Available online at www.sciencedirect.com
SCIENCE DIRECT
Biosensors and Bioelectronics 21 (2006) 1476–1482
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Waveguide excitation fluorescence microscopy: A new tool for sensing and imaging the biointerface

H.M. Grandin *, B. Städler, M. Textor, J. Vörös

BioInterface Group, Laboratory for Surface Science and Technology, Department of Materials, Swiss Federal Institute of Technology (ETH) Zurich, CH-8093 Zurich, Switzerland

Received 18 April 2005; received in revised form 14 June 2005; accepted 17 June 2005
Available online 30 August 2005

Abstract

A novel biosensing and imaging technique, the waveguide excitation fluorescence microscope, has been developed for the dynamic and quantitative investigation of bio-interfacial events in situ, ranging from ligand-receptor binding to focal adhesion formation in cell–surface interactions. The technique makes use of the evanescent field created when light travels in a mono-mode, planar optical waveguide to excite fluorescence in the near interface region. Advantages of the technique include high target sensitivity for fluorescence detection (femtomolar range), high surface specificity (ca. 100 nm perpendicular to the waveguide), large area analysis with submicron resolution, 'built-in' calibration of fluorescent light gain, and the capability to perform multi-colour imaging in situ and in real time. In this work, the sensitivity of the system has already been demonstrated through dynamic measurements of the streptavidin–biotin binding event to below 20 pM concentrations, signal to noise comparisons with conventional fluorescence microscopy have shown more than a 10-fold improvement, and surface specificity of the technique has also been illustrated in a comparison of fibroblast focal adhesion images. Thus, this new tool can be used to illuminate processes occurring at the interface between biology and synthetic surfaces in a unique manner.

Keywords: Fluorescence; Biosensor; Biointerface; Evanescent field; Proteins; Cells

1. Introduction

The ability to study molecular events occurring at the interface between biology, e.g. proteins and cells, and synthetic surfaces, e.g. metal oxides and model membranes, in the presence of a biologically natural, aqueous environment is of primary importance for advancements in biomedical technology (Kasemo, 2002). Applications ranging from medical implants and tissue engineering to biomimetic/biofunctional materials and medical diagnostics require an understanding of the fundamental processes involved in such biological–synthetic surface interactions. For this reason, (bio)sensing platforms, capable of sensing (bio)molecular interaction dynamics occurring at surfaces, have become an invaluable tool for discerning which events occur and when, as well as the kinetics and affinity of the interactions at a given biointerface.

Of the commercially available optical biosensors, the Biacore (Biacore AB, Sweden) is undoubtedly the market leader (Rich and Myszka, 2005). This is a label free technique whereby changes in the refractive index of the near-interface region, upon binding of a biomolecule to the surface, can be detected by monitoring the angle at which surface plasmon resonant excitation occurs. An alternative technique, also commercially available, is the optical waveguide lightmode spectroscopy (OWLS, Microvacuum Hungary) (Tiefenthaler, 1993). This technique follows changes in the incoupling angle of laser light into a planar waveguide via an optical grating due to changes in the refractive index upon binding of a biomolecule to the surface. As these are both label free techniques there are obvious advantages, such as ease of analyte preparation, on the other hand a disadvantage as compared to a fluorescence based technique

* Corresponding author. Tel.: +41 44 633 6148; fax: +41 44 633 1027.
E-mail address: michelle.grandin@mat.ethz.ch (H.M. Grandin).

0956-5663/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2005.06.011

is the limited sensitivity (Taitt et al., 2005; Brecht et al., 1998).

Correspondingly, one the most sensitive microarray readers on the market, the Zeptoreader, is based on fluorescence (Zeptosens, Bayer Tech. Services GmbH) (Duveneck et al., 1997). It makes use of the evanescent field, generated by light traveling in a waveguide, to excite and detect fluorescence in the near-interface region of a microarray chip and has a sensitivity down to picomolar concentrations which is equivalent to zeptomoles of captured antibody (Pawlak et al., 2002). Even more sensitive is the surface plasmon field-enhanced fluorescence spectroscopic method developed by Knoll et al., demonstrating attomolar sensitivity (Yu et al., 2004; Liebermann and Knoll, 2000).

