Sensors and Actuators B 134 (2008) 545–550

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Imaging SPR for detection of local electrochemical processes on patterned surfaces

Olof Andersson, Christian Ulrich, Fredrik Björefors, Bo Liedberg*

Division of Sensor Science and Molecular Physics, Department of Physics, Chemistry and Biology, Linköping University, SE-581 83 Linköping, Sweden

ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 20 May 2008
Accepted 23 May 2008
Available online 6 June 2008

Keywords:
Imaging surface plasmon resonance
Microcontact printing
Local electrochemical analysis
Surface analysis

ABSTRACT

Imaging surface plasmon resonance (iSPR) was used in conjunction with voltammetry to investigate the possibility of detecting local electrochemical processes in situ on chemically modified electrodes. More specifically, a pattern of self-assembled monolayers (SAMs) of thiocholesterol and 1-hexadecanethiol was microcontact printed on gold substrates, and the blocking characteristics on different parts of the pattern were investigated. The SPR images reflected the changes in the refractive index over the working electrode due to electrochemical processes, which in the present case showed the ability of the SAMs to impede faradaic reactions. The results show that differences in packing densities or porosity of SAMs in different regions of a patterned surface can be visualized as electrochemical images using iSPR. The strength of utilizing an optical detection method for electrochemical characterization lies in the ability to achieve lateral resolution in real-time. Electrochemical reactions can also be used to enhance the contrast in SPR images of thin layers of components with similar thicknesses and refractive indices.

1. Introduction

Patterned or otherwise heterogeneously composed surfaces serve important functions in biomimetic materials development and can be used as backbones in biochemical sensors. Microcontact printing (μCP), for example, is a commonly employed method of producing patterned surfaces suitable as platforms in microarray chips [1,2]. Using μCP, well-organized self-assembled monolayers (SAMs) can be obtained by transfer of alkanethiols onto gold substrates [3]. Surface plasmon resonance (SPR) sensing is an established technique for monitoring biochemical interactions at such surfaces [4,5]. Basically, the SPR technique is sensitive to changes in the refractive index in the close vicinity (a few hundred nanometers) of a thin free electron metal layer [6,7]. SPR can be further extended to enable mapping of the optical properties of two-dimensionally patterned surfaces, a technique commonly known as imaging surface plasmon resonance (iSPR) or surface plasmon microscopy [8,9]. In iSPR, the measured parameter is usually the reflectivity at a fixed angle of incidence. For a particular incident light wave vector, a shift in the reflected intensity from a certain sensing area is proportional to a change in the optical properties within that area. Using a two-dimensional array detector (e.g. a CCD camera), iSPR can be used to detect local changes in refractive index, caused by for instance chemical binding, at square millimetre-sized substrates incorporating hundreds or even thousands of different chemically modified areas.

Cyclic voltammetry (CV) is a frequently employed method for evaluation of the quality of self-assembled monolayers [10]. From CV measurements, qualitative information on the molecular ordering can be obtained via the blocking of faradaic reactions. In traditional CV experiments the collective current from the reactions at the substrate is measured, hence no lateral resolution is obtained. Scanning probe techniques, such as scanning electrochemical microscopy (SECM), on the other hand, provide spatial resolution of electrochemical processes at a micron to submicron level. SECM, however, typically requires scanning of the substrate, making it a less suitable technique for exploration of larger surfaces. Moreover, measurements in parallel over different regions of a sample can normally not be accomplished with SECM [11].

SPR and electrochemistry has been combined in a number of different applications, for instance for investigations of conducting polymer films [12,13], redox proteins [14], and enzymatic reactions [15,16]. Heaton et al. used SPR coupled with electrochemistry in an application intended for label-free DNA hybridization analysis [17]. A few examples also exist on the combination of electrochemical techniques with imaging SPR. Ertl and co-workers employed iSPR in a study of spatial potential wave patterns on bare gold electrodes [18]. Niwa and co-workers combined SPR with CV and showed that the SPR signal could be correlated to an electrochemical process on

---

* Corresponding author. Tel.: +46 13 281877; fax: +46 13 288969.
E-mail address: bol@ifm.liu.se (B. Liedberg).

0925-4005/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2008.05.042

the surface [19–21]. They also used imaging SPR to study the activity of an enzyme immobilized in a single spot on a gold electrode [22].

In this work, we combine iSPR and electrochemistry with the aim of visualizing the extent of electrochemical processes due to differences in the packing density or porosity of multicomponent, microcontact printed, thiol SAMs. As a model system, we printed a grid pattern of two different thiols, thiocholesterol (TC), and 1-hexadecanethiol (HDT) on gold substrates. The patterns were created in the micrometer size range, which is a common regime for biosensor array elements. TC is a molecule interesting in studies of for instance molecular recognition at biomembrane interfaces, and is known to form nanoporous monolayers on gold [23–25]. HDT, on the other hand, forms very well organized and dense layers with excellent blocking characteristics on gold [26]. The response in the electrochemical iSPR images presented in this work relies on differences in the refractive index between the oxidized and reduced forms of a redox specie, and, to a minor extent, also on variations in optical properties due to changes in composition of the electric double layer.

