A REPORT ON

Modelling and Simulation of Fluorescence based Optical Sensors using Microheaters

By

Vishwanath Guruvayur **2018A4PS1048H**

At

CENTRAL ELECTRONICS AND ENGINEERING RESEARCH INSTITUTE, PILANI

A Practise School – I station of

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

(June, 2020)

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Prepared in partial fulfilment of the

Practice School – I Course **BITS F221**

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ABSTRACT

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI (Rajasthan)

Practice School Division

Station: Central Electronics and Engineering Research Institute **Centre:** Pilani

Duration: Six weeks **Start date:** May 18, 2020

Date of Submission: June 24, 2020

Title of the Project: Modelling and Simulation of Fluorescence based Optical Sensors using Microheaters.

ID No. / Name / Discipline:

Vishwanath Guruvayur 2018A4PS1048H B.E.Mechanical Engg.

Name and Designation of the Expert:

Dr. Vijay Chatterjee Chief Scientist (Smart Sensors)

Name of the PS Faculty:

Dr. Noel Prashant R

Key Words: Microheaters, Fluorescence, Biosensing, Optical Sensors,

Project Areas: Optical Sensing, Biosensing, MEMS, VLSI

Abstract: This project review includes basic concepts required for developing an optical sensor based on the concept of fluorescence and also learning about the modelling and simulation of an optimized micro-heater required for the temperature maintenance for optimized biosensing.

Vishwanath Guruvayur Noel Prashant

May 24, 2020 May 24, 2020

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1. INTRODUCTION

Biosensors are smart devices which detect the presence of biological analytes and calculate its concentration. The biological analyte could be a biomolecule, a biological structure or a microorganism. The research and applications of biosensors is a rapidly growing field and it combines various sciences like biology, chemistry, physics, electronics and computer science. High specificity, portability, sensitivity and compactness are some reasons why biosensors have a high potential of replacing the currently existing analytical practices.

Fluorescence is by far the most widely used concept used for the detection and study of biological analytes in biosensors. The interrelations between fluorescence, optical properties and temperature is a very fundamental topic in biomedical optics. Thermal effects during these applications can often present confounding experimental factors. The exact mechanism by which temperature affects fluorescence is not completely defined or understood.

My project – Modelling and Simulation of Fluorescence based Optical Sensors using Microheaters is mainly related to the maintaining of a specific optimal temperature range for a specific amount of time so that there is a higher probability of fluorescence.
And the next part is to incorporate this microheater to optimimize the fluorescence and therefore improve the optical sensing based on fluorescence.

2. How do Biosensors work?

Biosensors works on the principle that the interaction between any two bio molecules proteins specifically causes a chemical change. This chemical change can be interpreted in many ways such as pH change, heat transfer, mass transfer, refractive index changes etc. Figure 1 below shows a schematic of basic biosensor and it's working principle.

Analyte is the particular molecule in the sample which needs to be detected and for that purpose the BRE (Bio-recognition Element) are made which can only bind the particular analyte. Both BRE and analyte are proteins or enzymes. The blue plate on which the BREs have been fabricated is the transducer which converts the chemical change into recognisable signals such optical, electrical etc. as shown in fig1.

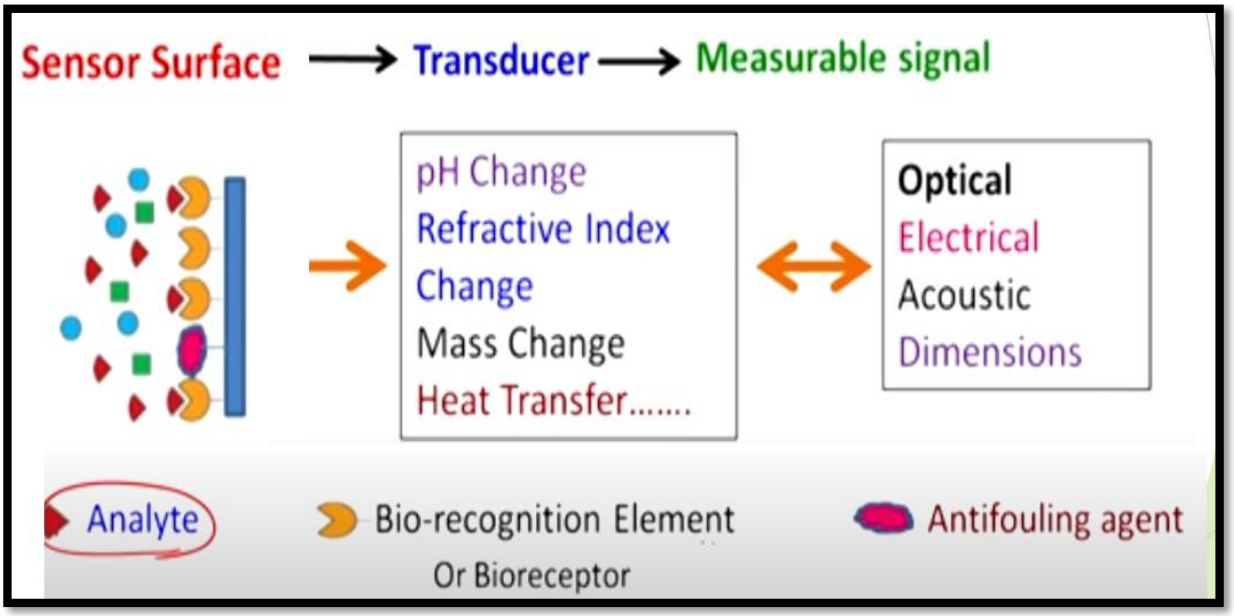

Fig.1 Components of Biosensors [2]

So, for the case of optical sensors the transducer converts the change into an optical signal which could be a change in phase, orientation, amplitude or wavelength of the incident light. The change in wavelength and amplitude causes the fluorescence detection to be possible.

