ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

DESIGN AND SIMULATION OF A MICROFLUIDIC BIOCHIP FOR OPTIC DETECTION WITH DERIVATIZED MICROBEADS AND THE BIOCHEMISTRY OF LEARNING

M.Sc. THESIS

TUĞÇE TÜYSÜZ

Nano Department of Nano Science and Nano Engineering

Nano Science and Nano Engineering Programme

JULY 2020

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Tuğçe TÜYSÜZ
(513171009)

Department of Nano Science and Nano Engineering

Nano Science and Nano Engineering Programme

Thesis Advisor: Assist. Prof. Dr. Onur ALPTÜRK

Thesis Co-Advisor: Assoc. Prof. Dr. Yıldız ULUDAĞ

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

TÜREVLENDİRİLMİŞ MİKRO KÜRELER İLE OPTİK BİYOSENSÖRÜ VE ÖĞRENME BİYOKİMYASI İÇİN MİKROAKIŞKAN BİYOÇİPİN TASARIMI VE SIMÜLASYONU

YÜKSEK LİSANS TEZİ

Tuğçe TÜYSÜZ
(513171009)

Nanobilim ve Nanomühendislik Anabilim Dalı
Nanobilim ve Nanomühendislik Programı

Tez Danışmanı: Dr. Öğr. Üyesi Onur ALPTÜRK
Eş Danışman: Doç. Dr. Yıldız ULUDAĞ

TEMMUZ 2020

Tuğçe Tüysüz, a M. Sc student of İTU Graduate School of Science Engineering and Technology student ID 513171009, successfully defended the thesis entitled "DESIGN AND SIMULATION OF A MICROFLUIDIC BIOCHIP FOR OPTICAL DETECTION WİTH DERIVATIZED MICROBEADS AND THE BIOCHEMISTRY OF LEARNING", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Dr. Öğr. Üyesi Onur ALPTÜRK** İstanbul Technical University	.....
Co-advisor :	**Doç. Dr. Yıldız ULUDAĞ** TUBITAK MAM	.....
Jury Members :	**Prof.Dr.Hüseyin Kızıl** İstanbul Technical University	..…….
	Prof.Dr.Neslihan Serap Şengör İstanbul Technical University	……..
	Prof.Dr.Numan Akdoğan Gebze Technical University	…….
Date of Submission :		
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To everyone who helped me write and delete*,

FOREWORD

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Tuğçe TÜYSÜZ
(Biomedical Engineer)

ABBREVIATIONS

Ab	**:**	Antibody
Ag	**:**	Antigen
BARC	**:**	Bead Array Counter
CE	**:**	Electrophoresis
DNA	**:**	Deoxyribonucleic acid
GOD	**:**	Glucose Oxidase
Ig	**:**	Immunoglobulin
LOC	**:**	Lateral Optic Chip
LTP	**:**	Long Term Potentiation
MIP	**:**	Molecularly Imprinted Polymers
NDMA	**:**	N-Nitrosodimethylamine
PBS	**:**	Phosphate Buffer Saline
PDE	**:**	Partial Differential Equation
PMMA	**:**	Polymethyl Methacrylate
PNA	**:**	Peptide Nucleic Acid
POC	**:**	Point of Care
PS	**:**	Polystyrene
RE	**:**	Reference electrode
SPE	**:**	Screen Printed Electrode
SPR	**:**	Surface Plasmon Resonance
SPW	**:**	Surface Plasmon Wave
WE	**:**	Working electrode
α-syn	**:**	Alpha-Synuclein

SYMBOLS

$\boldsymbol{d}$: Deciliter

$\boldsymbol{D}$: Pipe diameter in m

$\boldsymbol{h}$: Hour

$\boldsymbol{mg}$: Milligram

$\boldsymbol{ms}$: Millisecond

$\boldsymbol{ms}$: Meter per second

$\boldsymbol{m/s^2}$: Meter per second squared

$\boldsymbol{mV}$: Millivolt

$\boldsymbol{p}$: Fluid density in $\frac{kg}{m^3}$

$\boldsymbol{s}$: Second

$\boldsymbol{t}$: Time

$\boldsymbol{V}$: Fluid velocity in $\frac{m}{s}$

$\boldsymbol{\mu}$: Fluid dynamic viscosity in $\frac{kg}{m.s}$

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DESIGN AND SIMULATION OF A MICROFLUIDIC BIOCHIP FOR OPTICAL DETECTION WITH DERIVATIZED MICROBEADS AND THE BIOCHEMISTRY OF LEARNING

SUMMARY

Microfluidic systems are an important technology suitable for a wide range of applications due to their rapid response capabilities, low cost and, small amounts of sample need. Microfluidics tries to overcome difficulties in conventional assays in medical diagnosis. The combination of biosensors and microfluidic chips increases the analytical capability to extend the scope of possible applications.

In this thesis, two types of microfluidic modeling were designed for biomedical applications. The first design is a bead derivatized sensor in a microfluidic chip to detect biomarkers. The second model is designed to observe the effect of α-syn protein which constitutes the communication of two nerve cells through channels in the microfluidic system and the long term potentiation.

