A fluorescence based optical biosensor for detection of dye causing cancer

ANUPAM DAS[a], ARINDITA SAHA[b], BHASKAR BISWAS[c], DEBAYAN SEN[d], SOUMYA DEEP DAS[e], ABHIRUP CHAKRABORTY[f*], SUMAN DEBNATH[g]
[a]*Department of Electrical Engineering, NIT-Agartala, Tripura, India*
[b]*TMC & Dr. BRAM Teaching Hospital, India*
[c]*Tripura University,Tripura, India*
[d]*Department of EIE, NIT-Agartala, Tripura, India*
[e]*School of Energy Studies, NIT-Agartala, Tripura, India*
[f]*Primus Super Speciality Hospital, New Delhi, India*
[g]*Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India*

This paper deals with the design and investigation of a newly developed biosensor enacted for quantifying the fluorescence intensity and for sensing concentration of dye. Here the outcomes of the measurements of various dye concentration have been presented for the purpose of assimilating the constructed sensor in the form of a biosensor, for the purpose of its intended significance in diagnosis of fluorophores nicotinamide adenine dinucleotide and flavins that are heeded as metabolomic markers of cancer.

(Received April 27, 2015; accepted September 29, 2016)

Keywords: Diagnosis, Pressure Sensor, Strain Gauge, Sensor System

1. Introduction

Fluorescence spectroscopy has particularly been a preferred method for cancer diagnosis, which is considered to be the most deadly diseases that people came across in the past century [1-3]. A lot of research work has been done and many more are still under consideration for developing novel fluorescence probes and improving the prevailing ones that can be used for particular type of cancer diagnosis. Taking an instance of indigenous fluorescence attributes of human urine specimens were investigated by the use of exhilarated–emission matrices over a span of exhilarated and emission wavelengths accompanied by emission spectra at 405 nm excitation [4]. It was examined and proved that the agents for metabolomics markers of cancer might be contemplated as fluorophores nicotinamide adenine dinucleotide along with flavins [4]. At the same time, clinicians and researchers are in search of an uncomplicated and quick approach for acquiring data immediately for diagnosis of particular characteristics of the given specimen. As a result they are looking for biosensors that would enable the investigation of wide range of stuffs like blood, urine, serum and etc. Because of the ability to work simultaneously as an optical as well as sampling cells, that streamlines the setup for measurements, and also because of ability to easily couple with the light sources and detectors the capillary waveguides can serve as the optimum biosensor for the purpose [5].

There are mainly two ways of implementation of Capillary waveguides which includes liquid core based waveguide or it may be a wall based evanescent waveguide. The working of the sensor is totally dependent upon the construction and the refractive index (RI) of the substance used for filling up the core of the capillary. In case of the core propagating waveguides, the core is filled by a material with refractive index higher in comparison to the refractive indexes of the wall, accompanying the light to the core and preventing the light from propagating into the wall which is made up of BK7 glass [6]. This particular framework of the core propagating waveguide permits the light to travel within the waveguide by means of total internal refraction [7]. The core propagating waveguide biosensor produces high sensitivity because of the reason that the light propagates directly within the core which is filled up with solution over a longer distance. For evanescent waveguide based biosensor, excitement of the fluorescence is close to the interior surface of the capillary where there is the presence of maximum evanescent field. In this prospect a new capillary waveguide is being suggested that is composed of a number of capillaries, exciting the fluorescence signal using the aforementioned two techniques one which is as a liquid core based waveguide and another that is as a wall based evanescent waveguide [8-9].

2. Materials and method

2.1. Proposed biosensor: The structure

The quintessential manufacturing of waveguide biosensors presupposes the implementation of only one

capillary. Whereas in case of the proposed biosensor we improved the sensitivity of the biosensor by means of using several capillaries concurrently. In the Fig. 1 mentioned below the framework of the suggested capillary waveguide based biosensor is being introduced. The proposed capillary waveguide based sensor is mainly composed of 37 capillaries that are made from BK7 glass [10]. These capillaries are being organized in a hexagonal lattice. The suggested biosensor is of length L = 1 cm. The capillaries used in the proposed biosensor are having an inner radius of R_i = 159 μm and an outer radius of R_o= 265 μm. The capillary present in the center is having an inner radius of R_i = 211 μm and an outer radius of R_o = 289 μm. The total volume of the proposed capillary base wave guide biosensor is about V = 42.29 μL. Proper assemblage of the dimensions of the capillaries used in the sensor and by virtue of the fact of viscosity no other components were needed for safe guarding the solution from spilling [11] .