3. Fluorescence based Detection

Fluorescence is by far the most widely used concept used for the detection and study of biological analytes in biosensors. The interrelations between fluorescence, optical properties and temperature is a very fundamental topic in biomedical optics. Thermal effects during these applications can often present confounding experimental factors. The exact mechanism by which temperature affects fluorescence is not completely defined or understood.

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And the next part is to incorporate this microheater to optimimize the fluorescence and therefore improve the optical sensing based on fluorescence.

Before understanding the concept of fluorescence-based detection technique we need to understand another concept and that is FRET Fluorescence Resonance Energy Transfer

3.1 FRET

It is the transfer of energy from the donor protein (blue one in fig 2) which can emit fluorescence of a certain wavelength to the acceptor protein (yellow one in fig 2) which can emit fluorescence of another wavelength. So, when a light of certain wavelength is shined upon the donor protein the FRET takes place and this energy is released from the acceptor protein with a light of different wavelength in the form of fluorescence.

This fluorescence could be detected by the detector and since the acceptor here is the BRE or a BRE tagged with a certain dye of fluorophores (materials which can emit fluorescence) therefore it can give a qualitative analysis of whether the analyte we are looking for is present in the sample or not based on whether the fluorescence was detected or not.

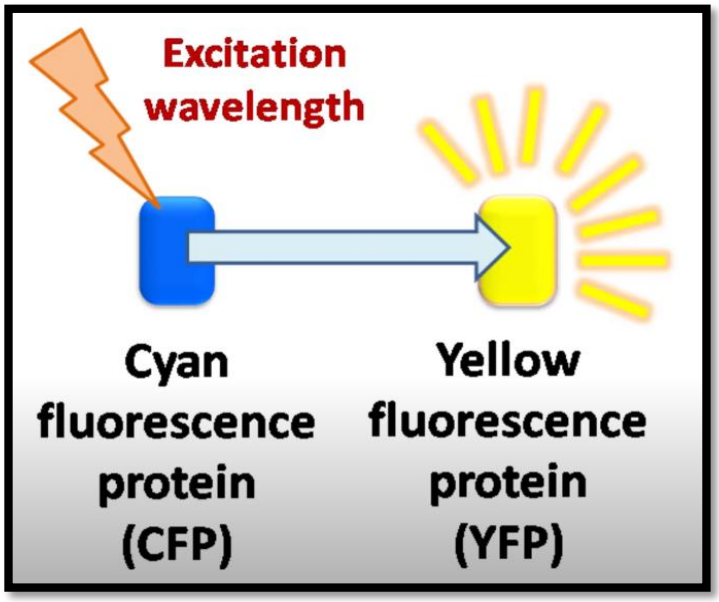


Fig. 2 Fluorescence Resonance Energy Transfer [4]

The donor and the acceptor protein must be bound to each other for the FRET to occur.

3.2 Understanding Fluorescence with an Example

This example has been taken from a research paper given in the reference. It is not required that the fluorescence sensing device should be fabricated in the form of a sensor only as discussed in 1. It could be in the form of a solution where the components of the solution represent the different components of a typical biosensor. To understand this concept here is an example of fluorescence-based detection of H2S (analyte) with the help of Mb (BRE and transducer). The FRET based approach will be chosen while exploiting the fact that the optical properties of Mb changes upon interaction with H2S. We will understand the concept with the help of two different dyes one of them is Cy3 which emits fluorescence in the 550-570 nm range while the other one is the Atto620 which emits its fluorescence in the 640 nm range.

The absorption spectrum of Mb exhibits a characteristic Soret band at 409 nm and two less-intense bands centred at 503 nm and 636 nm, H2S addition shifts the Soret band to 421 nm and quenches the 503- and 636-nm bands leading to

the appearance of three new bands centred at 543 nm, 581 nm, and 617 nm (see Fig. 3a). This change of the absorption spectrum of Mb will strongly modulate the fluorescence properties of a FRET donor–acceptor pair.

