

WAVEGUIDE EXCITATION FLUORESCENCE MICROSCOPY: A NEW TOOL FOR SENSING THE BIOINTERFACE

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INTRODUCTION: The ability to investigate interactions between a biological system and a synthetic surface is of critical importance to our fundamental understanding of biomaterials and their many applications in biosensors, medical implants, and tissue engineering. For this reason, (bio)sensing platforms capable of sensing (bio)molecular interactions have become an invaluable tool for discerning which events occur, when they occur, as well as the kinetics and affinity of the interactions at a given biointerface. We have recently developed a general sensing platform that combines the power of ultra-sensitive biosensing with the simultaneous ability for fluorescence microscopy imaging of bio-interfaces in their natural aqueous environment¹.

METHODS: The technique utilizes the evanescent field from a planar optical waveguide to excite fluorescence in the near interface region and is thus referred to as the Waveguide Excitation Fluorescence Microscope (WExFM). The final prototype of the WExFM is intended to be an add-on for a standard inverted fluorescence microscope (FM), thus ensuring ease of adaptability.

The technique is centered around a thin film planar optical waveguide coating on a glass substrate. Incoupling of laser light into the waveguide occurs via an optical grating and will only occur at a specific angle of incidence that is dependent on the wavelength, the grating properties, and the refractive index of the surface overlayers. Thus, as biomolecules adhere to the surface a change in the incoupling angle will occur and this can be converted into a change in mass and thickness.

Furthermore, once the light is traveling along the length of the waveguide, the evanescent field penetrating out from the waveguide into the overlying environment can be used to excite fluorescence in fluorescent molecules. Thus, by placing the waveguide onto a microscope it is possible to image while sensing mass adsorption all in real time and in-situ (i.e. in a liquid environment for example).

RESULTS & DISCUSSION: Studies of the streptavidin-biotin binding event have already demonstrated a sub-picomolar sensitivity for the WExFM technique. In the Fig. 1. a comparison of normal fluorescence and WExFM imaging is shown for a 10ng/mL solution of Alexa 633 labeled

streptavidin in contact with a 60x60 μ m biotinylated surface pattern. The WExFM image (Fig. 1b) illustrates the surface sensitivity of the technique whereby the background is measured to be essentially zero, as expected.

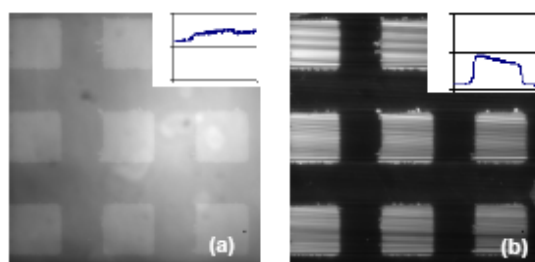


Fig. 1: a) normal fluorescence and b) WExFM image of fluorescently labelled streptavidin binding to biotin immobilised in a 60x60 μ m pattern and backfilled with a protein resistant PEG graft co-polymer.

In addition to biomolecular interactions, preliminary cell-surface studies have shown the improved signal to noise of the WExFM technique for the study of focal adhesions when compared to epi- and confocal fluorescence microscopy. In addition, some interesting insights into the processes occurring between lipidic vesicles and synthetic surfaces and subsequent bilayer formation have been obtained using this technique.

CONCLUSIONS: The WExFM provides a new and unique method for the dynamic, in-situ study of the biointerface. Advantages of the technique include high target sensitivity for fluorescence detection (<20 pM already demonstrated), high surface specificity (ca. 200 nm perpendicular to the waveguide), large area analysis with submicron resolution, 'built-in' calibration of fluorescent light gain, and the capability to perform multicolour imaging in-situ and in real time.

REFERENCES: ¹ HM Grandin (In Press, 2005) *Biosensors & Bioelectronics*, and references therein.

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