In Project 1, Integrated affinity sensors within microfluidic platforms show great interest in life environmental and science analytical science applications. They are generally placed in the base of a fluidic flow channel on which an analyte solution is passed. The analyte detection on the sensor depends on the event of a recognition-binding, most generally antigen-antibody, for which the recognition molecules are attached to the surface of the sensor for the analyte. The analyte -recognition molecule complex is detected on the sensor. The integration of bead-based immunoaffinity assays in microfluidic chips has recently become an area of interest for many researchers. Integrated affinity sensors inside of the microfluidic structures have many advantages which are low-cost, rapid, highly specific detection and sensitivity. In this study, the microfluidic system has been designed with different substrate patterns in the continuous flow of phosphate-buffered saline (PBS), and microbeads were examined. Functionalized microbeads have been used as biomolecules to enhance the affinity of biomarkers and for high sensitivity. Microchannel was patterned with square pyramid well array, conic well array, triangle pyramid array, and the each microbeads made of polystyrene were placed into the each microwell; PS beads were simulated with different flow rates. Initially, PBS was utilized to simulate blood serum, and PS nanoparticles, functionalized and fluorescently labeled nanoparticles that allow detection of biomarkers, were simulated for examination by fluorescence microscopy. As a result of three different geometric well chip patterns and three different bead size simulations, it was determined that the shape of the well should be conical and the bead size should be 150 μm. The lowest cross-section flow rate of the fluid sent from the inlet of the channel with a flow rate of 300 μl was determined in conical design. This indicates that there will be more interaction with the surface compared to other patterned arrays.

In Project 2, The purpose of this project is to create a biosynthetic neuron-on-a-chip to reproduce the activity of neuronal function. Neurons are the main important units of

the nervous system and brain. The target of neurons is to receive sensory input from the outside world and send motor commands to the muscles. They are also responsible for converting and transmitting electrical signals in every step that takes place in this cycle. Neurons communicate with electrochemical signals. Therefore, electrical and chemical events must occur together for the communication of two neuron cells. It transmits a neuron signal through the axons to the dendrites of other neurons to which it connects via the axons called synapses. Long Term Potentialization is a process in which synaptic connections between neurons are strengthened by frequent activation. LTP is thought to change the brain in response to experience, thereby providing a mechanism underlying learning and memory. In the process of learning, nerve cells, the basic computing units of any nervous system, are thought to exhibit digital and analog properties. Alpha-synuclein(α-syn) proteins are of high importance to sustaining LTP in the brain. In this thesis, the most suitable platform for communication between two yeast cells and the passage of α-syn proteins through channels is optimized and designed. It refers to nerve cells in the computer environment by yeast cells in the simulation program. A channel that enables the communication of two yeast cells was designed and these yeast cells were placed in the traps located at the entrances of the channels. The activating agent was sent to produce α-syn of yeast cells in the A channel. Alpha-synuclein protein, which is synthesized from yeast cells in the A channel, has passed through the channel and attached to the NDMA receptor in the other yeast cell in the B channel. Then, LTP was provided by activating the α-syn protein bound to the NDMA receptor in a balanced manner with Ca^{+}ions. Irregularity in the ratio of protein Ca^{+} and α-syn prevents the formation of long term potentiation and causes Parkinson's disease. Optimization studies were carried out in microfluidic chip design. The number of channels along with the microfluidic chip, the width of chamber A and B, the width of the communication channel, the distance between communication channels, the length of yeast cells chamber, the length of yeast cells communication channel, the inlet-outlet radius of chamber A and B were determined. As a result of these determinations, it was observed how each parameter affects diffusion. The greater diffusion indicates that the amount of α-syn protein passes more from chamber A to chamber B. It was also observed that some parameters started diffusion earlier. Therefore, it enabled more yeast cells to interact.

Computer modeling and simulation were applied as a very useful tool for improvements in the design of microfluidic chip geometry, as well as for the optimization of the technological and functional parameters. In this thesis, COMSOL Multiphysics, which is the most used in microfluidic systems, is used in two projects for microfluidic chip design and simulations within the designed chip.

TÜREVLENDİRİLMİŞ MİKRO KÜRELER İLE OPTİK BİYOSENSÖRÜ İÇİN VE ÖĞRENME BİYOKİMYASI İÇİN MİKROAKIŞKAN BİYOÇİPİN TASARIMI VE SİMÜLASYONU

ÖZET

Mikroakışkan sistemler, hızlı cevap yetenekleri, düşük maliyeti ve az miktarda numune ihtiyacı nedeniyle çok çeşitli uygulamalar için uygun ve önemli bir teknolojidir. Mikroakışkanlar, tıbbi teşhis koyarken geleneksel testlerdeki üstesinden gelmeye çalışır. Biyosensörler ile mikroakışkan çiplerin kombinasyonu, olası uygulamaların kapsamını genişletmek için analitik kabiliyeti arttırır. Mikroakışkan sistemler içerisine yerleştirilmiş olan biyosensörlerin geleneksel biyosensörlere göre bir çok avantajı olduğu görülmüş ve günümüzde yaygın bir şekilde kullanılmaktadır.

Bu tezde biyomedikal uygulamalar için iki tip mikroakışkan sistem içerisinde biyosensör modellemesi tasarlanmıştır. Tezde yer alan ilk tasarım, biyobelirteçleri saptamak için mikroakışkan bir çip içerisinde mikro küre ile türevlendirilmiş optik bir biyosensördür. İkinci model, iki sinir hücresinin mikroakışkan sistem içerisinde kanallar yoluyla iletişimini ve uzun süreli potansiyasyonu oluşturan alfa-sinüklein proteininin sinir hücreleri arasında geçişini gözlemlemek için tasarlanmıştır.