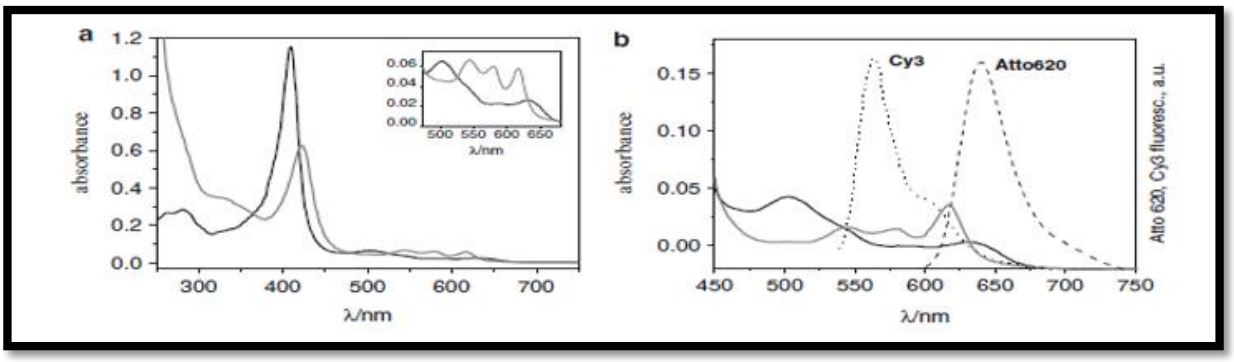


Fig. 3a [3]
absorbance spectrum of H2S free(dark) H2S bound(light) Mb

Fig. 3b [3]
absorbance spectrum of H2S free H2S bound Mb with emission spectra of Cy3 and Atto620

The idea is that using a dye label emitting in the 550-570 nm range, when the protein is in the H2S- free state, the fluorescence of the dye is essentially uninhibited, whereas as soon as H2S binds to Mb, the fluorescence of the label is quenched as a result of energy transfer to either the 543-nm or the 581-nm band (depending on the emission of the dye label chosen for labelling Mb). In other words, the H2S binding to labelled Mb turns off the dye fluorescence.

Differently, using a dye label emitting around 640 nm when the protein in the H2S-free state, the fluorescence of the dye is initially quenched as a consequence of energy transfer to the 636-nm band, whereas as soon as H2S binds to Mb, the 636-nm band disappears and all the energy absorbed by the label is emitted as fluorescence. It follows that the fluorescent label acts as a sensitive reporter of the H2S bound to the iron centre. The overlap of the Mb absorption bands (H2S-free and H2S-bound forms) with the emission spectrum of either Cy3 or Atto620 is shown in Fig 3b.

To test the system, the fluorescence intensity of labelled Mb was monitored as a function of time during a change from an H2Ssaturated to an H2S-free environment. Figure 5 shows a typical time trace of a solution containing 100 nm. of dye-labelled Mb when excited at the absorption maximum (l ¼ 550 nm) of the dye (Cy3).

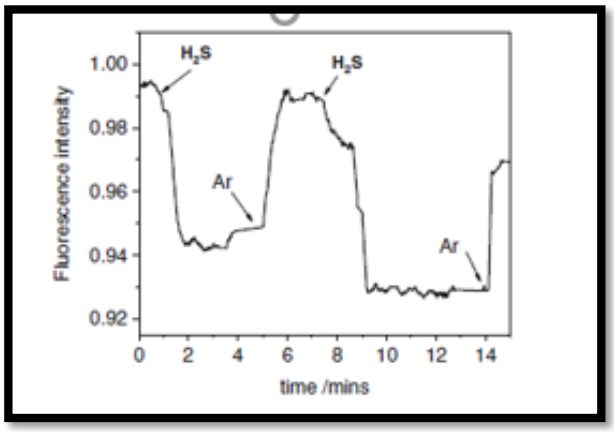


Fig. 4-time dependent fluorescence intensity with Cy-3 dye [3]

In this particular experiment each cycle was started by adding H2S to an end concentration of 50 mM (i.e., in excess over the Mb concentration) and completed by passing argon through the solution for the complete removal of H2S. The dye emission was followed at 570 nm. A fluorescence quenching of label emission was clearly observed upon each H2S addition. When bubbling argon through the sample solution to displace the H2S, the initial fluorescence intensity of the label was restored; the cycle could be repeated many times. This finding showed that, in the experimental conditions tested, the H2S-binding process is reversible, which is crucial for practical sensing applications.

When monitoring the Atto620 labelled Mb sensing construct under the same conditions, an opposite trend was observed (Fig. 5). An increase in label emission was observed upon each H2S addition. When displacing the H2S by bubbling through argon, the fluorescence intensity of the label diminished again.

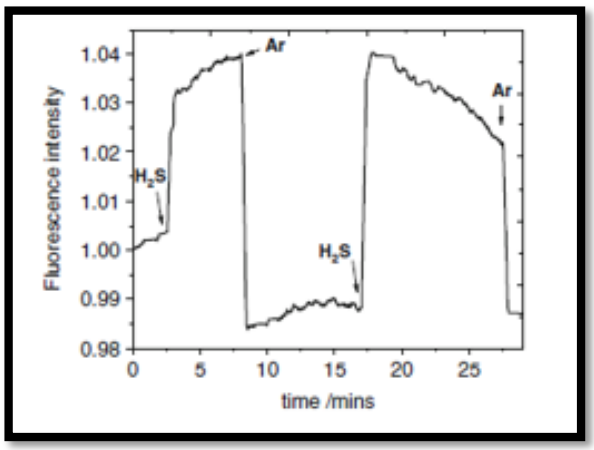


Fig.5 time dependent fluorescence intensity with Atto620 dye [3]

4. Role of Temperature in Fluorescence Sensing

The particles which are able to emit fluorescence upon absorption of light energy are called fluorophores and fluorophores quenchers are the particles which turn off or reduce the fluorescence effect of the fluorophores. It is sometimes possible that the sample being tested come with fluorophore quenchers which reduce the fluorescence effect hence compromising with the signal.

