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Development of a cost effective set up in a Surface Plasmon Resonance based Instrument using an USB interfaced Embedded Controller & GUI on a PC

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Abstract— In a typical Surface Plasmon Resonance based instrument, a right angled prism is used in the optics. A laser beam is shone on one side of the prism, which passes via the hypotenuse side of the prism by refraction. But, above a critical angle of incidence, the beam is totally reflected internally and passes out of the second side of the prism. A gold plated glass plate is kept on the surface of this hypotenuse. In a standard SPR, the positioning of the gold plate will be adjacent to the analyte solution, which will be a flow cell comprising of the liquid chamber. So, depending upon the flow cell component and refractive index (R.I.), the Surface Plasmon resonance taking place in the gold, causes a dip of the reflected light, which is sensed by a photo detector. The angle at which this occurs shifts if the flow cell fluid R.I. changes. It is a very sensitive change and is therefore used for detecting and analyzing biological samples, particularly proteins. An SPR prototype instrument setup is developed and its results are also verified with the simulation results. The SPR generated in real time is also plotted in a laptop computer by a suitably developed HID based visual basic program interfaced through a programmed PIC 18F2550 microcontroller. The design principles are explained bringing out the cost effectiveness of the same.

Index Terms— Surface Plasmon resonance, Laser & Detector, gold coated plates, sensogram, embedded controller, SPR Instrument.

1 Introduction

SURFACE Plasmon Resonance [SPR] has become an important bio molecular sensing instrument because of its fast, label free, non-destructive testing and real time measuring of samples [1], [2]. Because of these excellent features, the SPR instrument is very predominately used in many application areas such as DNA, RNA studies, pollution Detection, and for all bio molecular reactions, vide [1], [2], [3]. Besides, the SPR instrument was developed by many instrument developers all around the world with different techniques, but the most popularly used method is the so-called Kretschmen configuration [4]. The work in this paper is concerned with the development of such an SPR instrument based on the Kretschmen configuration, with modifications. In the development of the SPR instrument, we are very much particular about the cost effectiveness, since a low cost Instrument is generally beneficial to human society [5], [6].

The complete SPR instrument is controlled by a Programmable embedded controller (PIC microcontroller) and the output measured from the photo voltaic cell (PV cell) is plotted in a Personal computer through a Visual Basic (VB) program [7], [8]. Since the SPR is a fast sensing instrument, it is possible to detect even small changes in the molecules of the sample [9].

Three different SPR setups are developed and tested with different samples in the laboratory. In the testing of different samples, the gold layer generally gets washed through continuous use of samples [10], [11]. It is too expensive to replace it often between samples. Hence the gold washing out from the expensive coating can be prevented and protected by keeping a Teflon layer over the gold layer. Here, the SPR signal that is generated for different dielectric samples with and without Teflon layer are measured in a cathode ray oscilloscope, and also on a computer screen through the USB provision of the microcontroller. The final developed SPR instrument can be fully controlled by suitable controlled icons developed with visual Basic software and controlled through the programmed PIC board.

2 Kretschmen Configuration and Generation of SPR

In general three different methods are being used for the development of SPR instrument and they are gratings, prism coupler (Kretschmen and Otto configuration) and optical waveguides. The most simple and widely employed method used for developing the SPR instrument is by Kretschmen configuration. Hence, e the SPR instrument here was developed based on the Kretschmen configuration. According the Kretschmen configuration, a light source is passed through the one of the faces of the gold slide affixed prism. Above the critical angle, the light gets totally reflected and is measured on the photo voltaic cell kept on the other face of the prism as a detector. Based upon the total internal reflection principles, the whole laser

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beam is absorbed instead of transmitted, as it impinges all over the surface of the gold layer on the slide kept on the hypotenuse face. Free electrons in Gold are excited and a resonance phenomenon absorbs the incident light beam. When the reflected light is observed by the detector signal in the CRO, it shows a dip in the measured waveform with the angle of incidence being varied. The dip in the curve assures the generation of SPR. The figure 1 below shows the SPR generated based upon the Kretschmen configuration and the figure 2 shows the detector output in which the SPR is generated at an incident angle of the light called the resonance angle, which is somewhat above the critical angle. . Based on the dielectric sample, the SPR curve shifts from one angle to another. The SPR generated depends upon the sample's refractive index. Even for small changes in the refractive index of the sample, SPR curve shifts in angle considerably and that is how the instrument is known for its sensitivity in analyzing bioreactions.

