Optical immunosensor for endocrine disruptor nanolayer detection by surface plasmon resonance imaging

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ABSTRACT

Endocrine disrupting compounds (EDCs) such as bisphenol A (BPA) and female hormone Estrone are especially prevalent in surface and waste-waters in nano-molar concentrations and therefore, there is a need for sensitive analytical device for their monitoring. We have designed a miniature, low cost and fast surface plasmon resonance (SPR) imaging liquid sensor based on the angular interrogation using Kretschmann configuration with diverged incident monochromatic light. During this paper we present a surface plasmon resonance imaging (SPRI) biosensor to detect EDCs such as BPA and estrone. A pattern of SPR line which is dark intensity line on bright area was reflected at angles range depending on the dielectric constant of the analye: Rabbit Anti-Estrone polyclonal IgG + Estrone 11-MUA attached to the silver or non-specific sensing of BPA in water with nanoprecision. For analyzing the SPR signals we used an efficient detection algorithm based on Radon Transform with less sensitivity to laser speckle noise and nonuniformity of the illumination.

Keywords: Surface Plasmon Resonance (SPR) Imaging, biosensing, estrogenic disruptors, Bisphenol A, Estrone.

1. INTRODUCTION

The presence of chemicals, both natural and synthetic, in the environment with the potential to interfere with the endocrine system in both wildlife and humans has become a major international concern in the last decade. The presence of endocrine disrupting compounds (EDCs) such as Estrone which is a female hormone and bisphenol A (BPA) which is an industrial chemical in water and food sources has resulted from EDCs widespread usage. The consumption of such contaminated sources, even if they contain only very small amounts of these EDCs, can cause adverse effects on both humans and animals, therefore, there is a need for sensitive device for their monitoring. In order to determine the level of the estrogenic disruptors in the environment, such as water or food as well as in human urine and serum, a sensitive and rapid method is needed. Although high-performance liquid chromatography (HPLC)[1, 2] and gas chromatography-mass spectroscopy (GC-MS)[3-5] can be used for the analysis of BPA and estrone, several time-consuming sample pretreatment steps are required. Therefore, these methods are not suited for rapid analyses and also require a large volume of sample. When dealing with serum or urine, as the media of measurement, it would be very difficult to measure from the limited quantity available. To overcome these limitations, an enzyme-linked immunoassay (ELISA)[6, 7] has been widely used for determination of estrogenic disruptors due to its high selectivity and high sensitivity. However, ELISA requires more than one hour completing a single measurement.

Surface plasmon resonance phenomenon and nanophotonic based immunosensors have been receiving increasing attention in recent years, due to their potential as a label-free, real-time, rapid cheap and high-selective immunosensing techniques. During this paper we present a surface plasmon resonance imaging (SPRI) biosensor for detection of EDCs such as BPA and estrone.

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Molecular interactions at the surfaces between probe and target at the surface nanolayer shifts the SPR signal, thus indicating the presence of the substance. The high sensitivity and relatively low cost makes the SPR biosensors widely used in the field of low concentration sensing. Gold is usually used in SPR biosensors, although there are some studies that use silver as a metal for SPR sensing [8, 9] and even for the development of sensors based on extraordinary resonative transmission through the nano-gratings [10-13]. Due to its inertness, stability to water environment and simple bio-immobilization protocol, gold is usually used as SPR biosensing metal nano-layer. However, poor attachment of gold to the glass substrate needs additional layer of few nm of Cr or Ti which deform the SPR reflectivity signals. Silver, unlike gold, adheres very well to the glass and has very sharp SPR dip. Metallic bonding has its unique properties due to the free electrons that are at constant motion around the atomic nuclei of the metallic lattice. Oxygen which is an electrophile presents in air and soluble in water. When oxygen comes in contact with silver it receives some of its free electrons, deteriorating the metallic bond. Hydrogen sulfides (H_2S) are present in the air mainly because of the vehicles and manufacturing emissions. It has a thiolic group, which can form an uncontrolled covalent bond with the silver layer. To prevent these phenomena there is a need to cover the silver layer with a controlled self assembled monolayer (SAM). Since silver suffers from spontaneous sulfidizing and harmful to the silver layer oxidizing described in the previous lines, a thin protective layer (<15nm) is needed. The common protective layers used are variety of oxides (SiO_2, ZrO_2) and PMMA with 5-15nm thickness[11]. These layers make the immobilization more difficult. Moreover, gold is less sensitive and its signal is wider than those of silver especially in the visible spectra. We used a densed and packed thiol (11-mercaptoundecanoic acid (MUA)) layer which a) protects the silver from oxidation, b) sulfidizing the silver in controllable manner, c) enables immobilization of Rabbit Anti-Estrone Polyclonal IgG antibody to the thiol layer through reactive linker (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide (NHS)).