4.1 Fluorescence Quenching

Fluorescence quenching refers to any process by which there is a decrease in the intensity of emitted fluorescent light. The most common type of quenching is the one in which a certain molecule, or quencher, comes into contact with the fluorophore in its excited state. This is known as collision quenching.

This collision quenching results in the absorption of energy by the quencher which makes the fluorophore to come back to its ground state. This reduction in the fluorescence is described by the Stern-Volmer equation which is given as

$$\frac{F_0}{F} = 1 + \kappa_q \tau_0 [Q]$$

Fig. 6 Stern-Volmer equation

Here, F is the measured fluorescence, F_0 is the ideal unquenched fluorescence, K_q is the bio-molecular quenching constant (s^{-1}), t_0 is the unquenched fluorescence lifetime (s) and [Q] is the quencher concentration. The bio-molecular quenching constant depends on temperature and viscosity and can be calculating by using the Stokes-Einstein relationship. So, temperature plays a key role in the occurring of molecular quenching and the measured fluorescence.

4.2 Coagulation

Coagulation is the process in which blood converts from a liquid to a gel, forming a clot.

It is known that temperature past a certain threshold can cause coagulation. This leads to a change in the optical properties by means of scattering or increased opacity. These properties bring a change in the fluorescence and are a part of how the mechanism of fluorescence is affected by temperature.

So, the fact has been established that temperature plays a crucial role in the fluorescence properties a material shows. Hence, it is our task to maintain a specific temperature range for the high probability of occurrence of fluorescence rather than the alternative processes which can happen on the incidence of an energetic photon. This task of maintaining a specific temperature range is accomplished by the incorporation of a micro-heater.

5. What is a Microheater?

Microheaters are small high-power heaters, with precise control, that can offer temperatures up to 2000 ^{o}C. General heating method of microheaters is the conversion of electric work to high density heat. Microheaters are widely used in the field of Biosciences for fulfilling the various prerequisites required to test a specimen.

Microheaters are mostly made of platinum coils the platinum structures constitute a heater coil and a temperature sensor coil. The heater coil is supplied with an electrical excitation which increases the temperature of the micro-heater membrane. Fig. 8 shows a basic microheater.

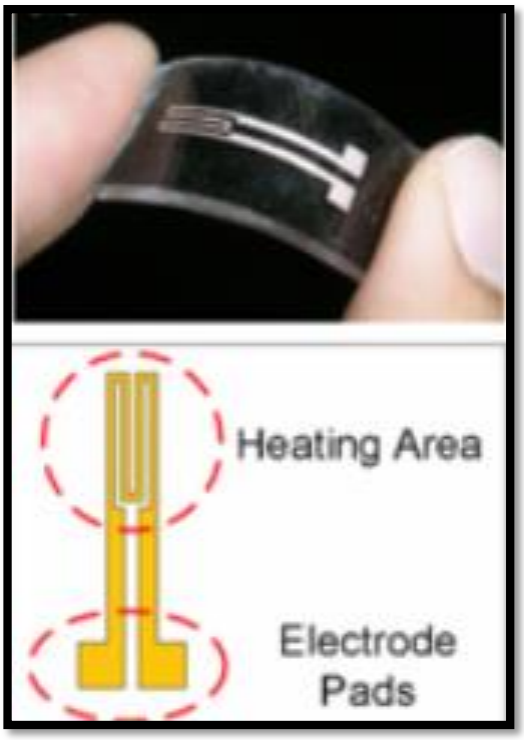


Fig.7 Basic Microheater [6]

6.Previous work done at CEERI in Microheater domain

Before dwelling into the design process of the microheater we can look into the work done at PS station discussions regarding which with the mentor were very helpful to us.

Fig shows the simulation done on a linearly arranged microheater strip by CEERI scientist Dr. Vijay Chatterjee with his fellow research scientists.

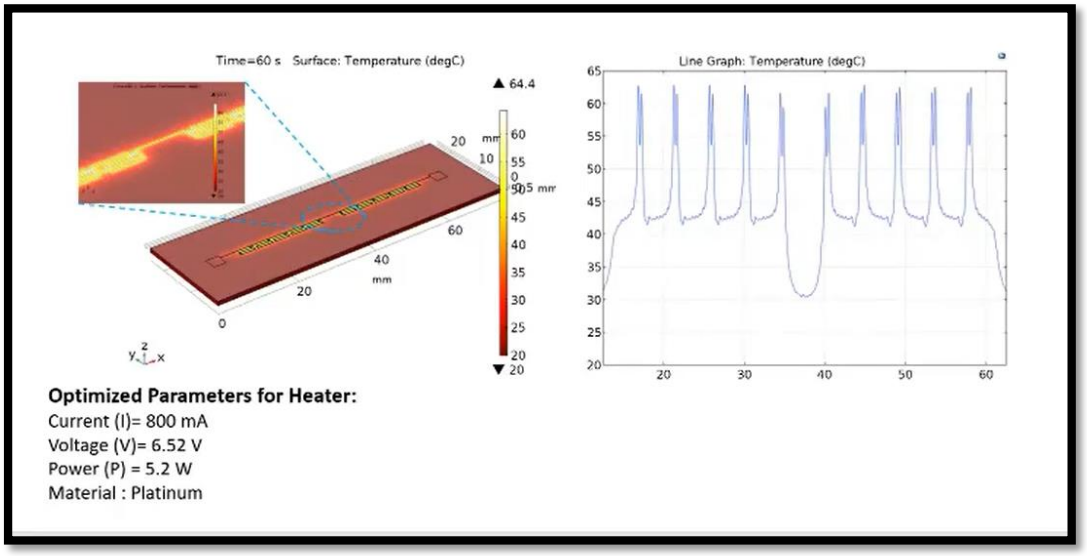


Fig.8 simulating linearly arranged microheater without fluidic interface [12]