Proje 1'de, mikroakışkan yapılar içindeki entegre afinite sensörleri yaşam bilimi ve çevresel analitik bilim uygulamalarına büyük ilgi göstermektedir. Bu afinite sensörleri genellikle üzerinden analit çözeltisinin geçtiği bir akışkan akış kanalının tabanına yerleştirilir. Analitin sensör üzerindeki tespiti, analit için tanıma moleküllerinin sensörün yüzeyine tutturulduğu bir tanıma bağlanma olayına, en tipik örneği olarak antikor-antijen ilişkisine bağlıdır. Tanıma molekülü-analit kompleksi sensörde tespit edilir. Mikro küre tabanlı immünoafinite deneylerinin mikroakışkan çiplere entegrasyonu son zamanlarda birçok araştırmacı için ilgi alanı haline gelmiştir. Mikroakışkan yapılar içindeki entegre afinite sensörleri, düşük maliyetli, hızlı, yüksek derecede spesifik algılama ve hassasiyet gibi birçok avantaja sahiptir.

Bu çalışmada, mikroakışkan sistem, fosfat tamponlu salinin (PBS) sürekli akışında farklı taban desenleri ile tasarlanmıştır ve mikro küreler incelenmiştir. Biyobelirteçlerin afinitesini artırmak ve yüksek hassasiyet için biyomolekül olarak fonksiyonelleştirilmiş mikro küreler kullanılmıştır. Mikrokanal tabanı kare piramit kuyucuk dizisi, konik kuyucuk dizisi, üçgen piramit kuyucuk dizisi ile desenlendirilmiştir ve polystyreneden(PS) yapılmış olan mikro küreler her bir mikro kuyucuğa tek tek yerleştirildi; PS tanecikleri farklı akış hızlarıyla simüle edilmiştir.Başlangıçta kan serumu simüle etmek için PBS kullanıldı ve biyobelirteçlerin saptanmasına izin veren fonksiyonelleştirilmiş ve floresan etiketli nanopartikülleri olan PS nanoparçacıkları, floresan mikroskopisi ile incelenmek üzere simule edildi.Üç farklı geometrik kuyucuk chip tabanı deseni ve üç farklı mikro küre boyutu simülasyonu sonucunda, kuyucuk şeklinin konik ve mikro küre boyutunun 150 um olması gerektiği belirlenmiştir. Bu belirlemede sadece simulasyon sonuçlarını değil, simulasyon verileri kullanılarak üretim aşamasına geçildiğinde en optimal tasarımın hangisi olacağı düşünülerek karar verilmiştir. Konik tasarımda 300 ul akış

hızına sahip kanal girişinden gönderilen sıvının en düşük kesit akış hızı belirlendi. Bu, diğer desenlendirilmiş tabanlara kıyasla yüzeyle daha fazla etkileşim olacağını gösterir ve optimal tasarım olarak kabul görür.

Proje 2'de, bu projenin amacı, nöronal fonksiyonun temel aktivitelerini taklit etmek için bir çip üzerinde biyosentetik bir nöron oluşturmaktır. Nöronlar, beynin ve sinir sisteminin temel birimleridir. Sinir sistemleri dış dünyadan duyusal girdi almaktan, kaslarımıza motor komutları göndermek ve aradaki her adımda elektrik sinyallerini dönüştürmek ve aktarmaktan sorumlu hücrelerdir. Nöronlar elektrokimyasal sinyallerle haberleşirler. Yani, iki nöron hücresinin haberleşmesi için elektriksel ve kimyasal olayların birlikte gerçekleşmesi gerekmektedir. Günümüzde elektriksel haberleşme yani beynin dijital kısmını anlamak için bir çok yapay zeka modellemeleri yapılmıştır. Ancak hem dijital, hem kimyasal kısmını birlikte ele alan çalışma sayıları azdır.

Bu çalışmada, beynin kimyasal olaylarının gerçekleştiği analog kısmın modellemesi üzerine çalışışmıştır. Bir nöron sinyalini akson üzerinden bağlandığı öteki nöronların dentritlerine sinaps denen bağlantı noktalarıyla iletiyor. Uzun Süreli Potansiyelizasyon, nöronlar arasındaki sinaptik bağlantıların sık aktivasyonla güçlendiği bir süreçtir. LTP'nin beynin deneyime yanıt olarak değiştiği ve böylelikle öğrenme ve hafızanın altında yatan bir mekanizma sağladığı düşünülmektedir. Öğrenme sürecinde, herhangi bir sinir sisteminin temel hesaplama birimleri olan sinir hücrelerinin dijital ve analog özellikler sergilediği düşünülmektedir. Alfa-sinüklein (α-sin) proteinleri beyindeki LTP'nin sürdürülmesi için büyük öneme sahiptir. Bu tezde, iki maya hücresi ve α-sin proteinlerinin kanallardan geçişi arasındaki iletişim için en uygun platform optimize edilmiş ve tasarlanmıştır. Simülasyon programındaki maya hücreleri bilgisayar ortamındaki sinir hücrelerini ifade eder. İki maya hücresinin iletişimini sağlayan bir kanal tasarlandı ve bu maya hücreleri, kanalların girişlerinde bulunan tuzaklara yerleştirildi. Aktive edici ajan, A kanalındaki maya hücrelerinin α-sin üretilmesi için gönderildi. A kanalındaki maya hücrelerinden sentezlenen alfa sinüklein proteini, kanaldan geçti ve B kanalındaki diğer maya hücresindeki NDMA reseptörüne bağlandı. Daha sonra LTP, NDMA reseptörüne bağlı a-sin proteini Ca^{+} iyonları ile dengeli bir şekilde aktive edilerek sağlandı. Protein Ca^{+} ve α-sin oranındaki düzensizlik, uzun vadeli potansiyel oluşumunu önler ve Parkinson hastalığına neden olur. Mikroakışkan çip tasarımı için optimizasyon çalışmaları yapılmıştır. Mikroakışkan çip boyunca kanal sayısı, A ve B kanalının genişliği, iletişim kanalının genişliği, iletişim kanalları arasındaki mesafe, maya hücrelerinin kanalının uzunluğu, maya hücreleri iletişim kanalının uzunluğu, A ve B kanalının giriş-çıkış yarıçapı belirlendi. Bu tespitler sonucunda her bir parametrenin difüzyonu nasıl etkilediği gözlenmiştir. Daha fazla difüzyon, α-syn proteini miktarının A kanalından B kanalına daha fazla geçtiğini gösterir. Difüzyonun daha erken başlaması, daha yoğun bir şekilde difüzyon gerçekleşmesi yorumları iki maya hücresi arasındaki etkileşimin arttığını gösterir. Bu nedenle, daha fazla maya hücresinin etkileşmesini sağlamak amacıyla farklı parametreler denendi ve optimizasyon sağlandı.

