Surface Plasmon Resonance Biosensor as a Tool for the Measurement of Complex Refractive Indices *

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***Abstract*— Optical characterisation of liquids through the measurement of their complex refractive index is critical in environmental monitoring, food industry and medicine. While surface plasmon resonance is widely used for measurement of the real part of the refractive index there have been few studies to date on measurement of complex refractive index with this method. We present a systematic study which highlights the challenges associated with this approach. Instrument design and data analysis techniques are presented together with preliminary experimental results.**

I. INTRODUCTION

Instruments that can measure analyte concentration from the change in refractive index of a solution are important for applications in various domains such as environmental monitoring, the food industry and medicine. In many of those applications, evaluating both the real and imaginary parts of the refractive index would provide more information than is available from the real part alone. Many samples of interest have a nonnegligible imaginary component; the cell is an entity with a complex refractive index [1] and multiple liquids are turbid in nature which imply a significant imaginary part due to strong scattering properties [2]. There is therefore a need for more instruments that can measure both the real and imaginary parts of the refractive index of liquids as well as more experimental studies of the role of the imaginary part for detection and monitoring purposes.

Surface plasmon resonance (SPR) biosensors have been extensively shown to measure low concentration of analyte [3]. Biomolecules of interest can be immobilized on the surface of the sensor with the use of biological recognition elements and their presence can be measured through the change in refractive index that they induced. SPR biosensors can be used for detection in gaseous samples [4], detection in liquid samples [5] and thin film characterisation [6]. In more recent years, they have also been used for the detection of whole cells [7,8]. They are popular for their real-time ability, their low detection limit, their biocompatibility and their versatility. In SPR biosensors, light is incident through a high refractive index prism on a thin metal film on the opposite side of the sample under investigation. The resonance conditions, where the reflectivity is minimised, occur at a precise incident angle and wavelength pair for a given sample and can be monitored with great precision. Since the plasmon is localised on the surface, this method is highly sensitive to any changes occurring at the interface between the metal and the sample. The device can be operated in different regimes: intensity-based, angular interrogation or wavelength interrogation [3]. The metal used is usually gold, especially for use with biological species. It also has been shown that the data analysis method can have a significant impact on the detection limit of the instrument [9,10].

In this paper, we present the effect of complex refractive index on the reflectivity curve of an SPR biosensor with angular interrogation both in theory and experimentally, we discuss the challenges associated with the choice of parameters for the instrument and their impact on the output and we suggest a method to analyse the data and extract the complex refractive index value of a sample.

II. MEASUREMENTS OF COMPLEX REFRACTIVE INDICES

A. Complex Refractive Indices

A complex refractive index is a refractive index that has both a real (n) and imaginary (κ) parts. The real part indicates the relative phase velocity as well as the refraction capacity of a medium through Snell's law. The imaginary part, or extinction coefficient, is related to the absorption of the sample. It can be calculated with the Beer-Lambert Law:

$$A = \frac{4\pi\kappa}{\lambda} l \qquad (1)$$

where A is the absorption, l is the path length, λ is the wavelength and κ is the extinction coefficient. Transparent samples often have a very small imaginary part, e.g. water [11]. The absorption increases for coloured and opaque samples, e.g. food colouring [12], or for turbid liquids [2]. Turbidity is the cloudiness of a fluid due to its non-homogenous content. It is often more challenging to measure the absorption of turbid liquids since the particles in the sample create a lot of scattering; therefore the knowledge of the incident and transmitted intensities are not sufficient to extract the absorption value and thus the extinction coefficient. It is essential to develop instruments that can characterise turbid liquids without the need to alter the samples prior to the measurement since these liquids are found in many applications. An important medium that has a complex refractive index is the cell. Cells have known refractive index around 1.35 RIU for the real part but they also have a nonnegligible imaginary part. Chabot et al. [1] clearly showed through surface plasmon resonance curves that cells should be modeled as complex refractive index

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media. This is another incentive to develop measurement techniques for complex refractive indices.