It is the simulation of a microheater with platinum as the material used and no fluid on the interface. The temperature graph is depicted on the right side of the image.

There was one more simulation done with a fluid interface with 1mm depth with the same microheater. This simulation was done to understand the effect of a fluidic interface of a specific width on the temperature along the length of the microheater. The image of this simulation is shown below.

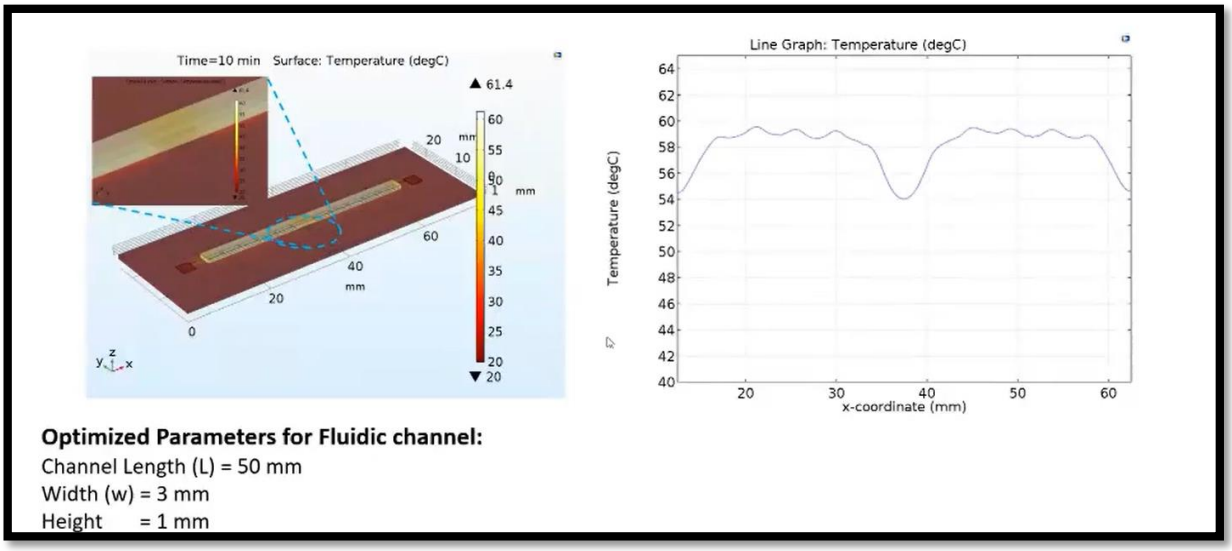


Fig.9 simulation of linearly arranged microheater with fluidic interface [12]

So, as we can observe, the peaks which are observed in the non-fluidic interfaced microheater have been mellowed down and those peak temperatures could not be reached. Possible explanation for this could be the absorption of the heat by the fluid.

One more factor which is very important to keep into account is the velocity of the interface of the fluid being tested. The velocity of the fluid will lead to the flow of heat emitted by the microheater leading to a complex distribution of temperature. Thorough study of fluid mechanics would help in determining the various parameters involved in the temperature distribution with respect to time.

7.Design Process of a Microheater

To achieve the final microheater product it could be divide into two parts one is the design which completely done on computers with different analysis and simulations, the other is the fabrication which done in labs using different equipment for the purpose. So, the on-system design which is the result of deep analysis and optimization could be divided broadly into certain number of steps which are discussed below.

7.1 Geometry Selection

The geometry of a microheater is basically a coil which is arranged in different orientations and shapes. There are a vast range of shapes that are currently in use for different applications (see Figure 1). These shapes however can be changed or arranged according to our own requirement their combination or more than one coil of the same shape could be used to make a completely different strip of a microheater.