Bilgisayar modelleme ve simülasyon, mikroakışkan çip geometrisinin tasarımındaki iyileştirmelerin yanı sıra teknolojik ve fonksiyonel parametrelerin optimizasyonu için çok kullanışlı bir araç olarak uygulanmıştır. Bu tezde, mikroakışkan sistemlerde en çok kullanılan COMSOL Multifizik simulasyon programı mikroakışkan çip tasarımı ve tasarlanmış chip içerisindeki simülasyonlar için her iki projede kullanılmaktadır. COMSOL aracılığıyla yapılan simulasyonlar sonucunda, programdan sonuç verileri alınıp grafiksel analizler yapılmıştır. Simulasyon programıyla çalışmamızın amacı;

gerçek sistemin modelinin tasarlanması ve bu model ile sistemin işletilmesi amacına yönelik olarak, sisteemin davranışını anlayabilmek veya değişik stratejileri değerlendirmek için deneyler yürütülmesini sağlamaktır.

1. INTRODUCTION

The main idea of my thesis is to modeling two bioapplications in microfluidic systems. There are two separate projects within the scope of this main idea. In Project 1, design a bead derivatized sensor in a microfluidic chip to detect biomarkers. In Project 2, the effect of α-syn protein, which constitutes the communication of two nerve cells through channels in the microfluidic system and the long term potentiation, was investigated.

1.1 Project-1: Design and Simulation of a Microfluidic Biochip for Optic Detection with Derivatized Microbeads

1.1.1 Biosensor

IUPAC definition of a biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element (IUPAC, 1996). A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal (IUPAC). In the combination of these sensor definitions, biosensors are biochemical sensors in which the acknowledgment framework uses a biochemical system. Estimation of the measure of organic composites such as glucose, urea, catalysts, hormones, and so forth is performed with the biosensor. These sensors change natural response with chemical or biomolecules to quantifiable signs that can be intensified and showed. The term biosensor has been differently used to various gadgets either used to screen living frameworks or consolidating biotic components (Bhalla et al., 2016).

Biosensors have been applied in many fields namely healthcare, life science research, marine sector, environmental, food & military applications. Further, these sensors can be enhanced as nanobiotechnology. Important features for commercialization of the biosensors are selectivity, sensitivity, stability, reproducibility, and low cost (R et al., 2019).

Every biosensor consists principally of basic components which are biological recognition elements, transducer, and a signal processing part is schematically given in Figure.1.1 (Luka et al., 2015).

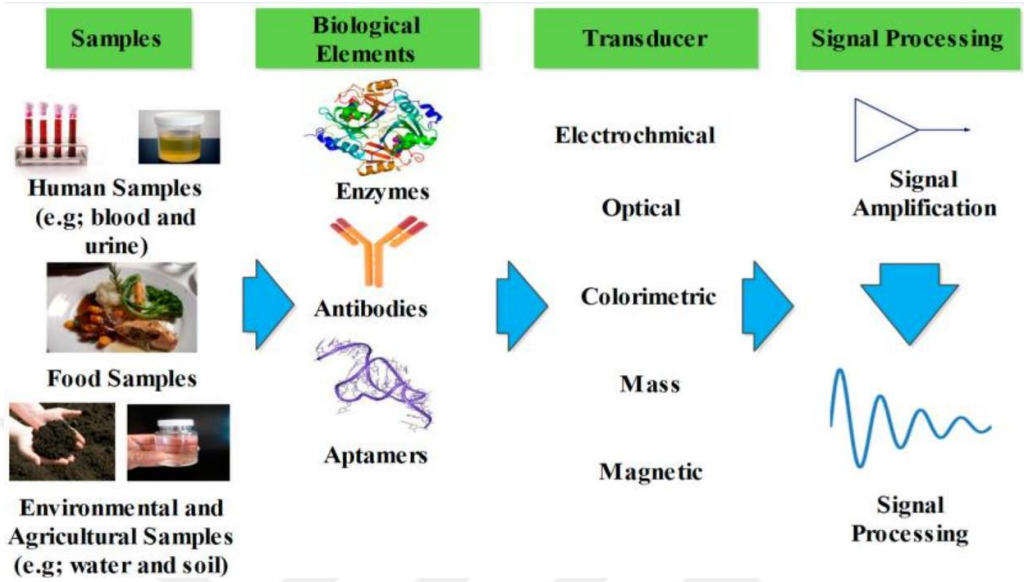


Figure 1. 1: Schematic representation of the basic concept of a biosensor (Luka et al., 2015).

