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Signal Acquisition and Processing in Surface Plasmon Resonance Instrument

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Abstract: The signal acquisition and processing aspects of the sensitive instrument of Surface Plasmon Resonance very much in use in industry for biomolecular reactions, biological antigen antibody affinity findings as well as water pollution estimation are handled in a novel way in our circuitry and software. The paper describes the principles and the technique of Moment sum evaluation to estimate minute changes in the Refractive index better than the resolution achievable by angle step refinement in the scan.
Index Terms: SPR, TIR, PIC18F embedded controller, Moment Sum, Signal processing, USB instrumentation.

I. INTRODUCTION TO SPR SIGNAL

A powerful scientific technique for analyzing any substance based on the change in its optical property viz., the refractive index, is the Surface Plasmon Resonance (SPR) Technique [1], [2]. In this paper, the circuitry and signal processing aspect for this technique are presented.

In order to generate SPR, a beam of light (laser or focused polarized light) which varies in angle from 90^{o} incidence angle to the critical angle for Total internal Reflection (TIR), the BK7 prism is kept with its hypotenuse face upwards and the light source with collimator is tilted over this angle in fine angular steps or the prism table is turned step by step in angle by a geared stepper motor [3],[4],[5]. This constitutes a single SPR scan. Such scans are repeated back and forth. In order to make this partial rotation of the light beam, a stepper motor with gear train is used. Now, the stepper motor shaft carries a beam at the tip of which the light source is held fixed. So, the reagent located on the capillary plate next to the gold coated plate will meet the rays at the various angles. At an angle beyond the TIR angle, there is a specific angle called the Surface Plasmon resonance angle. In this angle, the gold film, which is just about 50nm only thick [6],[7], will absorb almost all the light beam and hence the reflected light to the detector will become very low[8],. There is now a dip in the reflectance. The figure 1 shows the SPR generation and change in dip curve with respect to the dielectric sample.

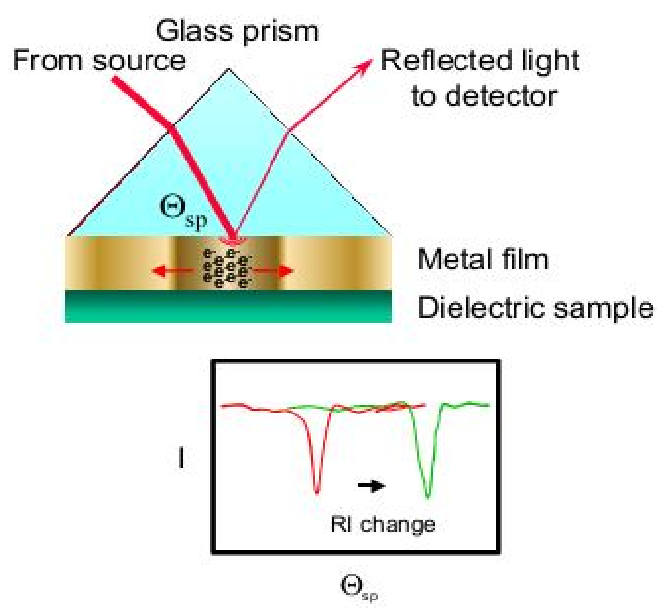

Fig.1 The set up of the SPR and the signal obtained from the reflected light.

The phase speed of light of a speific wavelength in any medium is given by

$$v = \frac{c}{\sqrt{\mu\varepsilon}} = \frac{c}{N}$$

When light form the prism of R.I. n1 goes to the outside medium of R.I. value n2, by Snell's law, for an incident angle of

$$\theta_i = Sin^{-1}\left(\frac{n2}{n1}\right) = Sin^{-1}\left(\frac{1}{n_{glass}}\right) \qquad (1)$$

the transmission of light just grazes the surface. Beyond this critical angle, there is total internal reflection of light.
The incident wave at angle θ_i has the propagation vector given by

$$\mathbf{k}_i = (2\pi/\lambda)\, n \,Sin\, \theta_i \qquad (2)$$

where n is the Refractive index of the medium where light is incident, which is the Glass prism, while the SPR propagation vector $\mathbf{k}_{plasmon}$ is given by [1]

$$k_{pl} = \frac{2\pi}{\lambda}\left(\sqrt{\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1 + \varepsilon_2}}\right) \qquad (3)$$

The wavevector of the plasmon wave, k_{pl}, depends on the refractive indices of the conductor, ngold, (being a constant complex number) and the sample medium, n2. When both these (2 and 3) are equal only, SPR can happen.
For gold, n_{gold}=0.17263+3.422i at 650 nm.
This gives $\varepsilon = n^2$ = -11.6803 + 1.1815i, which means a negative real part.
Let the light be red of lamda value 6.5000e-007, from a RED laser.The above equation gives k_{pl} as using ε_2=1 (air medium) 1.0104e+007 +4.7315e+004i
The value of sin θi is got by equating this gives θ_i,
$k_i = 2\pi/\lambda\, n \sin \theta_i$=(0.6042 + 0.0028i) Sin θ_i
The gives Θ_i after conversion to degrees from radians, as 33.1678.