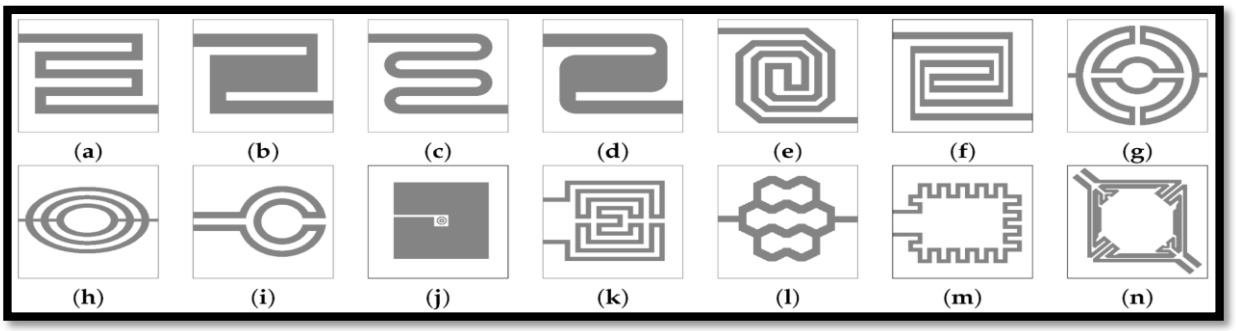
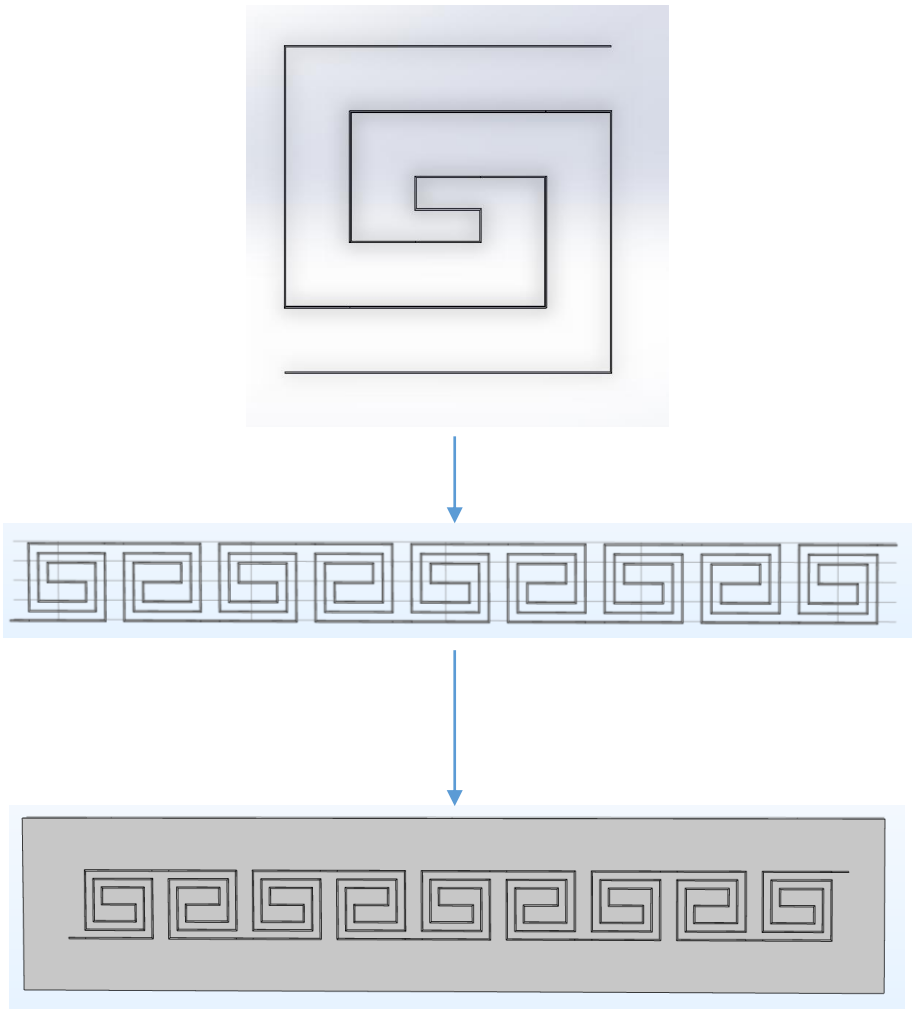


Fig.10 Microheater Shapes [7]

To get an understanding of how they can be used for making a new geometry out of it, Fig 2 shows the model that I have made for the microheater. The fan type shape has been used multiple times to form a long strip of microheater.



Double Square Spiral Microheater Coil Modelling Process

The selection of a new geometry is based on the analysis and idea of geometries already tried out before and altering it. An idea of the geometry was given by the mentor which was already tried and what new could be implemented for the shape and then intuitively the geometry was made.

7.2 Material and Electro-Thermal setup

Material selection for the microheater is based on the certain properties that are desired from the material. First, the material should not oxidise at high temperatures since a microheater could operate at temperatures as high as 2000 degrees therefore a common metal could oxidise at such temperatures further it is expected from the material to have stable temperature coefficient of resistivity. The conductivity or the resistivity of the metals is a function of temperature and to minimize this change in the material property while the temperature changes in the microheater it is necessary to keep in mind the temperature coefficient of resistivity in mind while selecting material. All these properties lead to a small number of options for the material thus making the task easier. Platinum is the most commonly used material for microheater and was also suggested by the mentor hence it was used as the material for the microheater strip.

Electro-thermal setup involves setting up the environment for the entire system i.e.- the microheater strip and the substrate for the simulation. This environment setup involves defining the boundary conditions defining which parts would be at room temperature, giving the value of the voltage or the terminal current whichever is applied, defining the material properties for the substrate and the microheater. It is necessary to ensure all the things for the setup otherwise the meshing could not be done and the result will not be computed.

7.3 Result Analysis

The result analysis is discussed completely on the basis of the research paper given in the reference.

