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Compact, high performance surface plasmon resonance imaging system

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Abstract

We report the construction and characterization of a new compact surface plasmon resonance imaging instrument. Surface plasmon resonance imaging is a versatile technique for detection, quantification and visualization of biomolecular binding events which have spatial structure. The imager uses a folded light path, wide-field optics and a tilted detector to implement a high performance optical system in a volume 7 in. × 4 in. × 2 in. A bright diode light source and an image detector with fast frame rate and integrated digital signal processor enable real-time averaging of multiple images for improved signal-to-noise ratio. Operating angle of the imager is adjusted by linear translation of the light source. Imager performance is illustrated using resolution test targets, refractive index test solutions, and competition assays for the antiepileptic drug phenytoin. Microfluidic flowcells are used to enable simultaneous assay of three sample streams. Noise level of refractive index measurements was found to decrease proportional to the square root of the number of pixels averaged, reaching approximately 5×10^{-7} refractive index units root-mean-square for 160 × 120 pixels image regions imaged for 1 s. The simple, compact construction and high performance of the imager will allow the device to be readily applied to a wide range of applications.

Keywords: Surface plasmon resonance imaging; Microfluidics

1. Introduction

1.1. Surface plasmon resonance imaging

SPR imaging was first demonstrated in the late 1980s (Yeatman and Ash, 1987; Rothenhausler and Knoll, 1988). It is an optical technique for detecting changes in refractive index near the surface of a thin layer of metal. Due to its high sensitivity and label-free operation, SPR has emerged as an effective method for quantitative analysis of biological and chemical binding reactions (Steiner, 2004). In a typical SPR imaging system, collimated monochromatic light is reflected from the sensor surface in the Kretschmann and Raether (1968) configuration and focused onto a two-dimensional image detector, allowing spatially varying binding events to be measured.

SPR imaging applications typically fall into the areas of genomics and proteomics. Some examples of genomics applications include DNA–DNA (Wark et al., 2005; He et al., 2000), DNA–RNA (Nelson et al., 2001) and DNA–drug (Wolf et al., 2005) interactions. For proteomics, the applications include determining protein thickness (Otsuki et al., 2005; Wilkop et al., 2004), conformational changes (Kim et al., 2005) and expression profiling (Usui-Aoki et al., 2005). Other diverse application areas for SPR imaging include toxin-binding inhibition in glycomics (Kanda et al., 2005), self-assembled monolayers (Pyo et al., 2005), detection of human choriogonadotropin (Piliarik et al., 2005) and fabrication of electrochemical switches (Arena et al., 2004).

SPR imaging systems have evolved over the past 20 years. The first published description of an SPR microscope included many moving parts (Yeatman and Ash, 1988). Since then, researchers have worked on simplifying and improving various aspects of the technique. Some examples include use of multi-spectral scanning (Otsuki et al., 2005; Yuk et al., 2006),

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use of an incoherent light source, such as an LED, in place of a laser to eliminate speckle (Wilkop et al., 2004), and use of a liquid crystal variable retarder to reduce spatial and temporal fluctuations from the light source (Wolf et al., 2005). SPR imagers designed for laboratory use are beginning to be commercially available, including the SPRimager II (GWC Technologies, USA) and FlexChip (Biacore, Sweden). The goal of our research is to take the next step by enabling leaps in instrumentation which will enable SPR imaging to be used outside of the laboratory, by relatively untrained users, for point-of-care medical applications. This will require improvements in performance and reliability, reduction in size and cost and simplification and automation of sample handling and assay procedures.

To this end, we are developing a prototype system capable of performing rapid (<20 min) multiple simultaneous assays of small molecules in liquid matrices. The system consists of a disposable microfluidics card that contains all of the chemistry and sample handling needed, mated to an optical sensing platform that allows highly sensitive surface plasmon resonance (SPR) imaging bioassays to be conducted in the sensing region of the microfluidic card. The instrument is designed to be rugged and compact, with few moving parts. In addition to its optical system, the instrument includes light shielding so that the instrument may be used in normal room lighting, a magnetic clamping mechanism for reproducible placement of the disposable card on the instrument, and a tablet PC for instrument control (Fig. 1).

