA02215*

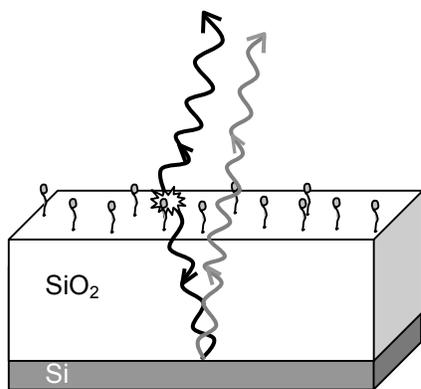
We present a new method of fluorescence imaging, which yields an unprecedented vertical resolution. The basic principles are related to an already well-established technique of using spatially modulated emission intensity from standing waves.<sup>1,2</sup> However, instead of spatial variation, our method uses the unique *spectral* signature of the fluorescent emission intensity above a reflecting surface to determine vertical position unambiguously. We demonstrate better than 5-nm vertical resolution by clearly distinguishing markers bound directly to the substrate from those bound on top of a single layer of streptavidin. Applications of this technique include intracellular imaging and screening for specific bacteria, virus or proteins, where discrimination between raised fluorescently labeled specifically bound markers from non-specific binding is crucial.

Emission spectrum of fluorescent markers is modified in the presence of a dielectric surface. When markers are on or above a reflecting surface, the self interference of emitted photons result in enhanced or suppressed emission (constructive or destructive interference) as schematically shown in Fig.1. The resulting *spectral* oscillations of the fluorescent emission intensity have unique wavelength dependence for a given. Even a very small height difference of several nm can be observed as a shift of the spectral peak positions and the period of oscillation.

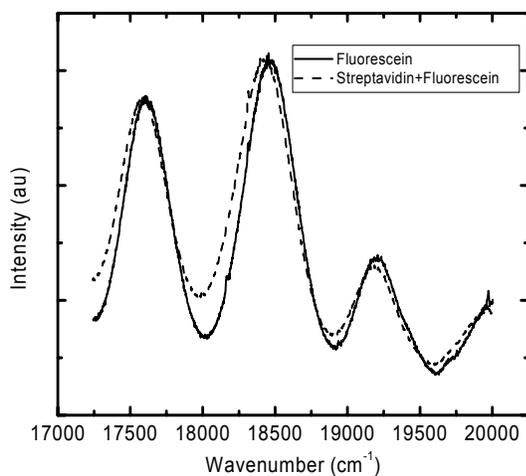
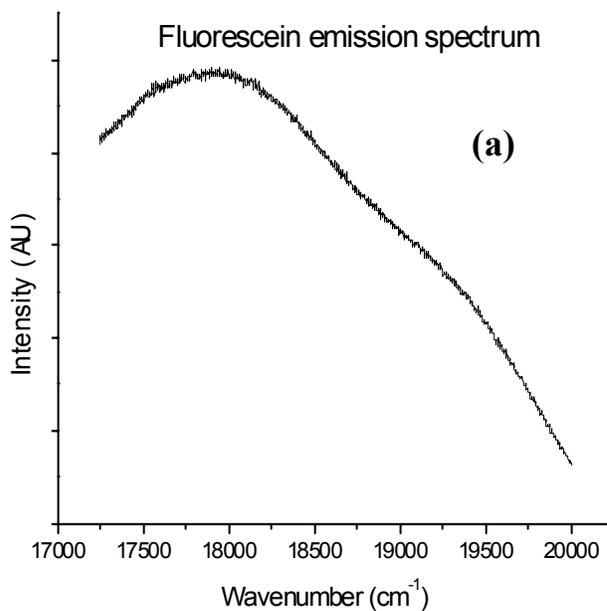
We have used a wedge-shaped substrate of a 5  $\mu\text{m}$ -thick  $\text{SiO}_2$  on top of Si with a slope of 70 nm/mm to follow the evolution of the spectra with increasing separation of the markers from the reflecting Si/SiO<sub>2</sub> interface (25% reflectance). Fluorescein is then immobilized on the wedge surface. Figure 2(a) shows the emission spectrum of fluorescein on a thick microscope glass slide without emission interference and Fig. 2(b) shows the emission interference on the wedge as a function of separation from the reflecting interface. The excitation wavelength used is 488 nm from an Ar ion laser, and the recorded part of the emission spectrum is 500 to 580 nm [displayed as wavenumbers ( $\text{cm}^{-1}$ )]. A 5X objective (NA=0.12) is used to avoid integration of a wide range of optical path lengths. We have applied the technique has been applied to measure the height difference between fluorescein immobilized directly on a substrate and on top of a protein (streptavidin-C). Figure 3 shows the two clearly differentiated spectra as a result of the small height difference. A simple analysis of our measurements yields an optical path length of 5 to 8 nm that agrees well with the known size of protein ( $\sim 5\text{nm}$ ). Currently, we are developing more sophisticated models for the spectral emission for dipole fields above dielectric interfaces which take into account chromatic dispersion and numerical aperture. We will compare experimental and theoretical results and evaluate the potential of this method for deconvoluting a spectral signal obtained simultaneously from markers at several different heights.

1. B. Bailey, D. Farkas, D. Taylor, F. Lanni, *Nature* **366**, 44, 1993

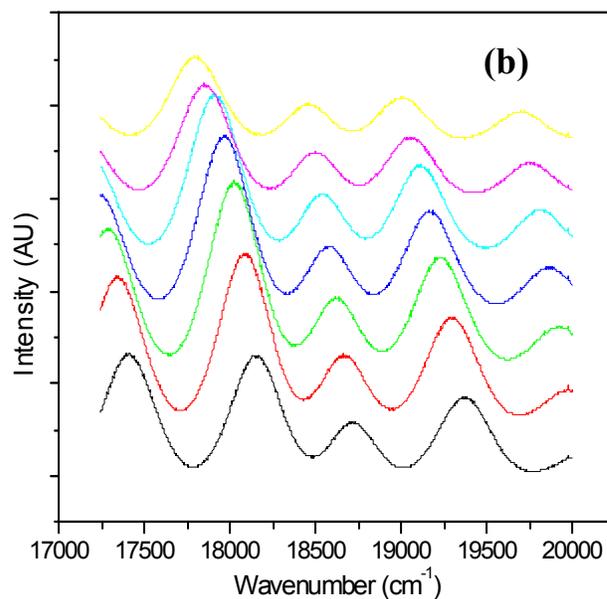
2. A. Lambacher, P Fromherz, *Appl. Phys. A* **63**, 207, 1996



**Fig. 1:** A schematic illustration of the self-interference of the emission spectra of a single fluorophore. For this particular example, the thickness, index, and wavelength are such that destructive interference occurs.



**Fig 3.** Experimental spectral response of fluorescein immobilized on the immediate surface (solid line) in comparison with that immobilized on top of streptavidin.(dashed).



**Fig. 2.** (a) Fluorescein emission spectrum without self-interference from buried mirror interface. (b) The spectral oscillations observed experimentally on a SiO<sub>2</sub>/Si wedge. As the SiO<sub>2</sub> layer increases, the peaks move towards lower wavenumber and the peak separation shrinks