From the simulation of a system many different results can be obtained and these results are to be analysed together to reach on to an opinion for a particular design. In this paper a microheater geometry has been developed which consists of small coils arranged in the form a matrix. The results have been obtained for two combinations one is for a 6 cross 6 matrix and the other is for a 3 cross 12 matrix. The temperature profile and the electric potential profile along with the two geometries could be seen in fig.11.

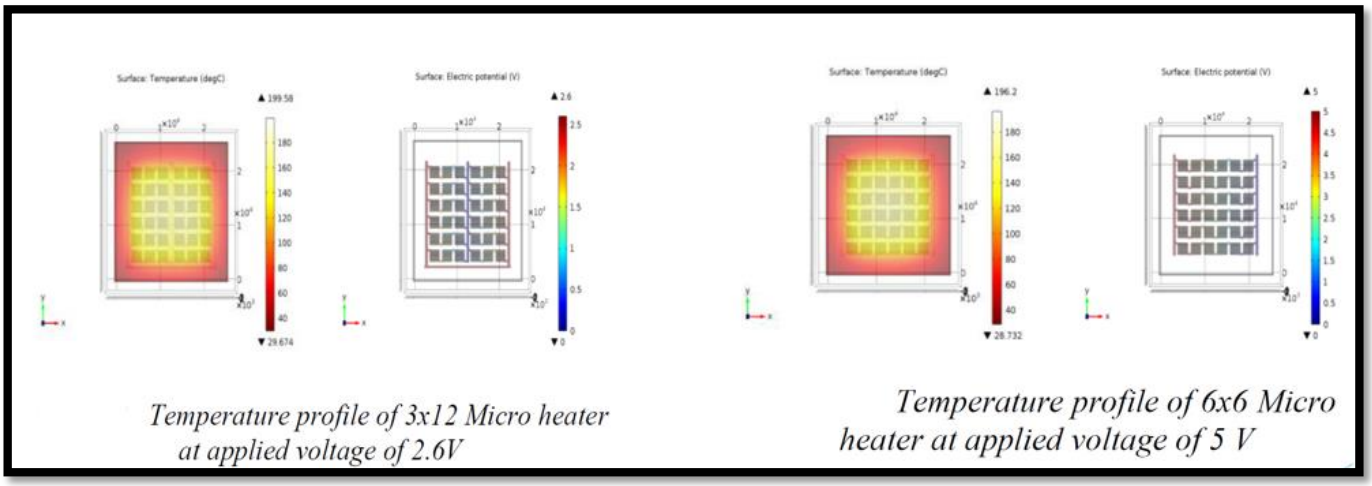


Fig.11 Temperature and potential profile for both heaters [11]

Since the geometry were different therefore to obtain the same temperature range different voltages needed to be applied for the 3 cross 12 it is 2.6 V and for the 6 cross 6 it is 5 V. Apart from these profiles many graphs could be obtained with respect to different parameters for each geometry. For example, if the microheater is to be optimized on the basis of least power consumption which is generally the case then a comparison for the power consumption could be made at the desired temperature ranges. In this case fig4 shows the graph of temperature vs. power consumed for each design.

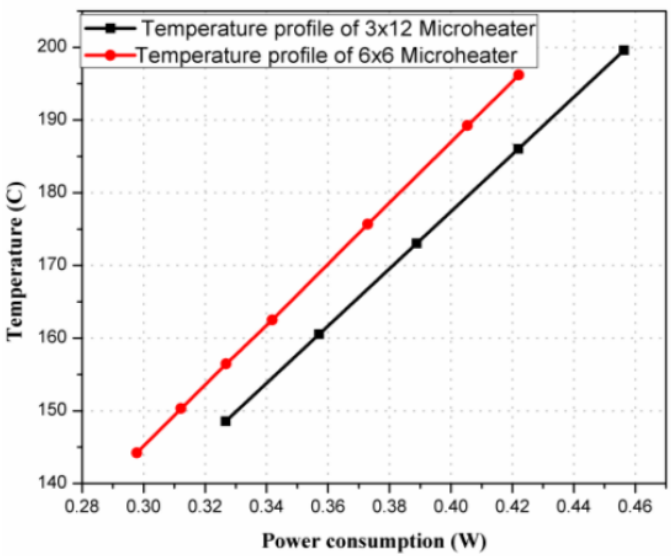


Fig.12 Temperature vs. Power consumption for both designs [11]

In fig4 the red line represents the plot for 6 cross 12 microheater and the black line represents the plot for 3 cross 12 microheater. The comparison could be made on the basis of the temperature range on wants to operate on and at that point a simple horizontal straight line could be drawn and the points of intersection would give the corresponding power consumption. The heater consuming less power could be classified as a better option for the case of least power consumption as the deciding parameter. Several other plots could also be observed according to the requirement fig.13 represents the graph of temperature vs. voltage applied for the two microheaters.