For analyzing the SPR signals we used an efficient detection algorithm based on Radon Transform (RT) with less sensitivity to laser speckle noise and nonuniformity of the illumination [8].

2. EXPERIMENTAL

2.1 Materials

Chemicals: All reagents were used as received without further purification. 11-Mercapto-undecanoic acid (11-MUA) 95%, 45056-1 Sigma Aldrich; Dimethyl Sulfoxide (DMSO) Merck Germany; Ethanol 97%; N,N'-dicyclohexylcarbodiimide (DCC); EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiiamide) GR1-SI- E,1769-0005 Sigma-Aldrich Co., Israel; luminiscence HRP antibody and luminol (Sigma-Aldrich Co., Israel); N-hydroxysuccinimide (NHS) GR1-FL- 56480-0100 Sigma-Aldrich Co., Israel; Phosphate buffered saline(PBS) pH 7.4; Estrone E9750 (Sigma-Aldrich Co., Israel); Rabbit Anti-Estrone polyclonal IgG 7030-0604 (AbD Serotec Co.). Silicon adhesive isolator s3560-25EA (Sigma-Aldrich Co., Israel).
Evaporation tools: Silver shot 1 to 3mm, 99.9999% (00906Dz Sigma-Aldrich Co., Israel). Tungsten boat (Kurt J. Lesker Co.)
Optical parts: EO Laser Diode 6mW 637nm (59-088 Edmund Optics); BK7 right angle uncoated prism (FRP1309, Foctek), 1/4" CCD FireWire.A Board Level Monochrome Camera (NT58-223 Edmunds Optics); 25mm FL Megapixel Finite Conjugate Micro Video Lens, F2.5 (NT58-207 Edmunds Optics).

Preparation of the silver samples: A thin film of silver on a glass substrate was grown. In evaporative deposition, the source and substrate were placed inside a vacuum chamber of 10^{-6} Torr, and the source was heated until it melted and began to evaporate. The resulting vapor then condensed on all surfaces inside the vacuum chamber, including our substrate which is a microscope BK-7 glass. We used a shutter to control the growth of the sample and to shield it from the initial burst of "crud" that comes off of our source when it first melts.

At the first stage of the research we demonstrated the concept of SPR imaging. For this purpose, silver layer of 70-nm thickness was deposited on BK7 glass slide. A thin layer of SiO_2 (~21 nm thickness) was deposited on top of the silver film as anti-sulfidization/anti-oxidation layer. Note: the parameters of the sensing element (metal and cover) are not ideal, but they are good enough for demonstrating the concept of the SPR imaging. To perform the experiment with a diverging beam, the optical arrangement shown in Figure 1 was used with 637nm TM polarized light. The beam incident on the prism cathetus has a diameter smaller than the cathetus width. The 25mm focal length Finite Conjugate Micro