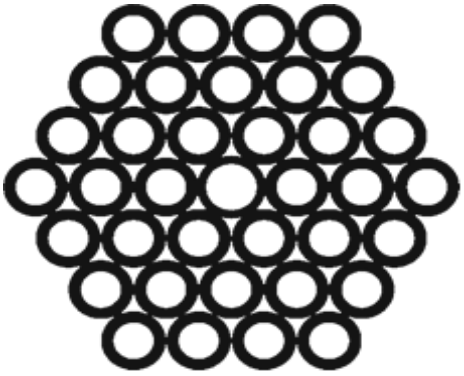

Fig. 1. Diagram of the Cross section of the investigated biosensor

The idea of the wave guide based biosensor is the outcome of the earlier implementation of the preform that was being developed in the course of designing a double cladding based cavity core fiber for the purpose of performing two photon fluorescence endoscopy. The unused preform in course of designing of the fiber was then cut and which in turn was implemented in the biosensor. As a result of which capillary present in the inner side of the sensor is of varying dimensions in contrast to the other used capillaries in the biosensor.

2.2. Working of the proposed biosensor

When a collimated beam of light irradiates the biosensor then, the light travels through both the glass and the solution exciting the fluorescence both in the solution and in the glass because of the presence of the of the evanescent field at the coalesce of the total internal reflection (TIR).Due to the presence of TIR, at optical coalesce when the light travelling through the waveguide with angle of incidence greater than φ, then the critical angle is given by [12]

$$\phi_c = sin^{-1}\left(\frac{n_1}{n_2}\right) \quad (1)$$

Here n_1 and n_2 are the refractive indices of the glass and the solution respectively, when n_1 is greater than n_2. The evanescent waves travels from the area of higher refractive index into the area of lower index. As the distance increases from the surface the strength of the evanescent field starts decreasing exponentially [16]. The length from the surface up to which the strength of the field is 1/e of its magnitude at the surface is defined as depth of penetration (d_p) [12].

$$d_p = \frac{\lambda}{2\pi\sqrt{\left(n_1^2\, sin^2\,(\varphi) - n_2^2\right)}} \quad (2)$$

As the wavelength increases, depth of penetration increases with increase in propagation angles (ϕ) which is relative to the normal perpendicular to the axis of the capillary being used in the biosensor, with the maximum at ϕ_{max}. If we consider that evanescent wave are being employed for the excitation of the fluorophores, then the fluorescence intensity can be given by [13]:

$$I_e = {}_{\alpha}\int_0^{\infty} \Phi c\, I_0 e^{(-\chi/\Lambda)}\, dx \quad (3)$$

Here I_o is known as the intensity of the incident light, c is known as the dye concentration and α is known as the molar absorptivity. The fluorescence quantum yield (Φ) is the ratio of photons emitted to the photons absorbed by the fluorophore which in turn provides the efficiency of the fluorescence process. Acridine orange (AO) is having quantum yield of 0.2 [14].

In the case of the proposed biosensor that is being irradiated by means of the collimated beam resulting in direct excitement of the fluorescence when light travels into the solution. Because of shorter path of the light in the sensor, we can surmise that the attenuation is imperceptible. Consequently, the fluorescence quantum yield Φ and the amount of light that is being absorbed is proportional to the intensity I_d:

$$I_d = k\Phi I_0\left[1 - 10^{(-abc)}\right] \quad (4)$$

Here b is the length of the path.

So, the total excited fluorescence achieved by the proposed biosensor can be defined as the addition of direct fluorescence and the excited fluorescence by means of evanescent wave [18, 19].

Fig. 3 shows the microscopic image of the part of the sensor and also fluorescence signals being generated by proposed biosensor. Solution of acridine orange (AO) is being used for filling up the biosensor. Here it can be noticed that the light travels with in the filled capillaries and through walls of the proposed biosensor. The circles depicted in the Fig. 3 illustrates the visible fluorescence in the biosensor.