B. *Instruments for the Measurement of Complex Refractive Indices*

Not all characterisation methods are able to measure both the real and imaginary parts of the refractive index at the same time. Some instruments where initially designed to measure one or the other: refractometry for the real part and absorption measurement for the imaginary part. For the latter, spectrophotometers are often used to measure the optical properties of a liquid. The spectrophotometer allows the measurement of reflected and transmitted light through a sample at multiple wavelengths. Using this information, the absorption coefficient and then the extinction coefficient can be obtained with the Beer-Lambert law. This technique and various related techniques have often been used to measure the extinction coefficient of water and heavy water [11,13]. The disadvantage of this method is that it does not take the scattering into account and it is therefore not a suitable instrument for turbid liquids.

When it comes to measuring both the real and imaginary parts of the refractive index simultaneously, one of the most widely used techniques is ellipsometry, which is used to measure the dielectric properties of thin films [14]. Ellipsometry can be used to analyse films from sub-nanometer to several micrometers thick. Multiple parameters can be extracted such as dielectric constant, thickness, roughness, etc; however, this technique requires prior knowledge of a few parameters to find the others. Additionally, ellipsometry assumes homogenous and isotropic samples and therefore might not be accurate for heterogeneous biological samples [15].

More recent techniques involve a modified version of well known techniques to extend their ability to measure both the real and imaginary parts of the refractive index simultaneously, to improve the limit of detection or to show their use in different applications, either by measuring more parameters at once or by reducing the dependency of the results on known parameters, for example to avoid the need for the thickness of the sample to be known [16]. Reflectometry is a method used to measure complex refractive indices; the critical angle of total internal reflection is obtained through the measurement of the reflected light for a large range of angles and the refractive index is extracted from the known refractive index of the prism and the measured critical angle. Using the reflectance at two perpendicular polarisation states, the real and imaginary parts of the refractive index can be measured [17,18]. This instrument has been extensively modified to measure turbid liquids like milk [19,20].

Lastly, SPR can also be used for complex refractive index measurements [21,22,20] and this technique will be discussed in the next section.

III. Surface Plasmon Resonance for the Measurement of Complex Refractive Indices

A. *SPR Curves for Complex Refractive Indices*

For SPR measurement in the Kretschmann configuration with an angular interrogation, the output is the reflected light intensity versus the incident angle. This SPR curve is then analysed to extract the refractive index. Typical simulated SPR curves for water (n = 1.333) and ethanol (n = 1.3604) are shown in Fig. 1. There are a few important points to notice on these curves: the location and intensity level of total internal reflection (TIR) and the location and intensity level of the minimum at resonance. The point of TIR, often called the "hook", depends on the refractive indices of the sample and the prism. This portion of the curve is interesting because it does not depend on the parameters of the gold film. At resonance, the location and intensity level of the minimum depend on all the parameters: the refractive index of the prism, the thickness and dielectric constant of the metal and the refractive index of the sample[23]. In Fig. 1, it can be seen that the intensity levels for water and ethanol are almost the same but the location is shifted. The location of the minimum is shifted due to the change in the real part of the refractive index while the intensity stayed the same because both samples are real only (imaginary part in the order of 10^{-8}).

This changes when we consider nonnegligible imaginary parts. Fig.2 shows the simulated SPR curves with the same real parts as before but with different imaginary parts. The notable features are the smoother hook, the lower intensity level of the hook, the different intensity level of the minimum and the larger full-width-at-half-maximum (FWHM) as the imaginary part increases. These significant changes show that SPR is well suited to detect changes in complex refractive indices. There are however, a few challenges associated with this. Firstly, a change in the imaginary part induces only a very small shift in the resonant angle but has a much larger effect on the FHWM and reflectance minimum. These are not as easy to track as the displacement of the resonant angle. The intensity level in itself is not meaningful but its variation can be evaluated. This is why the biosensor should be used

