Sensors and Actuators B 199 (2014) 488–492

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A novel algorithm based on the coefficient of determination of linear regression fitting to automatically find the optimum angle for miniaturized surface plasmon resonance measurement

Daeho Jang, Dayeseul Lim, Geun-hyong Chae, Jaechern Yoo *

College of Information and Communication Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea

A R T I C L E I N F O

Article history:
Received 28 November 2013
Received in revised form 13 March 2014
Accepted 27 March 2014
Available online 13 April 2014

Keywords:
Surface plasmon resonance
Fixed angle mode
Linear regression fitting

A B S T R A C T

We developed a novel algorithm which can automatically find the optimum angle for surface plasmon resonance (SPR) measurements based on fixed angle mode using MATLAB R2013a. The optimum angle is decided by the coefficient of determination of linear regression fitting on an SPR curve obtained from a miniaturized SPR system. The SPR biosensor based on the novel algorithm achieved a dynamic range of 0.00696RIU of and LOD of 3.1956×10^{-6}.

1. Introduction

Since its first observation by Wood in 1902 [1,2], surface plasmon resonance (SPR) has become one of the popular analysis methods for bio-molecules to detect changes in the refractive index or thickness of an adsorbed layer on or near an SPR-active surface with high sensitivity [3–9]. Activation of the surface with specific binding sites creates a biosensor that can detect bio-molecular interactions in real time without labeling requirements [10]. Most SPR measurement systems generally monitor bio-molecular interactions using two kinds of sensing modes: angle scanning mode and fixed angle mode. The angle scanning mode collects the shift of the SPR angle, and fixed angle mode reports the reflectivity change at a fixed angle or pixel of a 2D camera when the refractive index of a sensing surface is changed.

The angle scanning mode has generally been applied for SPR spectroscopy to measure bio-molecular interactions in real time, whereas, fixed angle mode, which is based on traditional fitting methods to find inflection points as a fixed angle or pixel on the left side of an SPR curve, has normally been used for SPR microscopy, which compares the adsorption at differently functionalized areas of a sensor surface at a single point in time. It is the nonlinearity of the SPR curve that makes the dynamic range of the traditional fixed angle mode insufficient for real-time monitoring of multi-layer formation in single experiments such as sandwich immunoassays. Despite the defects of the fixed angle mode, we think it has charming advantages such as simplification of optics and fast collection of data. These advantages will contribute to low consumption of battery power and allow use with low-level PC specifications. Thus, SPR biosensors based on fixed angle mode can easily be applied for miniaturized systems and point of care testing (POCT) systems, in contrast to SPR systems based on angle scanning mode.

In this study, we developed a novel algorithm to automatically find the optimum angle with maximum dynamic range for real-time monitoring of bio-molecule interaction as with traditional angle scanning mode using a miniaturized SPR system. The algorithm decides the optimal fixed angle with minimum reflectance intensity in the linear range of $R^2 = 0.990$ (R^2: coefficient of determination) on the left side of the SPR curve using linear regression fitting and differentiation of the raw curve.

2. Experimental

2.1. Miniaturized SPR system

We fabricated a home-made SPR system with flow cells of 2 channels and dimensions of $5\,mm(l) \times 1\,mm(w) \times 0.2\,mm(d)$ for use with MATLAB(R2013a), in order to compare the angle scanning mode and fixed angle mode. A schematic diagram of a simple and miniaturized SPR system is depicted in Fig. 1. The p-polarized

* Corresponding author. Tel.: +82 31 299 4591.
E-mail addresses: jangdh@skku.edu (D. Jang), imgenius@skku.edu (D. Lim), chkh86@skku.edu (G.-h. Chae), yoojc@skku.edu (J. Yoo).

http://dx.doi.org/10.1016/j.snb.2014.03.103
0925-4005/

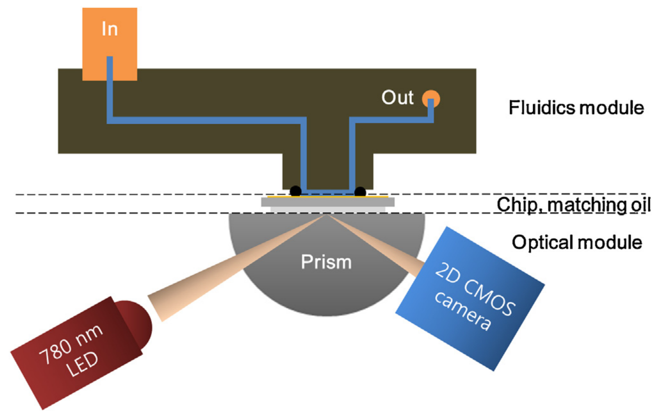

Fig. 1. Schematic diagram of a miniaturized SPR biosensor, which consists of flow cells of 2 channels, a 780-nm LED, a cylindrical prism, and a 2D-CMOS image sensor. A gold sensor chip is matched on the prism via immersion oil.

wedge-type incidence beam from a 780-nm light-emitting diode (Opnext Inc., Japan) passes through a band-pass interference filter (780 ± 10 nm) and is directed to the SPR sensor chip through a cylindrical prism (BK7). Then, the intensity of the reflected light beam is monitored using a two-dimensional complementary metal oxide semiconductor (2D-CMOS) image sensor (IDS Co., Germany), which has a 1/2-in. sensing area (752 × 480 pixels). The image sensor is located just in front of the prism and it allows the SPR system to be fabricated without any other lenses.

