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Real-time measurement of complex refractive indices with surface plasmon resonance

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ABSTRACT

Surface plasmon resonance (SPR) is an attractive method for the measurement of refractive indices due to its high accuracy and its broad range of applications. However, the use of this technique for the measurement of complex refractive indices in real-time has not yet been demonstrated. This article discusses and demonstrates how an SPR biosensor can be used to accurately measure both the real and imaginary components of the refractive index of a sample in real-time. The theory of the data analysis method, the calibration of the instrument as well as the application of the new technique to measure the complex refractive index of liposomes are presented.

1. Introduction

Surface plasmon resonance (SPR) is a well-established and sensitive biosensing technique for applications in environmental and biomedical fields for the detection and characterization of various analytes [1,2]. This optical technique involves the measurement of the intensity of light reflecting from a thin metal film through a glass prism as a function of the incident angle. Its success lies on the fact that the resonance condition, at which the reflection of light is minimized, is highly dependent on the refractive index of the sample adjacent to the metal. By tracking the changes in resonance conditions in real-time, small changes in the surface or bulk refractive index can be measured accurately. This is greatly relevant for biosensing as the change in refractive index can translate to the detection of a target analyte in a sample. Most biosensors only assess the real component of the refractive index but multiple samples, especially biological and environmental ones, have complex refractive indices with a non-negligible imaginary part. The real component of the refractive index represents the refraction capacity of a medium with respect to vacuum while the imaginary component, also called extinction coefficient, is linked to the absorption of that medium. Examples of samples represented by a complex refractive index are food colouring [3], ink [4] and cells [5]. Additionally, turbid liquids have a significant imaginary part [6].

There are several existing techniques to measure complex refractive indices. Notably, the components can be assessed separately; refractometry can be used to measure the real part while the imaginary part can be calculated with the Beer-Lambert law from the absorption measurements of a spectrophotometer [7,8]. The disadvantage of using absorption to calculate the extinction coefficient is that scattering is not taken into account and therefore it is not suitable for turbid liquids. One of the most widely used techniques to measure both the real and imaginary components of the refractive index simultaneously is ellipsometry which measures the dielectric properties of thin films [9]. Ellipsometry can be used to obtain multiple parameters such as dielectric constant, thickness and roughness but the sample interrogation take place on the sample side which can be inconvenient for liquid samples and real-time sensing measurements. SPR can perform the same measurements while interrogating the sample through a thin metal film and glass. Ellipsometry also assumes that the sample is homogenous and isotropic and therefore is not ideal for heterogeneous biological samples [10]. The use of SPR for complex refractive index measurements has also been reported previously [11,5,6] and this technique works with non-homogenous liquids. While other studies showed that changes in the real and imaginary components of the refractive index can be assessed with SPR, we demonstrate for the first time how this technique can measure complex refrac-

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tive indices in real-time. We present in this work a description of the SPR biosensor we implemented and the novel analysis technique used to evaluate complex refractive indices to offer kinetics measurement of the changes in both the dielectric constant and the absorption of a sample. The calibration of the instrument is detailed and the proof of concept of its application is demonstrated with liposomes as a model for complex biological systems.

2. Material and methods

2.1. Gold coated glass

SF11 glass slides coated with 2 nm of chromium and 40 nm of gold were purchased from Sydor Optics. Prior to each measurement in the SPR instrument, the slides were soaked in acetone for one minute, in isopropanol for one more minute followed by a rinsing step with distilled water. The surface was then dried with compressed air.

2.2. Ethanol and green food colouring samples preparation for system calibration

For the calibration of the instrument, solutions of anhydrous ethyl alcohol (Commercial Alcohols, Ontario, Canada) diluted in distilled water at different concentrations were prepared to assess the real component of the refractive index. For the calibration of complex refractive indices, solutions of powdered food colouring (Les chocolats Roxy & Rich Inc, Québec, Canada) in distilled water of various concentrations were used. The concentrations were defined as the weight percentage of either food colouring or ethanol in distilled water.