First of all, biological detection materials (other names in literature are biological recognition element, bioreceptor) part has a susceptible selectivity to the analyte that needs to be detected. This biological recognition element is immobilized to a transducer for getting efficient interaction between analyte and receptor. Cell, tissue, enzyme, organelles, antibody, DNA, aptamers are examples of biorecognition elements. Secondly, the transducers convert the biological recognition element and analyte interaction into measurable signals. The transducer part of a biosensor converts physical or chemical changes after interaction between biomolecules and analytes on the transducer surface. The conversion of changes in transducers is known as signalization. The third part of the biosensor is the detector (electronic part) which shows the results as a quantifiable and readable signal. The electronic part performs signal amplification and conversion signal from analog to digital platform. The output signal can be obtained as numerical, graphical, tabular, or an image based on user requirements (Bhalla et al., 2016).

The interactions between analyte and receptor molecules occur physical, chemical, or electrical changes on the transducer part of the biosensor. The changes became after

these interactions can be exemplified as increasing or decreasing electroactive materials, changing temperature, light, mass, pH, and viscosity. There is a correlation between these changes and the concentration of the analyte and transducer part of the biosensor transmits this correlation to the electronic parts of the biosensor as the quantifiable signal. This signal is an interpretation of the concentration of an analyte. Thus, the analysis of biomolecules could be getting fast, easy, and economic after analysis of the concentration of biomolecules/analytes by using biosensors (Thévenot et al., 2001).

In 1962, the first biosensor was once used for the detection of glucose concentrations in blood samples using the usage of an enzyme-coated oxygen electrode. Clark *et al*. placed glucose oxidase enzymes on the Platinum electrode by help a membrane. Glucose and oxygen molecules are converted to gluconic acid and peroxide through the glucose oxidase enzyme (Equation 1.1). After a chemical reaction between glucose and oxygen molecules, electrons pass through Pt electrodes (Equation 1.2). It provides that quantifiable signal to read the concentration of glucose molecules in an oxygenated medium because passed electrons are correlated with glucose molecule concentration (Goodsell, 2004), (Hoffmann et al., 1984), (Clark & Lyons, 1962). Glucose biosensor which detects glucose from one drop of blood was developed later on. To provide standardization of oxygen-peroxide conversion, the oxygen level has to be retained instability. So that a mediator molecule inserted in the oxygen-glucose medium. Articles about biosensors started to become widespread at the beginning of 20. Century. In the beginning period of Bio-Sensing research-development activities in 1955-56, Clark investigated the oxygen electrode and introduced glucose biosensor (*A Personalized History of the Clark Oxygen Electrode: International Anesthesiology Clinics*, n.d.). With the innovation of biosensors after that time, the number of diagnostic equipment and emergency checks have been multiplied and grew to become widespread (Malhotra & Chaubey, 2003).

$$glucose + O_2 \ \ glucose\ oxidase \rightarrow \ gluconic\ acid + H_2O_2 \qquad (1.1)$$

$$H_2O_2 \ \rightarrow \ O_2 + \ 2H^+ + \ 2e^- \qquad (1.2)$$

The basic and important characteristics of biosensors consist of selectivity (or specificity), sensitivity, response time, renewability, and simplicity.

1.1.2 Classification of Biosensors

When the biosensor literature is examined it can be seen that, there are three types of biosensor denotations based on bioelement, transducer type, and analyte. The glucose biosensors as mentioned above are the most recognized biosensors classified by the analyte type. In literature, the classification of biosensors is focused on bioelement and transducer types (Kissinger, 2005), (Poloju, 2007). Bioelement based biosensors are enzymatic biosensors, immunosensors, and nucleic acid-based biosensors, microbial (whole cell) biosensors, and carbohydrate biosensors (Koyun et al., 2012). Transducer based biosensors are electrochemical, optical, acoustic, thermal, and microcantilever biosensors. They are summarized and classified in detail in Table 1.1.

1.1.2.1 Classification of biosensors based on transducer

Electrochemical Biosensors

Electrochemistry has a vital function in the research and improvement of biosensors. During the electrochemical reaction loss of electrons or obtain of electrons occurs between electrolyte components. These oxidation and reduction reactions are known as redox reactions. After redox reactions, some prominent electrochemical analyzer supply information in the matter of the concentration, reaction mechanisms, chemical status, kinetics, and other behavior of a chemical species in an electrolyte solution. The determination of electrochemical changes in biosensing investigations is provided by relating to electrochemical reactions in nature and biological systems. Electrochemical biosensors are designed with an electronic platform and bioelectrochemical component. Bioelectrochemical component/bioelement such enzyme is an element of data transmitter to transducer part of a biosensor which converts the signal to readable and quantifiable results. In electrochemical biosensors, there has to be an electroactive bioelement or any intermediate produced from the enzyme-catalyzed chemical reaction. In other words, electrochemical interactions have to have occurred for the construction of any electrochemical biosensors (D'Orazio, 2003),(Chaubey & Malhotra, 2002). In electrochemical biosensor applications, various bioelements such as enzymes, microorganisms, proteins, oligonucleotides are used and especially enzymes are mostly utilized in the development of electrochemical biosensors because of their specificity and bioanalytical capability abilities. Electrochemical biosensors are categorized as amperometric, voltammetric, potentiometric, conductometric and impedimetric biosensors (D'Orazio, 2003). Electrochemical transducers are

commonly used in biosensor applications because of the ease of use, low cost, fast analysis, portability, miniaturization, small analyte volume and simplicity of fabrication (Orozco et al., 2011).