2.2. Image processing

A final image for curve fitting is acquired (normalized) from 3 images: a dark image, reference image, and sample injected image. The dark image is obtained when the incident light is turned off, while the reference image is obtained from the light-on mode when the media is air on a gold sensor chip before the sample is injected. The sample injected image is obtained when the media such as running buffer is injected.

We processed these three images by the following methods. Firstly, the intensity of the dark image is subtracted from the reference and sample injected image. Then, the final image is derived by dividing the subtracted sample injected image by the subtracted reference image:

$$\text{Final image} = \frac{\text{sample injected image} - \text{dark image}}{\text{reference image} - \text{dark image}}$$

The image processing was done to remove noise come from dust or incident light. Fig. 2 shows four images obtained from a 2D CMOS image sensor. The horizontal pixels (0–752) of the image sensor matched the sensing angle range for 64.8–72.2°. The intensity of the final image was normalized to have a range of 0–100% and used as the alternative unit of reflectivity.

2.3. Fitting algorithm

The SPR curve is acquired by plotting the intensity (*X*-axis: pixel, *Y*-axis: %Intensity) of a region of interest (ROI) in the final image, which is processed using the method in Section 2.2. The ROI is made of 90 rows of pixels, which belong to a column in the final image.

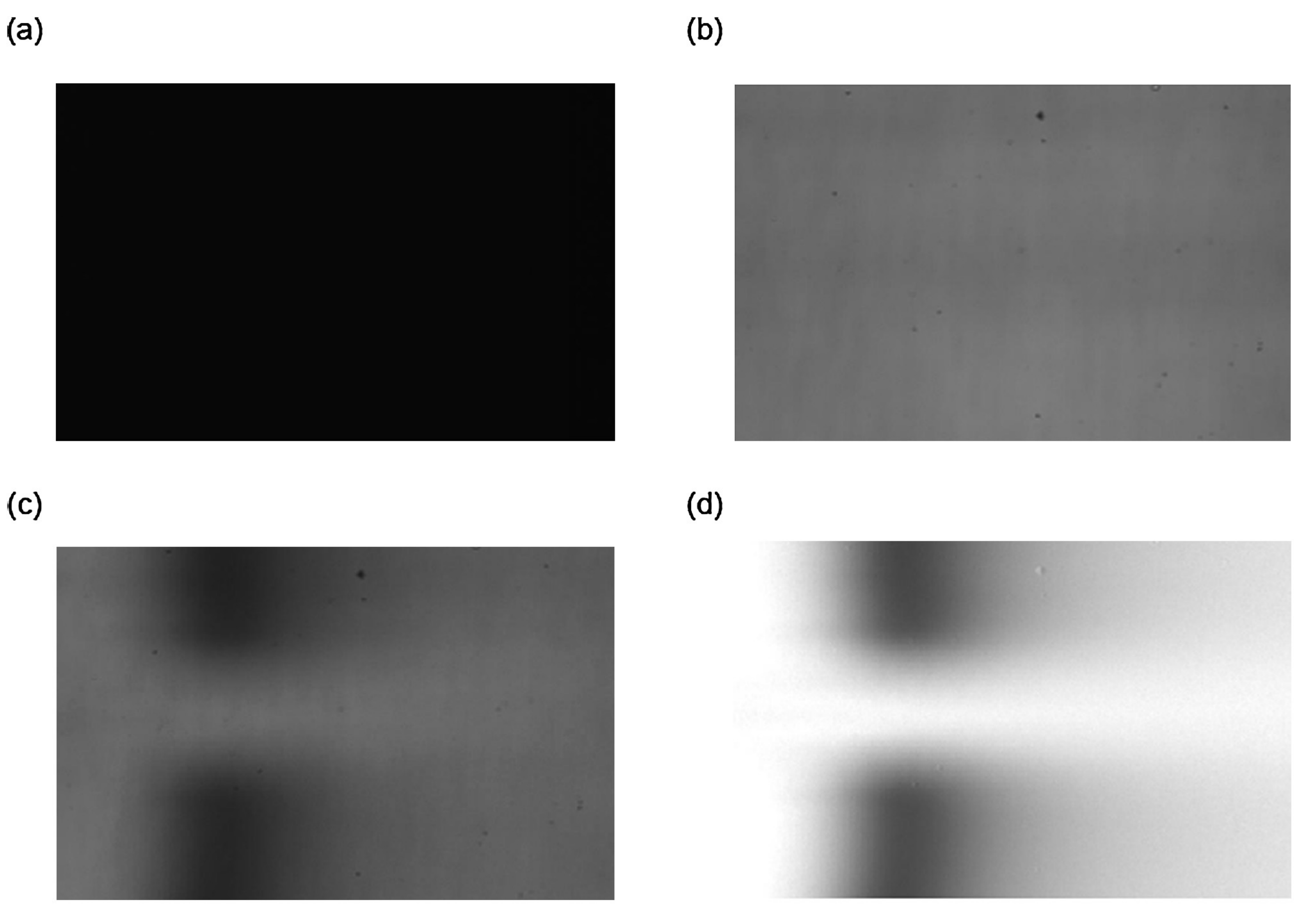

Fig. 2. Images from a 2D CMOS image sensor of 752 × 480 pixels: (a) dark image: incident light off mode, (b) reference image: incident light on mode when media is air, (c) sample injected image: media is running buffer, (d) final image: after image processing using (a), (b), (c).