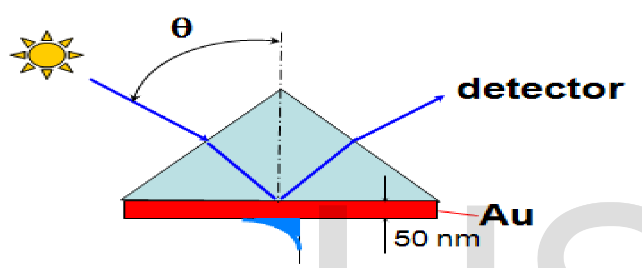

Fig.1 The Kretschmen configuration for SPR instrument.

The figure 1, explains the generation of SPR based on the Kretschmen configuration and the figure 2 explains, SPR curve generated at an angle greater than the critical angle.

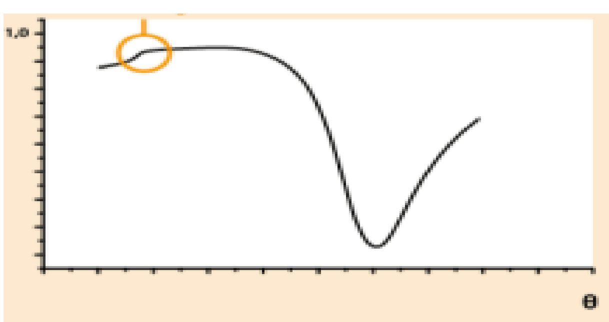

Fig.2 The dip in the SPR curve occurs above critical angle

3 PROPOSED SYSTEM

The figure 3 below shows the proposed systems used for the development of SPR instrument. The proposed system basically consists of three units 1. Kretschmen configuration based setup: He-Ne laser gun (whose power is > 20mw and wavelength is 635nm), polarizer and optical setup with photo voltaic cell as detector. 2. Controller unit: PIC microcontroller 16F73 and PIC 18F2550 is used to control the full SPR instrument operation. 3. Real time plotting of data from SPR instrument USB to Laptop through PIC microcontroller using Visual Basic program. The graphical user interface created by visual basic software for controlling the SPR instrument operation such as forward, reverse operation.

In the figure 3, prism with layers is zoomed to show the layers involved in the generation of the SPR. The layers are as follows: 1. Prism 2. Matching oil over the prism which is applied so as to remove the air molecules present and fix the glass plate very tight with the prism; .3. Glass plate. 4. Gold layer 50nm, 5. Dielectric sample (water is taken as the dielectric sample).

To prevent the gold layer over the glass plate is very important so as to cut the extra expenses. Therefore, Teflon layer of micron meter is stretched & placed over the gold layer. This Teflon layer is hence protecting the gold coating from washing out due to frequent use of samples. The dielectrics of the Teflon layer will have an impact over the SPR generated.

Here the SPR curve is repeated all over the angles. These curves to be integrated so as to obtain a single curve so as to compare with the standard SPR curve

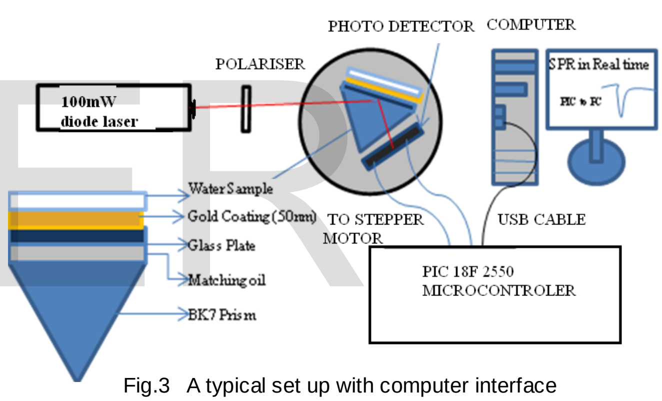

Fig.3 A typical set up with computer interface

4 EXPERIMENTAL SETUP

Based on the Kretschmen configuration the experimental setup is built, so as to generate the Surface Plasmon. Three different setups were tried out. The First setup is built such that the prism is kept stationary and laser beam is made to move back & forth. Second setup is build with laser beam made stationary and the prism is moved back and forth by stepper motor. Third setup (ϴ- 2ϴ) is built, such that both laser beam as well as prism is allowed to move back & forth. Among, these three different setups, it is tested that the second setup is very efficient for the generation of Surface Plasmon. Thus, the second setup is used and shown in the figure 4. The experimental setup as shown in the figure 4 indicates how the laser is directed over the prism and detected signal is measured in CRO and also in laptop. The DC power supply unit of 12V and 5V are used both for the stepper motor and microcontroller's board. The SPR generated is measured in CRO as well as in laptop in real time through USB from the SPR instrument. The real time SPR curve is plotted in laptop as time varying curve