Type of electrochemical biosensors as potentiometric, amperometric, voltammetric, conductometric, impedimetric. Potentiometry gives information about ion activity in electrochemical reactions utilizing measuring the difference of potential between reference electrode(RE) and the working electrode (WE). The potential differentiation in the electrochemical cell occurs due to the fact of free energy change that would become if the chemical phenomena had been to exist until the satisfied equilibrium condition. The potential difference between electrodes is correlated with analyte concentration, so this principle is using the development of potentiometric biosensors. Modification in the current generated from the electrochemical oxidation or reduction in an electrochemical cell is observed whilst a constant potential is maintained at the working electrode between the reference electrode. An amperometric biosensor is an electrochemical biosensor that measures continuous current during oxidation or reduction of electroactive species that occurres in biochemical reaction at an appropriate applied potential value to the electrochemical cell (Luppa et al., 2001). Voltammetric biosensors measure the occurred current and a sharp rise/fall of the current by applying increasing/decreasing potential to the working electrode versus reference electrode in an electrochemical cell until the oxidation/reduction of the substance to be analyzed on the working electrode. This current is electrolysis current and is limited by the mass transport rate of molecules to the electrode. There is a correlation between the concentration of the electroactive material and the height of the peak current, this working principle creates voltammetric biosensor devices. In voltammetry, three electrodes that are working, auxiliary, and reference electrodes are employed to obtain electrolysis current, unlike potentiometry measurements that employ only two electrodes (working, reference). The use of three electrodes system allows accurate application of potential functions and the measurement of the resultant current. Conductometric biosensors measure conductivity changes between reference nodes and electrodes. Conductivity changes occur after interaction between analyte and bioelement on conductor or semiconductor surface/active layer (polymeric thin film) or mediator (wire) between reference nodes and electrodes. Conductivity changes would be provided with enzymatic reactions whose charged products result

in ionic strength changes, antibody-antigen interaction, and DNA hybridization which obtain resistance on the conductive surface substrate (*Comprehensive Biotechnology*, 2019). Impedimetric biosensors which are also known as capacitance biosensors measure capacitance change between electrodes in an electrochemical cell with utilized alternating voltage, amplitudes from a few to 100 mV are used. Impedimetric biosensors have recently gained popularity because of the advantages such as it is high sensitivity. Redox mediators have been used in the detection to be limited by the mediator's mass transfer rate in impedimetric biosensor applications.

Electrochemical biosensors have electrodes that convert the chemical signal into an electrical signal. The electrochemical biosensor ion selector can consist of glass, metal, and carbon electrodes. The electrochemical signal can be produced directly or indirectly. If direct production is produced by the presence of analyte on the electrode surface or the formation of electroactive species by the target molecule, or indirectly, by combining a biorecognition event with a redox probe or a mediated enzyme electrode. Electrochemical sensors can detect different biomolecules such as glucose, cholesterol, uric acid, lactate, DNA, hemoglobin, blood ketones, and others in the human body (Ronkainen et al., 2010).

Detection of a specific analyte is usually done by protein-based and enzyme electrochemical biosensors. Proteins or enzymes are immobilized on the transducer and the specific analyte can be measured utilizing electroactive and measurable by-products in these types of biosensors. Protein or enzyme-based electrochemical biosensors, which can release drugs in the treatment of different diseases, are used. For instance, the xanthine oxidase enzyme catalyzes the hypoxanthine production, and xanthine and excessive production of these products causes kidney failure. Xanthine oxidase is inhibited by allopurinol; a biosensor system that detects hypoxanthine and xanthine overproduction and releases allopurinol as a reaction could be an important case of an electrochemical biosensor for drug delivery (Chen et al., 2016), (Grieshaber et al., 2008).

In an other example; a disposable electrochemical DNA biosensor has been developed for the identification of toxic aromatic amines. The device is based on the electrostatic or intercalative collection of aromatic amines followed by a chronopotentiometry analysis on a layer of immobilized dsDNA or ssDNA. The anodic signal of the guanine bases of DNA coated screen-printed electrodes (SPEs) is intensely affected by

conformational or structural modifications of the DNA layer accumulated from DNA–analyte association. Therefore, the diverseness within the oxidative signal of guanine is taken as the molecular recognition index (Chiti et al., 2001).

The amperometry, potentiometry, conductometry, and impedimetry are measurement modes.

Optical Biosensor

Optical transducer-based biosensors convert chemical or physicochemical changes because of analyte-bioelement interactions on optic transducers to quantifiable signals. Chemical and physicochemical changes on transducer are converted to signal by light reflection, light polarization, light intensity, phase, peak position, and angular wavelength. Photon counter, fluorescence, luminescence, adsorption spectroscopies are used as a signal transducers in optical biosensors. Ellipsometric biosensors, Surface Plasmon Resonance (SPR) biosensors, fiber-optic biosensors, absorption spectroscopy, interferometer-based biosensors, RAMAN & FTIR can be improved for label-free detection. Fluorescence spectroscopy, luminescence spectroscopy generally employs by labeling analyte or bioelement with another material such as florescent dyes (Šípová et al., 2012).