Video Lens in front of the camera was added to collect the beam so that it fits the active area of the camera sensor. The sample is held on the base of the prism horizontally for convenience while adding liquids as shown on Figure 1. Output images acquired by the camera were processed using MATLAB. The processing algorithm of the captured image using 70nm silver layer covered by ~21nm of SiO2 on BK7 glass is shown on Figure 2. Input image is transformed to the Radon space using Radon Transform. Median filtered Radon space of the input image subtracted from the Radon space of the original image to suppress the background and to reduce unwanted minimal points. Once the detection of the minimal values was done, SPR line was extracted and shown as a smooth line along the whole image on Figure 2. In the following lines we will explain the mathematics behind the diagram proposed on Figure 2.
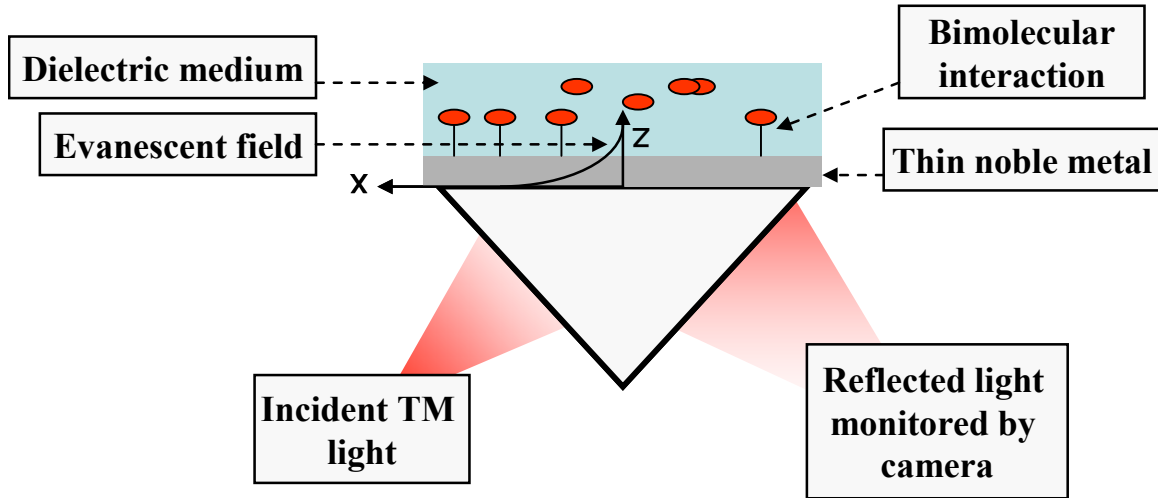

Figure 1. SPR imaging arrangement scheme for improved angular interrogation. The evanescent wave is generated at a single incidence-angle among the angular range of diverged TM polarized monochromatic laser beam through a prism on a thin noble metal layer. Reflected light is captured by the CCD camera. When the momentum of the photons matches the momentum of the metal electrons, the dark line appears on the bright area of the captured images which is related to the resonance of surface plasmons.

2.2 Method

In order to locate the SPR dark line in highly speckled low contrast SPR image, which is an input image to the diagram on Figure 2, we proposed an algorithm based on Radon Transform (RT)[8, 9] which is not sensitive to the speckle noise. The RT in polar coordinates is defined as follows [14]:

$$
(\Re f) \equiv g(\rho, \theta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) \delta(x\cos\theta + y\sin\theta - \rho) dx dy
$$
$$
= \int_{-\infty}^{\infty} f(\rho\cos\theta - l\sin\theta, \rho\sin\theta - l\cos\theta) dl
$$

(1)

Where $f(x,y)$ is a two dimensional function which represents the image, $\rho = \sqrt{x^2 + y^2}$ and $\theta \in [-90°, 90°]$ are the radial and the polar coordinates respectively. The Dirac δ-function converts the two-dimensional integral to a line integral along the line: $x\cos\theta + y\sin\theta = \rho$; (x,y) are the pixel coordinates in the image: $x \in [0, m]$ and $y \in [0, n]$ with $m \times n$ being the dimensions of the camera sensor in pixels. The function $f(x,y)$ represents unknown reflection from sensing element, and then $g_{min}(\rho, \theta)$ (in Radon space) represents the parameters of the SPR line at given analyte. Due to the integration along the line, RT is sensitive to line patterns. In addition, the integration along the line partially averages the speckle noise. The algorithm scheme is presented on Figure 2.