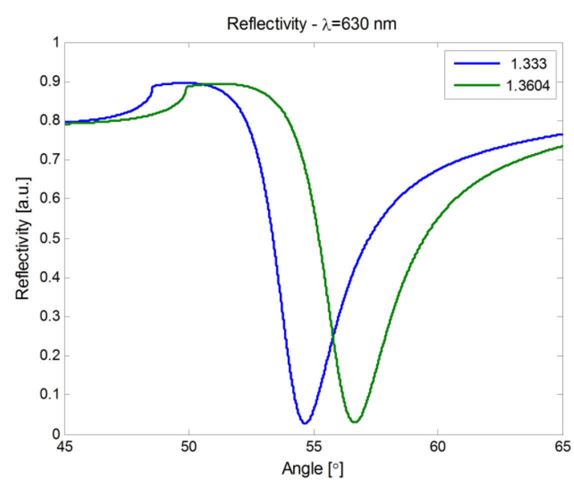

Figure 1 - Simulated SPR curves for two refractive indices: 1.333 (water) and 1.3604 (ethanol). The wavelength is 630 nm, the refractive index of the prism is 1.7789 and the gold is 50 nm with a dielectric constant of -11.5+1.5i.

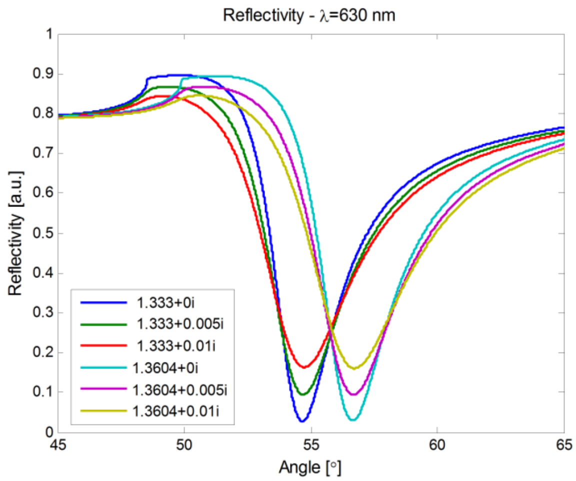

Figure 2 - Simulated SPR curves for complex refractive indices. The wavelength is 630 nm, the refractive index of the prism is 1.7789 and the gold is 50 nm with a dielectric constant of -11.5+1.5i.

to measure changes in the refractive index rather than absolute values.

Another challenge is the influence of the parameters of the metal layer on the change in intensity at resonance. These parameters have a significant impact on the SPR curve as shown in Fig. 3 where the intensity at minimum with respect to the imaginary part of the refractive index of the sample changes for different gold thicknesses. It is important to understand that the intensity level at resonance does not always increase with this imaginary part but the variation in intensity strongly depends on the thickness and dielectric constant of the gold. It means that for a given gold film there is an optimum complex refractive index of the sample for which the minimum level intensity is the closest to zero. It implies that as the imaginary part of the sample increases the intensity level at minimum could first decrease then increase. This phenomenon occurs for gold dielectric constant with smaller imaginary part and/or smaller metal thickness. Even when the intensity level at minimum does not decrease, the slope is still different for different thicknesses and dielectric constants of the gold as seen in Fig. 3. Appropriate calibration is therefore required to measure the imaginary part of the refractive index of the sample properly.

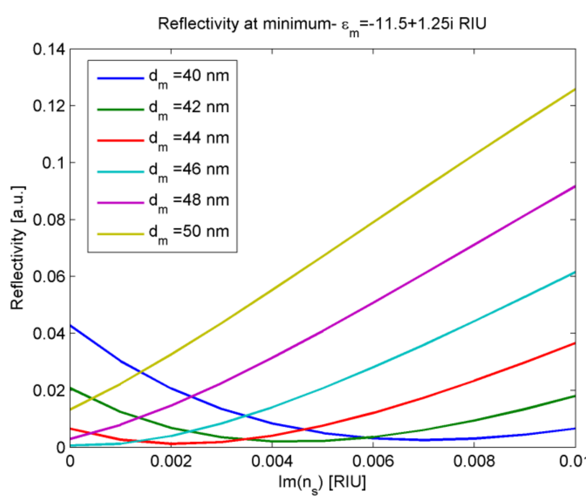

Figure 3 - Simulated reflectivity at minimum with respect to the imaginary part of the refractive index of the sample for different gold thicknesses (in nm). The wavelength is 630 nm, the real part of the refractive index of the sample is 1.333 and the dielectric constant of the gold is -11.5+1.25i.