Optical biosensors are used in the fields of healthcare, environmental analysis, and biotechnology. The advantages of the optical biosensors include low concentration and required analyte amount, rapid completion of the analysis, and reusability of the sensor chip are the advantages of these applications (Damborský et al., 2016).

An optical biosensor is a compact analytical system with a biorecognition component structured with a transducer system. They are sensors that emit an optical signal directly proportional to the concentration of the analyte. The biorecognition components which are biological materials, such as antibodies, enzymes, antigens, receptors, nucleic acids, whole cells, and tissues are used are commonly in the biosensor. Based on the signal transmission approach method used, biosensors can be divided into different groups as thermometric, optical, magnetic, piezoelectric, and electrochemical. Optical biosensors make use of the interplay of optical fields (i.e., the electric field) with the analyte for optical detection. (Damborský et al., 2016).

Ellipsometric biosensors measure changes of light polarization or the polarization states after and before reflection after interaction between analyte and bioelement

(*Ellipsometric-Based Novel DNA Biosensor for Label-Free, Real-Time Detection of Bordetella Parapertussis | SpringerLink*, n.d.). Parts of ellipsometric biosensors are polarizer, compensator, analyzer, and detector. The incident light from the light source reflects and refracts more at each interface in the chip composed substances passing through a linear polarizer, a compensator in order and the ellipsometric data obtained includes information for investigated material within the penetration depth of the light, change of the polarization and refractive index values of a sample (Figure 1.2). In ellipsometric biosensor applications, sensor modeling is also required due to their low refractive indexes and nanometer range thicknesses (*(PDF) Fast and Sensitive Ellipsometry-Based Biosensing*, n.d.).

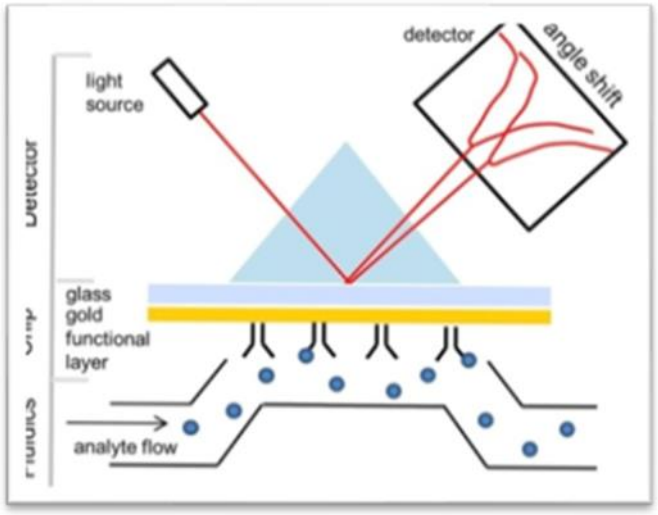


Figure 1. 2: General schematic of an ellipsometric biosensor **(Damborský et al., 2016)**.

Surface plasmon resonance (SPR) biosensor was first introduced in 1983 by Liedberg *et al*. (Liedberg et al., 1983). Measuring the interaction between a wave vector induced by the incident light beam and a wave vector in a metal film surface constructed by a dielectric material coating on gold or silver generates the basic principle of detection by SPR biosensor. Surface plasmon wave (SPW) is an electromagnetic wave that occurs at the interface of a metal and dielectric media. SPW generates an electromagnetic field at the interface of a metal and dielectric media. There are four basic methods to set SPR principles which are prism coupling, waveguide coupling, fiber optic coupling, and grating coupling methods. SPR via the coupling prism method is configurated to clarify the SPR principle in Figure 1.3. Also, a light wave passes through a high refractive index prism and is reflected at the interface and

generates an evanescent wave at the interface in Figure 1.3. This evanescent wave creates an evanescent field penetrated the metal layer and excites molecules near the interface. This evanescent wave propagates along with the interface with a propagation constant. The propagation constant of the SPW is sensitive to the refractive index of the dielectric. In SPR biosensor, after immobilized bioelements (antibodies) on the surface of metal recognize and capture analyte (antigen), a local increase in the refractive index at the metal surface occurs. The Refractive index increasing at the metal surface generates an increase in the propagation constant of SPW propagating along the metal surface. The magnitude of SPW propagation constant change and refractive index change after bioelement-analyte interaction can be optically measured and related analyte concentration in SPR biosensor applications. By the other SPR structures, changes of some light properties such as; light amplitude, spectral distribution, polarization can be correlated with the propagation constant of the SPW. Thence, changes in the refractive index at the sensor surface after biosensing can be determined by the change of SPW propagation constant via measurement of the above-mentioned light characteristics (Homola, 2003).