After the demonstration of SPR imaging concept, the set of experiments was designed to allow for a general test of detection of different concentrations of BPA in water. Note: The solubility of BPA in water is 120-300 mg/l

(http://www.bisphenol-a.org). Therefore to dissolve BPA in water, first it was dissolved in di-methoxy sulfoxide (DMSO) then added to double distilled water (DDW) - (1:5 v:v).
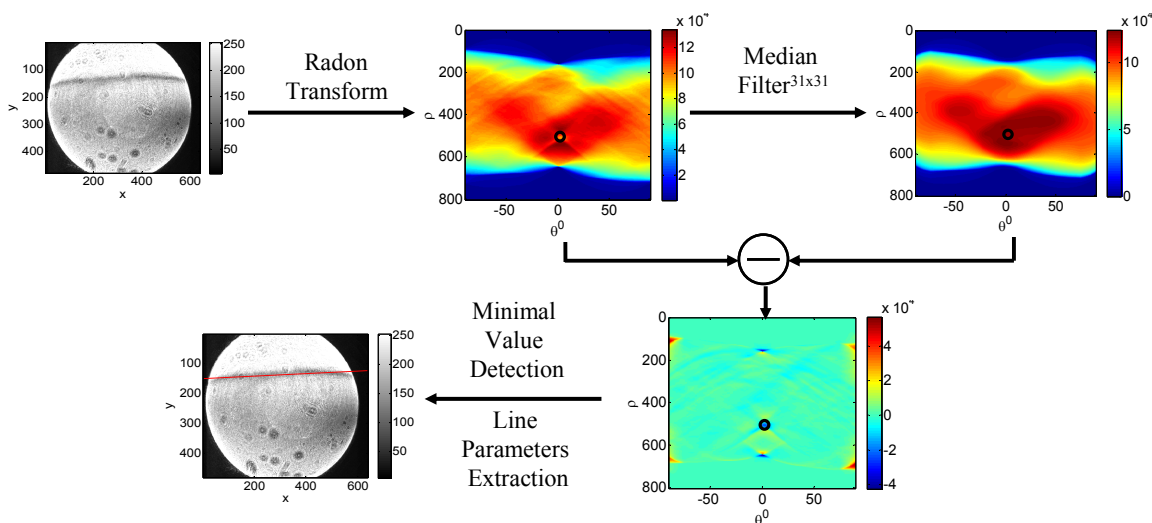

Figure 2. Scheme of the SPR pattern (location) extraction algorithms. The decrease in the reflected laser beam (dark intensity line) characterizes evanescent wave formation. The sample is 70nm silver which was deposed on BK7 glass and covered by 21nm of SiO_2.

3. RESULTS AND DISCUSSION

Different concentrations of BPA dissolved in DDW and DMSO were prepared for SPR imaging experiment while the sample was 47nm Ag on SF11 glass. Figure 3 shows some of the images for lowest concentrations of BPA in water captured while extracted SPR line appears along the images.
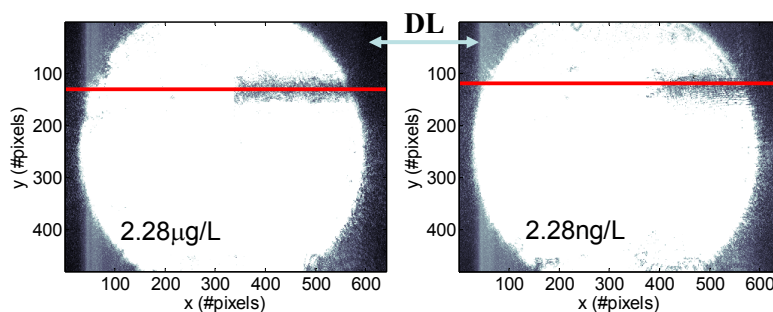

Figure 3. Some of the reflected signals captured by the camera with extracted SPR line along the images. Concentrations of BPA dissolved in DDW and DMSO are written on images. DL abbreviates (experimental) detection limit.