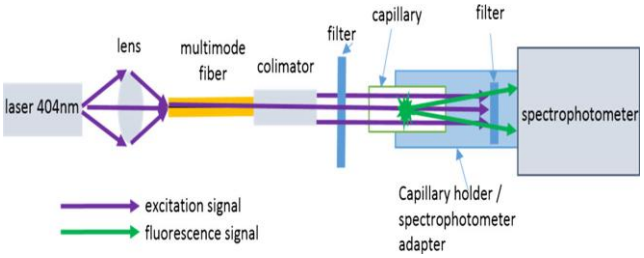

Fig. 2. Lay out of the setup for the purpose of measurement

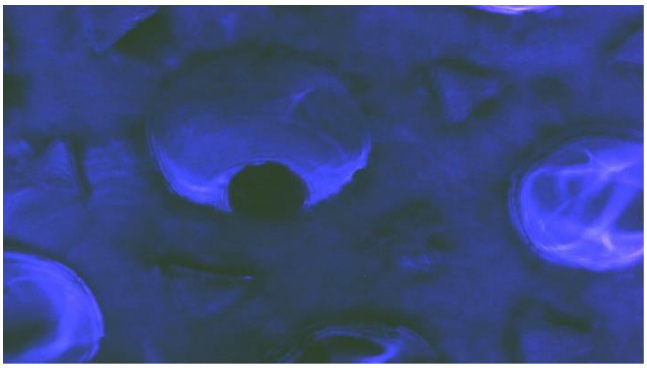

Fig. 3. Microscopic image of the fluorescence signals generated by proposed sensor

2.3. Setup used for the purpose of measurement

The capillary waveguide based sensor is being filled by liquid AO in a solution of water and then is was being irradiated by means of collimated light at a wavelength of 405 nm [20]. In order to make the process irradiation of the sensor simpler, multimode fiber was being used. For the purpose of irradiating the capillaries used in the biosensor at equal intensities a collimator is being used. To reject out the unnecessary irradiance developed from the multimode fiber, filter 1 was being used. The biosensor is kept in a holder in order to prevent the impact of light coming from the surroundings and concurrently helps in using the filter 2 to cut out the excited signals (Fig. 2).

3. Results

While experimenting the whole process, we got three biosensors from the perform of the fiber, which we used and organized a three sets of experiments with these three biosensors. To maintain the accuracy of the whole process, a syringe is used to pour the solution into the capillary. The capillary was being positioned in a holder in order to maintain the similar input state of the light. The capillary was cleaned many times with water and then it was dehydrated by using compressed air each and every time the solution was changed from the capillary. It is to be mentioned that this study has been done and tested from lowest to the highest molar concentration of the solution. Fig. 5 represents the mean values of the measured intensity of fluorescence for several molar concentration of the solution of AO in water as a contrast between observed fluorescence from field of the capillary with the solution of AO and that of the capillary field with in the water.

Fig. 4 represents the normalized mean intensities of fluorescence for several molar concentration of AO. The standard deviation is represented by the error bar. From the figure it can be well understood that the intensity of fluorescence is proportional to that of the dye concentration. It can be observed from the Fig. 4 that the error bars are small which is due to the repeatability of the measurement [17].

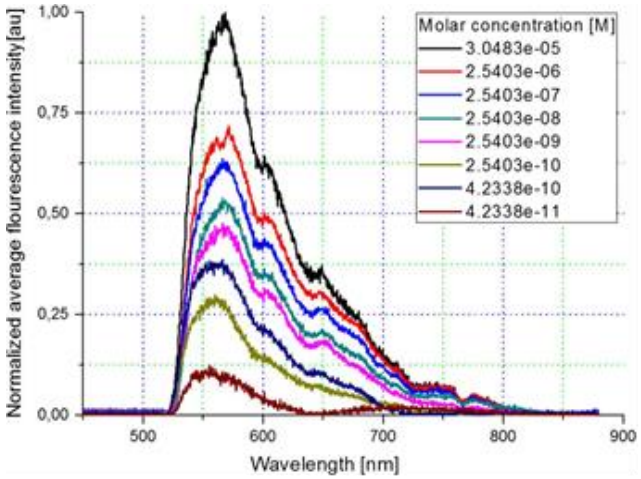

Fig. 4. Graph for various molar concentration of acridine orange (AO) solution in water accompanied by maximum normalized average fluorescence intensity

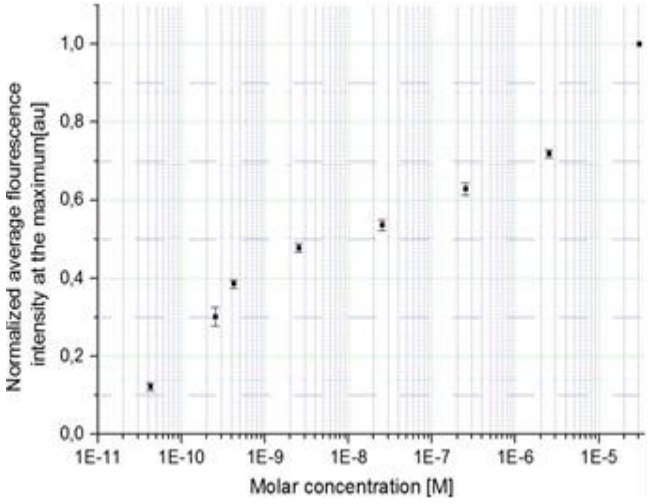

Fig. 5. Graph for various molar concentration of acridine orange (AO) solution in water accompanied by normalized average fluorescence intensity