This behaviour can be seen experimentally as well. Fig. 4 shows the SPR curves for deionized water and liquid green food colouring samples of two different concentrations. It can be noticed that the intensity level at the hook decreases for larger imaginary part of the refractive index (higher concentration of food colouring) - this is always the case for different thicknesses and dielectric constants of the gold - while the minimum also decreases.

B. Data Analysis of SPR Curves to Measure Changes in the Complex Refractive Index of the Sample

To measure the change in the real part of the refractive index using SPR, tracking the position of the resonant angle was usually sufficient and efficient. This is however not sufficient to measure the real and imaginary parts simultaneously. This method cannot distinguish if the shift is due to the real part, the imaginary part or both. We have recently introduced a novel projection method to accurately extract data from experimental SPR measurements [9]. This method consists in creating a reference set with simulated SPR curves for different refractive indices across the dynamic range of the system and comparing the curve obtained experimentally with each simulation. The best match corresponds to the refractive index of the sample. An advantage of this technique is that the step in refractive index between the SPR curves of the reference set does not need to be as small as the limit of detection of the system. We have

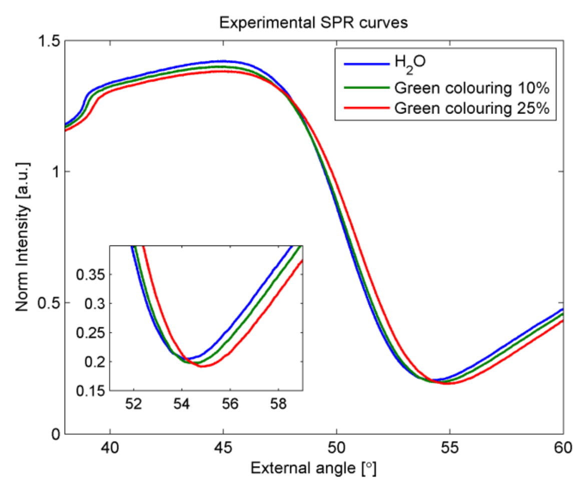

Figure 4 - Experimental SPR curves for deionized water and liquid green food colouring mixed with deionized water at concentration of 10% and 25%. The inset shows a close up of the resonance. The external angle is the incident angle outside the prism.

shown that changes on the order of 10^{-7} Refractive index unit (RIU) can be measured with a reference set containing simulated SPR curves every 10^{-3} RIU across the dynamic range [10,24]. This method not only works for bulk refractive indices but can also be used for practical applications where there are two layers: a surface layer and the bulk region. The reference set can be extended to include two unknowns, the refractive indices of both the surface layer and the bulk. It is also possible to characterise the surface layer and include its parameters in the reference set. This is relevant if the same surface chemistry will be used for multiple experiments. In this case the reference set has again one unknown which is the bulk refractive index.

Another advantage of our SPR system is its real-time ability. The instrument is built without any moving parts since the angular range is obtained through focusing light with a cylindrical lens onto the gold surface. The reflected angular spectrum is measured on a camera and the image is processed in real-time with the pre-calculated reference set. This data analysis technique is ideal to also measure complex refractive indices as both the angular shift and the change in the shape of the SPR curve will be captured in the reference set. The challenge really lies in the calibration of the system, as was discussed before.

IV. Conclusion

In this paper, we showed that surface plasmon resonance is an appropriate method to measure complex refractive indices. It is useful especially for turbid liquids which cannot be characterised with most instruments while SPR would allow the measurement of those liquids without alteration (such as dilution or filtration). We also discussed the challenges associated with the calibration of the instruments due to the great influence of the gold parameters on the SPR curve and on the changes in intensity upon changes in the imaginary part of the refractive index. We are confident that our instrument and our data analysis technique is relevant for the measurement of complex refractive indices.

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