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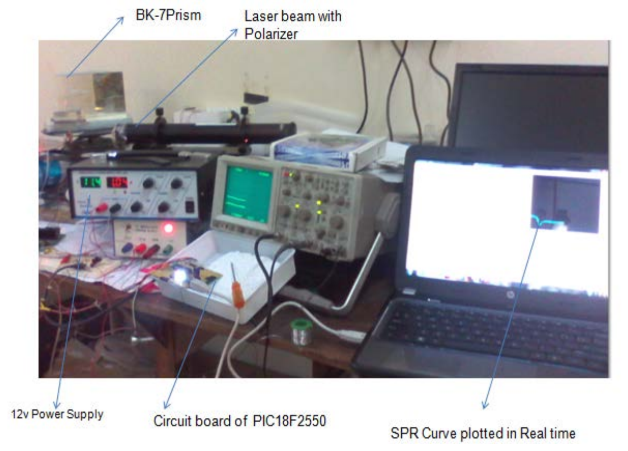

Fig 4.Experimental setup used for SPR generation

through USB from the SPR instrument with the PIC 18F2550 and it is visible in the figure 4.

5 SPR CURVE ANALYSIS

The Winspall is freeware software meant only for the analysis purpose of SPR. With this software it is a very simple procedure to generate SPR for different layers and also easy to find the best incident angle, Surface Plasmon resonance angle. With the use of this software, the experimental setup output is verified and also used in finding the critical angle, SPR resonance angle, for different layers causing the surface Plasmon resonance.

5.1 SPR Curve Layer Analysis

In the experimental setup, the layers used to generate the SPR are: BK7 prism, matching oil, glass plate, gold layer, and dielectric sample (water). These layers were common in all SPR instrument, but only the materials and refractive index will vary. Based upon these the materials & refractive index the SPR curve will changes in position if there is a change in the refractive index of the materials. Thus it is very important to discuss the layer properties in detail, so as to get a well defined SPR.

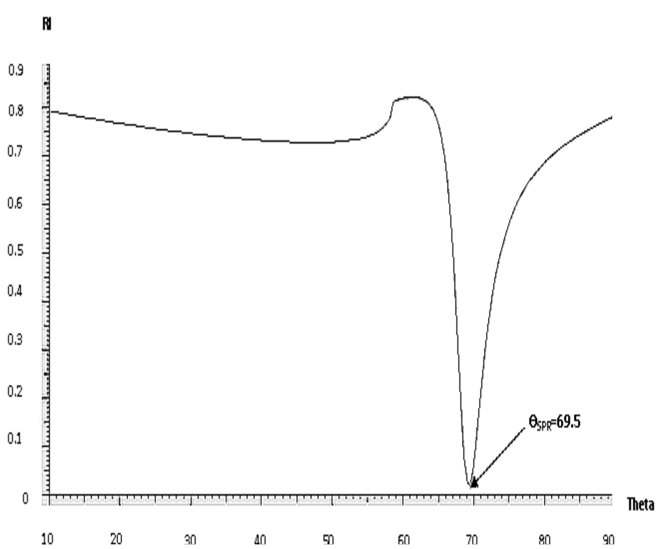

Fig 5.SPR generated at angle 69.5 degree using simulation software with water as a dielectric sample, whose refractive index is n=1.3

Materials	Thickness, nm	Refractive index, n	Absorption coefficient, k
BK7 Prism	0	1.62	0
Matching oil	1000000	1.62	0
Glass plate	5000000	1.62	0
Gold layer	50	0.1995	3.6425
Water(as sample)	0	1.3 to 1.33	0

Table 1 The materials used for the generation of SPR

The above table 1 clearly shows the materials used and their respective thickness values, refractive index and absorption coefficients, for the generation of SPR. For the above mentioned table parameters, the SPR curve is plotted and shown in the figure s 5 & 6 using Winspall software. The same could be noted in the experiments as well.