4. Conclusion

So it can be concluded that the proposed and investigated capillary biosensor is a straight forward and economical piece of equipment that is able to detect fluorophores in a given solvent. In this prospect AO solution was used in water for detecting the principal properties of the proposed capillary based biosensor. The experiment manifested that the fluorescence signal can be measured for concentration of dye contained in a range of 10^{-10} M which is very small, with in a small volume of solution of V = 30.1 μL. This measurement of very low concentration of dye was possible because of the use of several short capillaries accompanied by the elevation of fluorescence directly and also by an evanescent wave. The proposed biosensor can also be used for detecting very little concentration of NADH present in urine by presuming the quantum production of NADH is thrice the lesser than that of the acridine orange (AO). Moreover, the measurement of the florescence signal can be further improved by the application of spectrophotometer adapter [15]. The spectrophotometer adapter can be employed for effective detection of signals from the biosensor and also as an integrating sphere.

References

[1] M. Panjehpour, C. E. Julius, M. N. Phan, T. Vo-Dinh, S. Overholt, Lasers Surg. Med. **31**(5), 367 (2002).
[2] E. Borisova, P. Troyanova, P. Pavlova, L. Avramov, Quant. Electron. **38**, 597 (2008).
[3] Z. Volynskaya, A. S. Haka, K. L. Bechtel, M. Fitzmaurice, R. Shenk, N. Wang, J. Nazemi, R. R. Dasari, M. S. Feld, J Biomed Opt. **13**(2), 024012 (2008).
[4] R. Rajasekaran, P. R. Aruna, D. Koteeswaran, L. Padmanabhan, K. Muthuvelu, R. R. Rai, P. Thamilkumar, C. M. Krishna, S. Ganesan, Photochem. Photobiol. **89**, 483 (2013).
[5] B. K. Keller, M. D. DeGrandpre, C. P. Palmer, Sens. Actuators B **125**, 360 (2007).
[6] Zourob, Mohammed, Lakhtakia, Akhlesh (Eds.). Optical Guided-wave Chemical and Biosensors II, Springer; 2010 edition.
[7] R. Ulrich, Nouvelle Revue d'Optique **6**, 253 (1975).
[8] María Espinosa Bosch, Antonio Jesús Ruiz Sánchez, Fuensanta Sánchez Rojas, Catalina Bosch Ojeda, Sensors (Basel).**7**(6), 797 (2007).
[9] M. A. van Eijkelenborg, M. Large, A. Argyros, J. Zagari, S. Manos, N. Issa, I. Bassett, S. Fleming, R. McPhedran, C. M. de Sterke, N. A. Nicorovici, Opt. Express **9**(7), 319 (2001).
[10] A. Bossi, L. Castelletti, S. A. Piletsky, A. P. F. Turner, P. G. Righetti, Electrophoresis **24**, 3356 (2003).
[11] L. Gorton. Biosensors and Modern Biospecific Analytical Techniques (Comprehensive Analytical Chemistry), 375-421, Elsevier, 2005.
[12] E. D. Paprocki, B. K. Keller, C. P. Palmer, W. R. Laws, M. D. DeGrandpre, Sens. Actuators B Chem. **135**(1), 145 (2008).
[13] A. J. Demello, B. Crystall, G. Rumbles, J. Colloid Interface Sci. **169**, 161 (1995).
[14] B. Soep, A. Kellmann, M. Martin, L. Lindqvist, Chem. Phys. Lett. **13**(3), 241 (1972).
[15] Y. S. Li, W. P. Liu, X. F. Gao, D. D. Chen, W. G. Li, Biosens Bioelectron. **24**(4), 538 (2008).
[16] Harshini Mukundan, Aaron S. Anderson, W. Kevin Grace, Karen M. Grace, Nile Hartman, Jennifer S. Martinez, Basil I. Swanson, Sensors **9**, 5783 (2009).
[17] Das, Anupam, et al. International Journal of Biomedical Engineering and Technology **17.1**, 83 (2015).
[18] Saha, Saptadip, et al. Recent Developments in Control, Automation and Power Engineering (RDCAPE), 2015 International Conference on. IEEE, 2015.
[19] Das, Anupam, Arindita Saha, International ournal of Telemedicine and Clinical Practices **1.4**, 293 (2016).
[20] Saha, Arindita, Anupam Das, Abhirup Chakraborty, Technology and Health Care **24.2**, 241 (2016).

*Corresponding author: apsuraabhi@gmail.com