To accurately calibrate the SPR instrument, the refractive indices of the samples must be known. For the ethanol dilutions, the refractive index as a function of concentration is available in the literature [12,13]. To measure the refractive indices of green food colouring solutions a spectrophotometer (NanoDrop 2000, UV–vis spectrophotometer, ThermoScientific) was used. This instrument measures the absorption of a medium through transmission. Since it cannot distinguish between scattering and absorption, it was important to use a non-turbid liquid such that the loss in transmission could be attributed to absorption which then could be translated to the extinction coefficient. This fact motivated the use of food colouring for the calibration of the SPR biosensor. Additionally, green food colouring has a linear relationship between its concentration and its imaginary component [3]. This was important since the spectrophotometer saturated quickly as the concentration of food colouring increased. Consequently, the four largest concentrations could not be evaluated with the spectrophotometer and were found by extrapolating a linear fit with five lower and different concentrations (which are not all shown in the calibration data presented in this article).

2.3. Liposome sample preparation

The liposomes were prepared using the thin film lipid hydration method. In brief, 20 mg/ml of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Corden Pharma, Germany) and 5 mg/ml cholesterol (Sigma-Aldrich, Ontario, Canada) were dissolved in a chloroform-methanol mixture 4:1 (Fisher Scientific, Ontario, Canada). The solution was placed in a round bottomed flask and the solvent was removed by rotary evaporation. The resulting lipid thin film was then rehydrated in MilliQ water with constant agitation at a temperature of 43 °C for 45 min. The formed liposomes were extruded by polycarbonate membranes with 200 nm (Whatman Nuclepore Track-Etched Membranes, 19 mm) and 800 nm pores (Osmonics inc, 19 mm). The final sizes were measured by dynamic light scattering (ZetaPALS, Brookhaven Instruments Corporation) and were found to be 209 nm and 723 nm. The various concentrations were defined by the DPPC component and were performed by diluting the extruded 20 mg/ml solutions with MilliQ water.

2.4. Experimental conditions

For the calibration samples, the injection volume was 1200 μl and the flow rate was 100 μl/min. For the liposome samples, the injection volume was 600 μl and the flow rate was 60 μl/min. All experiments were performed at 25 °C.

3. Results & discussion

3.1. Surface plasmon resonance biosensor

The developed surface plasmon resonance biosensor consists of a collimated fibre-coupled light emitting diode (LED) centered at 630 nm, a polariser (orientated to transmit TM light), a cylindrical focusing lens (Lens 1) with a focal length of 50 mm, an SF11 glass prism, an SF11 glass slide with a thin gold layer, a cylindrical collimating lens (Lens 2) with a focal length of 50 mm and a charge coupled detector (CCD) camera (Fig. 1). The interrogation wavelength for this instrument was chosen to support the use of a high resolution camera which is essential to obtain more precise measurements with the data analysis introduced in the next section [14]. The focusing lens creates a range of angles incident on the gold surface which is then imaged on the CCD camera. This range of incident angles determines the dynamic range of the instrument and therefore the scope of samples that it can analyse. The image obtained is the reflection intensity as a function of the incident angle in one dimension and the lateral position on the gold in the other dimension. This second dimension permits the measurement of refractive index changes in multiple flow cells simultaneously. The advantage of this configuration is that the instrument does not have any moving parts and can therefore operate in real-time where the sampling rate is only limited by the frame rate of the CCD.

Fig. 1 represents examples of curves recorded by the SPR instrument as a function of the reflected light versus the incident angles. When the change in refractive index is purely real, the curve maintains the same shape but translates over to a different resonant angle which corresponds to the minimum reflectivity. When a shift occurs in the imaginary component, the resonance position remains unchanged while the shape and intensity of the curve is altered. As the imaginary part increases, the typical sharp transition at the critical angle (around 48° for water) becomes smoother and presents lower intensity while the intensity at resonance varies. The direction and magnitude of the change in intensity at resonance depends greatly on the thickness and dielectric constant of the gold [15].

3.2. Data analysis method for the measurement of complex refractive indices

To evaluate in real-time the complex refractive index of a sample, an efficient data analysis method has been developed. This method, which was modified from a version previously reported [16,17,14], is based on projection. It analyses every SPR curves captured by the camera and outputs the complex refractive index it represents. It can detect small variation in the resonant angle as well as in the intensity. In this method, a captured SPR curve is represented by the vector $\mathbf{v}$ in which each entry is the reflected light intensity measured at a pixel on the camera. The vector is projected on the pre-calculated reference set $\mathbf{A}$ using the equation $\boldsymbol{s} = \boldsymbol{A} \cdot \boldsymbol{v}$.