II. ANALYSIS BASED ON VERY SMALL CHANGES IN REFRACTIVE INDEX OF SAMPLES

When the R.I. of the medium changes, the angle of the dip also changes. By very small changes in R.I., as small as 10^{-4}, the angle change can be measured to indicate the change in R.I. of the substance.(fig.2). This is the principle behind the analysis of substances of even biomolecular species, based on Fig.2 For substance R.I. values 1.33 and 1.34, the angle change is from 66 to 64 degrees. The amplitude of the dip is not varying much. very minute refractive index changes. The SPR reflected signal dip is constant and only angle changes with Specimen R
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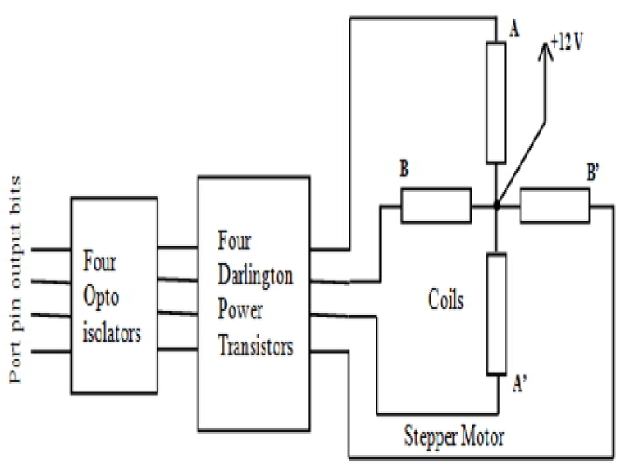

I.(fig.2)

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III. THE CIRCUIT FOR SPR SIGNAL GENERATION

On a disc mounted upon a turn table, a right angled prism of about 10 cm side is kept. Light from a semiconductor Diode laser is directed on one of its face to fall near its centre. The table is mounted on the shaft of a vertically fixed stepper motor with a gear box, which gives a very small angle shift of rotation for each step. This could be as small as 0.05 degree. The other side of the prism which receives the TIR light should be detected and converted into an electrical voltage. For this purpose, we have employed, among other choices, a Photovoltaic Solar panel of same size and it is held facing the other side of the prism. The voltage change caused by SPR phenomenon is very small and is therefore amplified by a D.C. coupled Operational amplifier circuit, with a provision for adjusting the DC offset due to the steady light.

The turn table has to be rotated under program control from an embedded controller. The number of steps can be as much as needed to scan the angle of incidence over a range of 30 to 45 degrees. For this, the program generates the stepping with a time delay between steps of about 0.1 s. Microstepping is employed. The stepper motor is a two winding four terminal one, with a centre junction. The circuit of the stepper motor is shown in fig. 3.

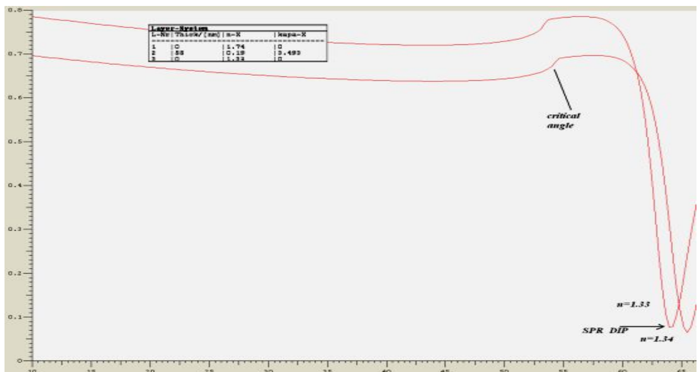

Fig.3 Circuit for driving the stepper motor.

Since we have to process the data of the optical signal for comparative analysis, we had to develop an interface between the embedded controller (fig.4) and a laptop. This was an usb interface.

The signal from the photovoltaic panel is amplified and given to the ADC pin of the embedded controller. The data is read after each step so that the value of the reflected signal can be tracked as the scan of the angle proceeds.