2. Experimental

2.1. Imaging SPR

SPR images were acquired using an in-house custom built instrument based on the Kretschmann configuration [27]. A schematic outline of the imaging SPR instrument and the cell used in the electrochemical experiments is shown in Fig. 1. The setup consists of two vertically oriented synchronously movable rotation stages (Newport Corp., Irvine California, USA), each carrying an optical rail, and an optically centred 60° prism (BK7, Melles Griot Inc., Carlsbad, CA, USA). A discharge lamp (300 W Xenon, Oriel Corp.) was used as a fixed white light source. The light was collimated, p-polarized and filtered (760 nm) before being guided towards the prism using mirrors mounted on one of the movable arms. A refractive index matching oil (Cargille-Sacher Laboratories Inc., Cedar Grove, USA) was used to achieve optical contact between the prism and the substrate. A CCD camera (1 MP, Retiga EXi, Qimaging Corp., Surrey, Canada) with imaging optics, mounted on the second movable arm, was used to measure the intensity of the reflected light. The rotation stages enabled scanning of the incidence and reflection angles in the range 50–80°. 12 bit images from the CCD camera were transferred to a computer by means of a LabView (version 8, National Instruments) controlled frame grabber card at a rate of 5 Hz. Each pixel in the images depicted an area of $\sim 2\,\mu m \times 4\,\mu m$ of the substrate. Acquisition of SPR curves by angular scanning was possible at a rate of 0.1 Hz. The LabView-based control software enabled monitoring of the SPR image intensity as a function of time. The measured data was later synchronized with the applied potential to give iSPR voltammograms. All acquired SPR images were normalized by subtraction of an image taken at the open circuit (Fig. 3) or at the starting potential (Figs. 4 and 5), resulting in images where the pixel values represent the shift in intensity upon changing the potential.

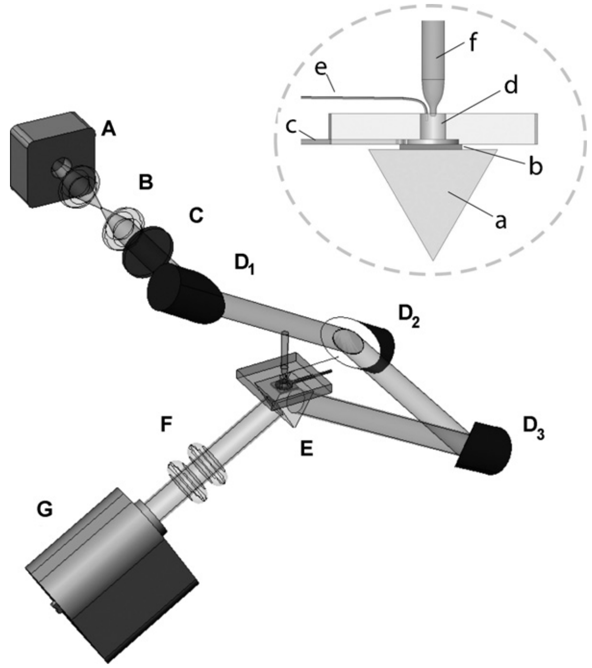

Fig. 1. Schematic view of the imaging SPR instrument. The components are light source (A), collimator (B), p-polarizer and interference filter (C), mirrors (D_1–D_3), prism (E), imaging lenses (F), and CCD detector (G). The inset shows the prism (a) and gold surface (b) with the electrochemistry cell on top: contact pad (c), liquid cell (d), counter electrode (e), and reference electrode (f).

The SPR sensor surfaces (simultaneously used as working electrodes) consisted of 12 mm × 12 mm glass slides coated with a 45 nm thick film of gold. The gold surfaces were obtained as a generous gift from GE-Healthcare (Biacore), Uppsala, Sweden.

2.2. Electrochemistry

Electrochemical iSPR was performed in a 150-μl PTFE cell, tightly sealed to the working electrode using a nitril rubber o-ring. A counter electrode (Pt wire) and a reference electrode (Ag/AgCl, saturated KCl) were also immersed in the cell. The SPR surfaces/working electrodes were contacted using gold plated pads pressed onto the gold film. An Autolab PGSTAT20 (Ecochemie, Utrecht, The Netherlands) was used in all experiments. Hexacyanoferrate(III) ($K_3Fe(CN)_6$, Merck) was used as the model redox specie. In all solutions 100 mM KNO_3 (Merck) was used as supporting electrolyte. The formal potential of the hexacyanoferrate redox-couple was found to be around 0.2 V (vs. Ag/AgCl). During the CV experiments the potential was scanned between 0.6 V and −0.2 V, with a scan rate of 50 mV/s.

2.3. Surface chemistry

The gold surfaces were patterned with thiol monolayers using microcontact printing. Prior to stamping, the surfaces were cleaned in a mixture of 25% ammonia, 30% hydrogen peroxide, and Milli-Q water at a ratio of 1:1:5 at 85 °C for 5 min. A silicon wafer, photolithographically patterned with structures from a negative photoresist (SU-8), was used as master for the molding of the polydimethylsiloxane (PDMS) stamp. The structure consisted of 200 μm wide stripes separated by 200 μm wide grooves. After curing, the PDMS stamp was pealed off from the master and cleaned by ultrasonication in ethanol (99.5%) for 3 min. It was then incubated for 60 s with alkanethiols dissolved in ethanol, thoroughly dried under a stream of nitrogen gas, and finally put in conformal contact with the surface. After a contact time of 45 s, the surfaces were rinsed and ultrasonicated in ethanol for 3 min. Patterning of multiple thiols was achieved by rotating the samples 45° and repeating the incubation and stamping procedure. The substrates were first stamped with HDT in a horizontal pattern. After rinsing and ultrasonication, an additional diagonal pattern of TC was transferred on top of the previous HDT pattern. Through diagonal patterning, the orientation of the surface could be used to distinguish the different areas from one another. The TC adsorbed predominantly to the bare gold areas, leading to a final two-dimensional surface structure consisting of