It can be seen from Figure 3 that BPA detection is presented with nano-precision.

At the last stage of the research we developed an immobilization protocol using an organic solvent DMSO which enabled protection of the silver samples and attachment of anti-Estrone antibody. Figure 4 shows captured images from two samples, both were immersed in 11-MUA dissolved in DMSO for 24hr, while the left image were achieved from the sample with immobilized anti-Estrone antibody and attached to its antigen - Estrone.
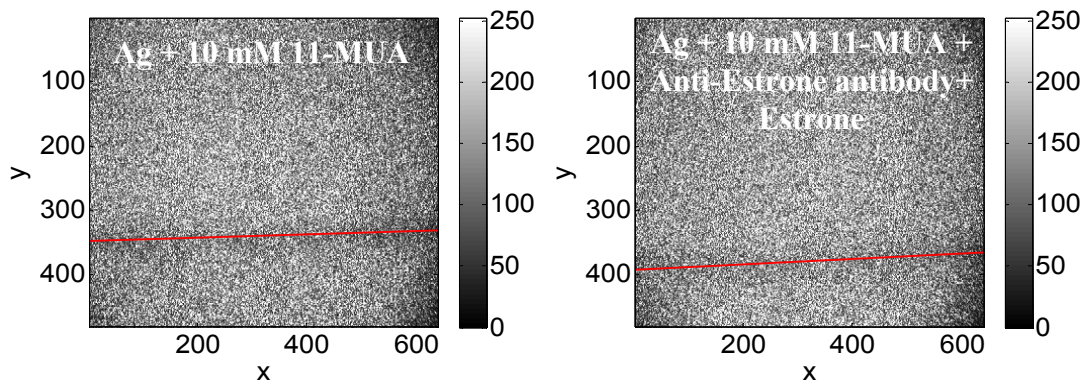
Figure 4. Extracted experimental results using SPR Imaging extraction algorithm (Figure 2) from 69nm Ag on bk7 glass covered with 10 mM 11-MUA layer and 69nm Ag on bk7 glass with immobilized anti-Estrone antibody on it and attached its anti-Estrone. Note: to improve the signal to noise ratio we guided the incidence laser beam through the optical fiber.

4. CONCLUSIONS

The combined simplicity and high sensitivity of SPR imaging measurements make them ideally suited for biosensing applications such as recognition of estrogenic disruptors. We show detection of the industrial chemical - Bisphenol A and natural occurring hormone – Estrone using single channel SPR imaging. Attachment of antibody was prepared using developed protocol for immobilization of receptors on silver surfaces based on the covalent bond of thiols with the silver layer. Experimental SPR imaging results of immobilized Rabbit Anti-Estrone polyclonal IgG and the attachment to its antigen - Estrone were presented. A pattern of dark intensity line (perpendicular to the edge of the prism cathetus) on bright area was reflected at angular range depending on the dielectric constant of the analye: Rabbit Anti-Estrone polyclonal IgG + Estrone 11-MUA attached to the silver or non-specific sensing of Bisphenol A dissolvrd in DMSO and DDW.

For analyzing the SPR signals we used an efficient detection algorithm based on RT with less sensitivity to laser speckle noise and nonuniformity of the illumination[8]. Such sensor may find variety of applications: 1) detection of thickness of the surface – metal thickness differences will appear as changes of the intensity of the black color of the SPR line; 2) degree of homogeneity of the ambient – chemical compound (for non homogeneous compounds the SPR straight line will appear as a curved line); 2) evaporation time of materials – SPR line will change its location with time; 3) microbial and enzyme detection for environmental monitoring; 4) monitoring of immunoreactions; 5) olfactory device (odor sensing system) which can replace or complement human sensory tests in fields of food, drink, cosmetics and environmental control using odorant binding proteins (OBPs), etc.

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