The figure 5 explains the surface plasmon is generated at an angle θ_{SPR}=69.5 degree with n=1.3 water as a dielectric sample. Figure 6, explains that surface plasmon resonance is generated at an angle θ_{SPR}=74 degree with n=1.33 as a dielectric sample. From these SPR curves, it is visible that the resonance angle shifts from 69.5^o to 74^o with respect to the refractive index change from 1.3n to 1.33n, whereas the other parameters and the absorption coefficients are constant. It is very clear that only the angle shift occurs whereas the others remain same.

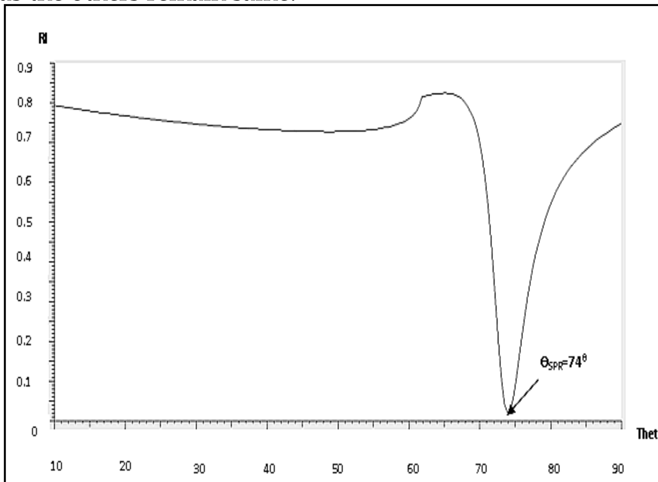

Fig 6.SPR generated at angle 74 degree using simulation software with water as a dielectric sample, whose refractive index is n=1.33

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This inference shows that SPR instrument is very sensitive even for small changes in the molecules in terms of refractive index.

Thus, by using the Winspall simulation software the SPR angle can be very easily identified and used for experimental setup. The Real time SPR, plotted in the laptop using Visual Basic through PIC microcontroller as shown in the fig.9 is also very similar to the simulation results. Thus the simulation results and real time SPR output match with each other.

6 Software Tools Used

In development of SPR instrument, three software were used. Oshon software for PIC programming, Visual Basic for graphical user interface for controlling the SPR instrument by laptop through PIC 18F2550. Basic complier for calculating the optimum incident angle for generating SPR and Eagle software for circuit layout for PIC microcontroller board. Winspall software is for investigating SPR for different layer parameters.

Oshon software V.691 was used for PIC programming of the chips 16F73 & PIC 18F2550. The figure 7 below shows the development of program as well as the verification made using Oshon software. The PIC does the running of stepper motor back & forth for moving the prism in our experimental setup.

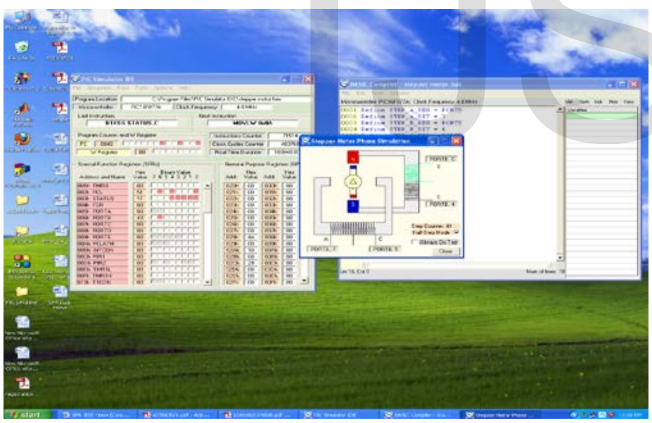

Fig 7 .Shows the PIC program simulated using Oshon PIC simulator.

The Visual Basic software is used for the developing the program for getting the USB data from the SPR instrument through PIC 18F2550. With the help of VB software, it is programmed in such manner that the SPR instrument can be controlled from the Laptop through labeled icons like forward, reverse etc

7 Circuit Board description

The whole SPR instrument setup is controlled by the embedded controller as the heart. Here, PIC 16F73 & PIC 18F2550 microcontrollers are programmed in such ways that for the generation of SPR at fast and higher resolution,PIC 16F73 microcontroller is mainly used to control the stepper motor motion back and forth in micro stepping. PIC 18F2550 microcontroller is used for USB data transfer from the detector PV cell to laptop through USB cable. PIC 18F2550 along with Visual basic program controls and plots the SPR in real time in the laptop.