In this paper, we describe the optical sensing platform we have developed. The other critical component of our sensing system, the microfluidic cards containing the sample handling and assay chemistry, will be described in detail in a separate publication. Briefly, these cards are constructed from laminated layers of laser-cut adhesive-coated mylar and polydimethylsiloxane (PDMS), in structures designed to implement: (1) sample loop for storage of the injected sample; (2) diffusion-based "H" filter for extraction of low molecular weight compounds (Brody and Yager, 1997); (3) herringbone micromixer (Stroock et al., 2002) for combining filter sample with antibody solution; (4) assay channels for flowing sample–antibody mixture over the gold-coated surfaces monitored by SPR imaging; (5) storage of waste solutions produced during the course of the assay.

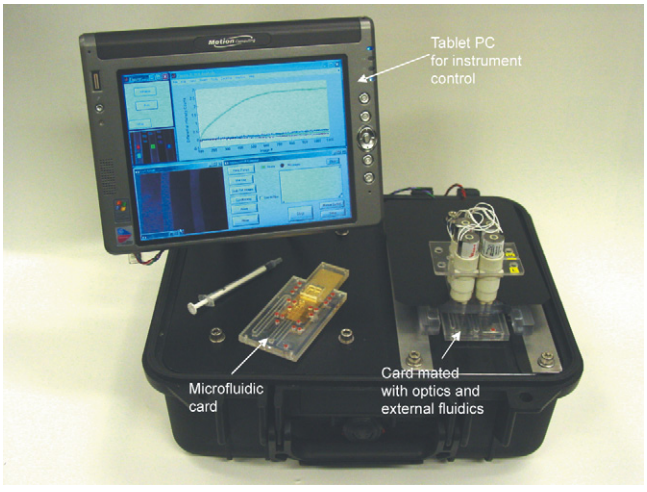

Fig. 1. Photograph of the compact SPR imager, showing optical platform, control computer, microfluidic card and external fluidics fixtures.

1.2. Principles of SPR imaging

SPR is a well established technique for observing biomolecular binding reactions. As typically implemented, light traveling through a high refractive index (RI) substrate (here BK7, $n = 1.51$) reflects from the substrate surface, which is coated with a thin layer of gold (~45 nm). An aqueous sample, generally contained in a flowcell, contacts the opposite side of the gold. For certain wavelengths and angles of transverse-magnetic (TM)-polarized incident light, part of the incident energy will couple into a surface plasma wave traveling between the sample and the gold layer. The loss of this energy is observed as a decrease in reflectivity. Because the coupling conditions vary strongly with the refractive index of the sample, observations of reflectivity may be used as a surface sensitive measure of sample refractive index. To make an SPR sensor for detection of specific substances, the gold surface is chemically functionalized such that substances of interest will bind to the surface while other material will tend not to bind. Imaging the reflectivity of the surface makes it possible to obtain a measurement of binding at each point on the surface. As biomolecules bind to the surface, the surface RI will increase roughly proportional to the quantity of the substance that has bound. Observation of the RI over time will give a binding curve which reveals the quantity of bound material in real-time. If the functionalization layer on the gold surface is patterned such that different regions of the surface tend to bind different substances, the changes in reflectivity which result as the surface is exposed to a sample may be analyzed to determine which of those substances are present in the sample, and in what concentration.

2. Instrumentation

2.1. Design goals

The goal of the research described in this paper is to develop a compact high performance SPR imager suitable for use in medical diagnostics. Optimization of such an instrument is not straightforward because tradeoffs must be made between four main instrumental attributes: refractive index resolution, spatial resolution, refractive index linear range and mechanical and optical simplicity and cost (Chinowsky et al., 2004). The optics of all SPR imagers are somewhat similar: light emitted from one or more light sources passes through collimating optics and filters, passes through the side of a prism, and strikes the gold-coated sensing surface at angles appropriate for observation of SPR. The reflected light passes through imaging optics and is focused onto a detector which records the image. In implementing each of these components, we sought to achieve state-of-the-art performance while avoiding complicated, expensive construction.