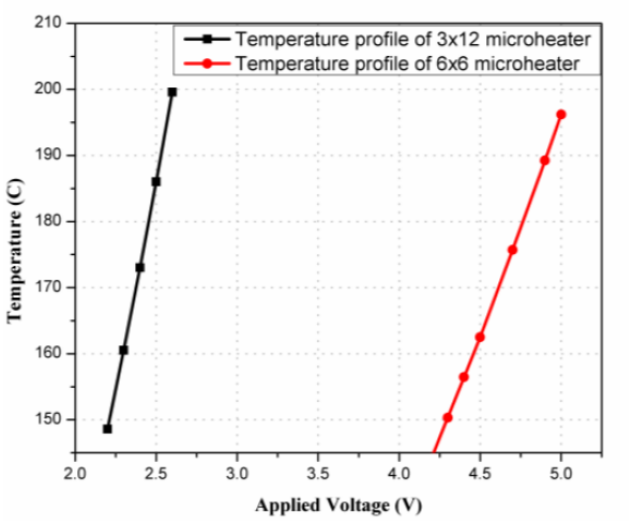


Fig.13 Temperature vs. Voltage for both coils [13]

Now if the deciding parameter is the limitation on voltage then it can be seen that the two coils operate at two different voltage range to give similar temperature profile so a selection could be made on the basis of the applicable voltage.

Graphs between temperature and current, temperature and resistance could also be obtained and the selection could be made on the required deciding parameter.

The analysis of results helps to compare between two geometries so a new geometry could be iterated and compared with the previous one and this process could be repeated until an optimized design is obtained.

8. Mathematical Analysis

Microheater works on the principle of joule's heating but there is one more phenomenon that happens in it and that is heat transfer in solids. The equations of both of these phenomenon work simultaneously and gives the temperature profile over the geometry.

The following equations are involved for the Joule heating

$$Q = JE = J\left(\frac{J}{\sigma}\right) = \frac{1}{\sigma}J^2 = \rho J^2 \qquad J = \frac{V_{in}}{RWd} = \frac{V_{in}}{Wd}\frac{A}{\rho L}$$

Fig.14 Heat equation [10] Fig.15 current density [9]

Where E = Electrical field w = width A = area of cross-section R = Resistance J = current density d = thickness rho = resistivity whose reciprocal is the conductivity(sigma) given by: -

$$\sigma = \frac{\sigma_o}{1+\alpha(T-T_0)}$$

Where,

σ_o =Conductivity

T_0 =Reference temperature

α =Temperature coefficient of resistivity

Fig.16 temperature function of conductivity [9]

The power consumption can be expressed in terms of either current and resistance or voltage and resistance and it is given by Power P = I^2*R where I is current and R is the resistance of the microheater the equation could be expressed in terms of voltage also given P = V^2/R where V is the applied voltage. Which expression is to be used depends on whether voltage or current has been applied at the two ends of microheater.

The next equation is for the heat transfer in solids module which is given by

$$\rho C_p \frac{dT}{dt} - \Delta(k\Delta T) = Q$$

Fig.17 Heat transfer equation [10]

The heat generated due to the conversion of electrical energy is used by the heat transfer equation to give the temperature profile using the equation in Fig. 17

9. Fluid Interface with Microheaters

As our main objective is to fulfil the temperature related prerequisites for optimal fluorescence emission, it is important to study the temperature related behaviour of a fluid on interfacing it with a microheater.

There have been various approaches for the integration of fluidic components like the hybrid-package approach, direct integration of IC compatible process, etc.

Integration of microheaters in microfluidic systems has important applications like the control of chemical reaction rates, temperature cycling, fluid flow measurement and control, etc.

As our main task is to maintain a specific temperature range of a substrate under study by fluorescence optical sensing, we need to simulate these microheaters with various different types of fluid environments and understand the variation of temperature of the fluid through the conduction and convection process.

For advanced optical sensing, the fluid under study maybe in motion and this motion maybe laminar or turbulent in nature. Simulation with the fluidic interface with various velocities will bring out various important results.

In Biomedical sensing, common fluids under study are blood, mucus, etc.

10. Scope of Design Improvements

By changing few parameters of the microheater, the results also change and hence they give a base for comparing various different applications. Few things which can be changed about a microheater coil are its width, length, thickness, material used, shape, combination of various shapes, etc.

Design improvements can also be done after assessing the results found from a specific design of the same type of microheater coil. But, the comparison should be done by keeping one of the factors varied and all the other factors the same.

This will help in getting a clarity of the impact of change in that varied factor irrespective of the other factors.

11. CONCLUSION

After reading this report, one should be able to understand the concept behind bio sensing on the basis of fluorescence based optical sensing and the manner in which the signals are presumed and analysed in order to test the sample with this technique.

This report also gives the reader the idea how temperature plays a key role in optimizing the probability of the occurrence of fluorescence by avoiding the unfavourable conditions possible. It hence emphasizes on the importance of a microheater for fluorescence based sensing.

The report discusses about the design modelling a microheater and also about the simulation with various environments. It gives insight of how the iterative method works on the basis of results obtained in order to get the optimized design of microheater. This report also draws the connection between the microheater and the fluorescence detection and the use of microheater in general.

The complete simulation of the model which was made wasn’t successful due to technical limitations and will be rectified in the upcoming days.

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12. https://www.youtube.com/watch?time_continue=3502&v=D6L9XZDRY1g&feature=emb_title

GLOSSARY

Analyte – a substance whose chemical properties are being identified.

Fluorophore- a substance which possesses fluorescence properties.

Transducer- a device that converts various different forms of physical quantities into electrical signals, or vice versa.

Fabrication – the process of manufacturing a product

FRET – Fluoresence Resonance Energy Transfer