The VB program developed used Visual Studio 6.0. A Program was developed using the Oshon Support files for USB interface to the PIC chip. The entire program is written in BASIC language makes for simplicity. The Visual Basic program was chosen because it has an interface in BASIC language for the HID interface. We installed GUI for the same which has provision for data and graphical display. The graphical display shows the SPR curves while the text displays the angle and analog detector value. Thus, we could step finely or fast and reverse the motor for repeated measurements. Also, keeping the angle constant, if the sample solution is varied, as it causes the detector output to change, that can also be plotted on graph.

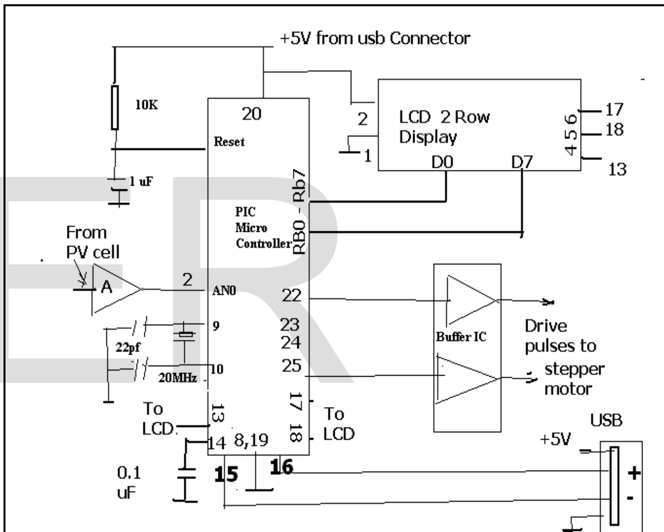

Fig. 8 Circuit Diagram of the PIC USB controller.

In the figure 8, PIC 18F2550 is used to control the stepper motor though ULN2003 IC. Where RA0 is used as inputs for the photo detector amplifier and RB0 to RB3 is used as output to control the motor through a buffer IC. The output from the RB port input to ULN2003 buffer and output from the ULN 2003 is fed to stepper motor coils. Pins 9 & 10 are used for crystal and pins 8 & 19 for ground. Pin 20 is given 5V DC power supply.

8 Discussion on the Result

The SPR instrument prototype is developed based on embedded controller (PIC 18F2550). The whole setup is controlled by software icons via Visual Basic program (graphical user interface) and PIC microcontrollers. The SPR is also plotted directly in laptop is shown in figure 9. The experimental result is also compared with Winspall simulation SPR results with same parameters as followed in the experiment. Thus the figure 9. Shows the SPR generated in real time is plotted in the laptop using PIC microcontroller and VB software. Thus SPR instrument prototype is built and we obtained good results. As the SPR instrument is used in bio-molecular applications, good accuracy and resolution are essential. These can be take care of by selection of stepper motor with gear ratio and using high intensity 100mW laser light source.

9 CONCLUSION

Thus the SPR instrument prototype has been developed based on the Kretschmann configuration and is capable of generating the SPR curve. Depending upon the dielectric samples, the SPR curve shifts very sensitively and results in an SPR output which is plotted directly in screen on a laptop with the use of embedded controller PIC 18F2550 and Visual Basic's graphical user interface. By simply clicking the labeled SP icons in the screen, it would be possible to obtain the SPR curve for any dielectric sample. Herein, SPR instrument results are also verified with the SPR simulation results. Hence, it is possible to detect the instantaneous changes in the molecular interactions. Thus, a simple, cost effective, user friendly, real time based SPR instrument prototype as developed for laboratory purpose has been described.

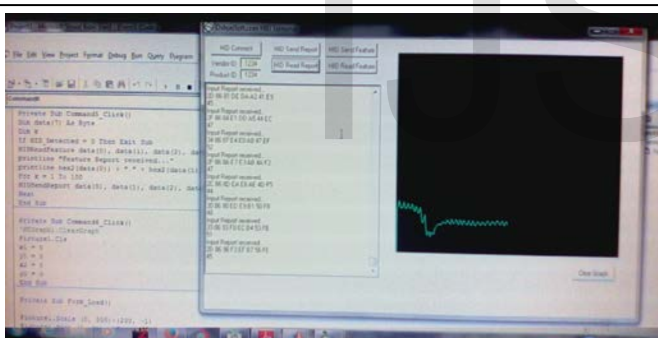

Fig.9 The graphical user interface used for plotting the SPR in

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