After the scan is completed in the direction of increasing incidence angle, the motor is turned backward by exactly the same number of steps to bring back to the reference angle

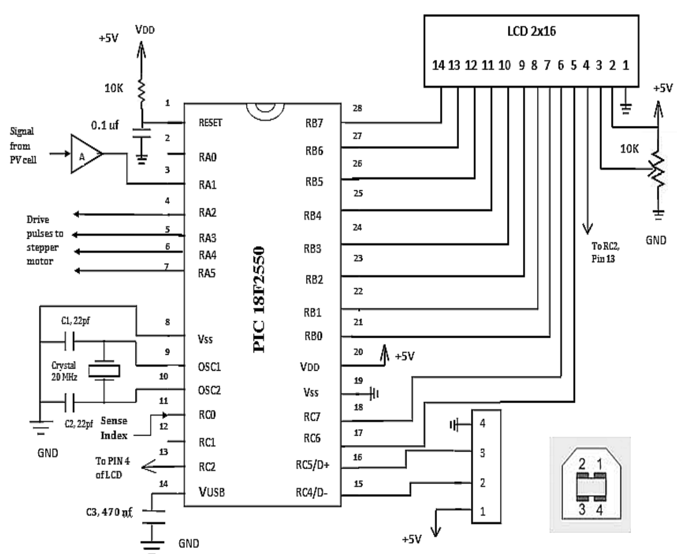

Fig.4 Circuit of PIC 18F2550 used for signal acquisition and USB transfer as well as data processing with LCD display.

position. The reference angle position is about 25^o because there will be no substance that will cause an SPR below this angle. For water, the angle is about 60^o.

By counting the steps it is possible to relate the angle of reflected signal versus its amplitude. This is a curve shown as the SPR curve. (fig.5).

IV. SIGNAL PROCESSING

The detected signal samples are read by the embedded controller and saved in its internal RAM. The PIC18F2550 has six channels of ADC of 1 microsecond sampling time but since we step the motor only at a much slower rate, we collect only once every step, about 0.05 s. The data is 8 bits only. We can use a PIC18F4550 for 10 bit results, if need be.

The program was developed for the device to perform the following :

A. Entry of steps required
B. Upon switch control, start the scan and collect the data
C. The LCD display indicates the number of the step and the data value of photo signal
D. When forward scan is over, upon reverse switch being pressed, to turn back to reference angle.
E. When commanded from the GUI program on the laptop, to send the scan data of intensity signals.

The graphs of dip curves are displaced between two samples of different R.I. values. Rather than determining the angle difference and estimate the change of the substance, we resort to the following signal processing technique.

V. MOMENT CALCULATION

From the reference angle point, the amplitudes are known as the signals of intensity variations during angle scanning. Let $y(\theta)$ be these values for all the θ in the range of scan, viz., $\theta1$ to $\theta2$.

The θ values are actually sequential numbers from 1 to the total steps N. Therefore, we calculate the moment of each *y* value with these numbers and sum them up. $\text{Moment sum} = \sum y(\theta)\, d\theta$

This comprises of N multiplications. This could be done both in the PIC embedded controller as well on the program in the laptop.

The value of this moment changes much more than the angle change noted by the number of steps change for the minimum point. hence, it is much more sensitive to changes in R.I.

If only we step at 0.05degree angles, the point of the change due to R.I. may dip in between the steps, i.e., within 0.025 degrees, there is no possibility of detecting the change in the substance analysed.

But, when we employed the moment sum, the change is much more and hence this is a high resolution technique that we have employed to improve the angular change method of SPR.

Suppose we have a very small change in the R.I. of the water sample, from 1.33 to 1.335. The angle change is found to be 0.9 degree.

When the moment sum is found for the two. Sample curves, it gave a difference -49 as an index value, which is a much more resolved result for comparison.

Table 1. Moment calculation for SPR signal with respect to the refractive index

Refractive index of sample	Angle value	Moment value
1.33	66	13585
1.335	65.1	13536

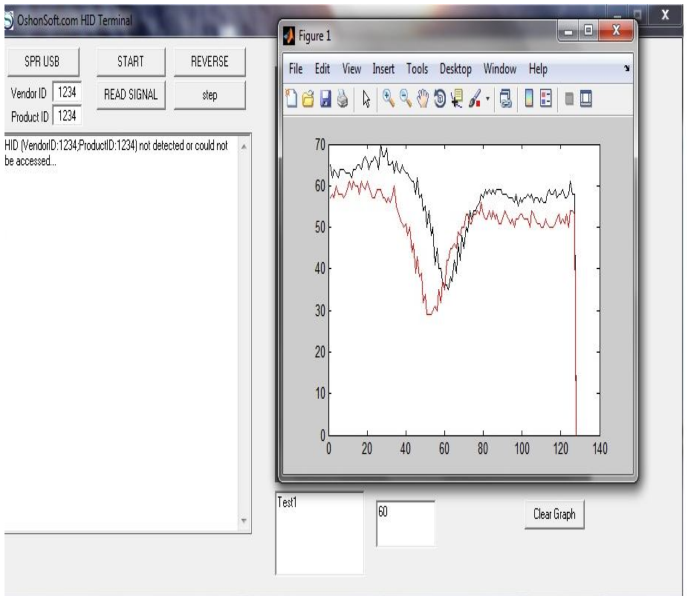

Fig.5. The GUI on computer showing the three curves for different water samples, indicating changes due to pollution content.

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