Although each of these techniques has contributed a significant amount to our understanding of bio-surface interactions (Voeroes et al., 2002; Fivash et al., 1998; Duveneck et al., 2002; Yu et al., 2003), it is only the Zeptoreader that provides spatial information, though limited to low magnification. As images with high spatial resolution combined with the dynamic sensing of biomolecular interactions can provide new insight into the processes of bio-surface interactions, we have designed and built a new sensing platform based on fluorescence excitation by an evanescent field that is compatible with an inverted microscope.

This so called 'waveguide excitation fluorescence microscope' (WExFM) is a novel technique, based on an evanescent field excitation of fluorescence from a planar optical waveguide, capable of highly surface sensitive investigations of bio-interfacial phenomenon from ligand-receptor to cell–surface type interactions. Further advantages of the WExFM technique include: high target sensitivity for fluorescence detection (femtomolar range), the capability to perform multicolour, in situ, temporally resolved experiments, as well as large area analysis with submicron resolution, and 'built-in' calibration of fluorescent light gain. In this paper, the experimental set-up of the WExFM will be described followed by first results that illustrate the sensitivity of the technique. In addition, the improved imaging capabilities compared with conventional fluorescence will be demonstrated. Finally, a brief discussion of future areas for which this newly combined sensing and imaging microscopy technique should contribute interesting new insights will be presented.

2. Experimental

2.1. Instrument set-up

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 for researchers already equipped for fluorescent measurements. Therefore, the initial construction has involved the adaptation of our own inverted Zeiss Axiovert 100 TV FM for this purpose. A schematic and photograph of the WExFM are shown in Fig. 1.

Central to the technique is the planar optical waveguide (WG) substrate, which has a grating relief pattern embedded into the WG layer (see Fig. 1). These are commercially available, e.g. Microvacuum, Zeptosens. It is via this optical grating that mono-mode laser light can be efficiently incoupled, but only at a specific angle of incidence that is dependent on the wavelength, the grating properties as well as the refractive index of the substrate, the waveguiding layer, the adlayer and the surrounding solution above the grating. Slight changes in the refractive index above the grating, due to molecular adsorption or changes in the surrounding solution, can be determined by the corresponding shift in the incoupling angle. The information obtained for both the transverse electric and the transverse magnetic modes can then be used to determine the mass and thickness of the adsorbed material via de Feijter's formula; this is in essence the OWLS technique and is also incorporated into the WExFM. More details on the use of planar waveguides as sensors are described by Lukosz (1991).

In the WExFM, the evanescent field generated by the incoupled light is also used to excite fluorescence in the interfacial region. The penetration depth of the exponentially decaying evanescent field is roughly 100–200 nm for visible light traveling in a thin (ca. 200 nm) mono-mode waveguide. Thus, as the evanescent wave extends from the incoupled light along the length of the incoupled light beam, the fluorescence sensitivity of the WExFM also occurs along the length of the incoupled light beam (typically 1 mm × 20 mm). In contrast, the mass sensitivity of the OWLS method occurs only on the grating area illuminated (ca. 1 mm^2) whereby it is only upon mass adsorption onto the grating that a change in in-coupling angle can be detected.

In our set up, the laser light sources are coupled into mode-preserving fiber optic cables (KineFlex Broadband Fibre System, Point Source) equipped with a collimating lens, fitted onto an angular scanning goniometer, and directed onto the optical grating. Thus, the light source can be scanned to determine the angle at which incoupling occurs, while the sample itself remains stationary. For imaging this is an advantage over the OWLS technique in which the sample is rotated while the laser remains fixed. Furthermore, co-incident laser beams of different wavelengths enable multicolour experiments with ease. The angle of maximum incoupling is monitored by the photodiode (Hamamatsu) placed at the end of the waveguide.

The WG forms the base of a controlled flow-through cell designed for the instrument in order to investigate interfacial processes occurring at a solid–liquid interface. Relevant biomedical thin films of TiO_2 and Nb_2O_5, for example, can also be coated onto the WG, provided that they are optically transparent. Surface immobilization of protein receptors, antigens, DNA, or other relevant biomolecules is also possible via many different immobilization techniques (Huang et al., 2002; Peelen and Smith, 2005). Thus, a number of surface events can be monitored, such as binding interactions (either by introducing a second molecule that is fluorescently labeled or via quenching), release of fluorescent dye from