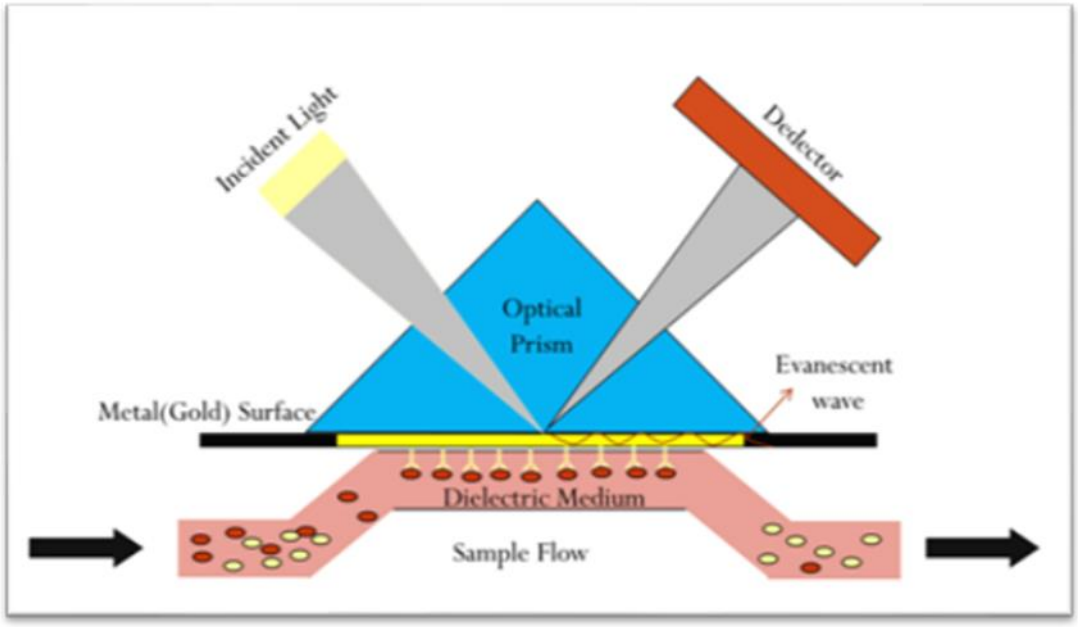


Figure 1. 3: SPR biosensor via a prism coupling configuration (Homola, 2003).

Fiber-optic biosensors generally use optical fibers as a transducer and generally include sensitive layer on the distal end of optic fibers. Light propagates by total internal reflection through fiber and this propagation includes two parts: the guided field in the core of the fiber and the exponentially decreasing evanescent field in the

cladding layer of fiber. Evanescent field decays to almost zero within the cladding layer in a uniform-diameter cladded fiber. Interactive relation between light and analyte-bioelement reaction can be measured by absorbance, luminescence spectroscopy, polar isolation, and change of refraction index (Leung et al., 2007).

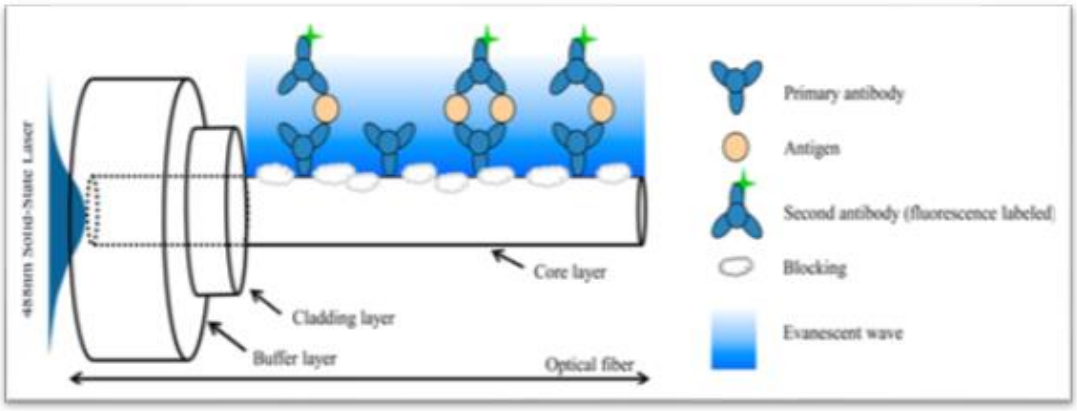


Figure 1. 4: Fiber optic biosensor working principle based sandwich-type immunosensor labeling by fluorescence tagged second antibody **(X. Li et al., 2018)**.

Optical spectroscopic measurements in biosensor applications give a change of absorbance of light, fluorescent light emission, and luminescence light emission after interaction between analyte and bioelement. An FTIR spectrometer equipped with a real-time evacuation apparatus was applied to determine analyte concentration. A RAMAN spectrometer measures the vibrational energy spectrum depends on the chemical composition of the sample so that gives information about chemical specificity and represents a chemical fingerprint of the sample. These two spectrometers measure the interaction of energy with the molecular bonds in a sample of an unknown material. These spectrometers have been used in biological recognition in biosensor applications (Notingher, 2007).

Acoustic biosensors

Acoustic transducers measure mass changes on the biosensor's surface. Piezoelectric crystals are the most common acoustic transducers which generate electric currents from a vibrating crystal because of absorbed mass on its surface after interaction between immobilized bioelement and analyte. Piezoelectric materials have the ability of acoustic waves production and propagation. All acoustic sensors are sensitive to changes in many chemical or physical parameters such as film thickness, force, concentration, mass, viscosity. Piezoelectric elements can be found in various

electronic devices, like computers, radar, mobile phones, electronic blood pressure monitor, and so on. Acoustic biosensors easily allow the improvement of label-free detection because of responding to mass accumulation on the sensor surface. Another important advantage of the acoustic sensor is that it can offer real-time measurement of surface interactions as an alternative to optical biosensors such as surface plasmon resonance and optical waveguides (Araya-Kleinsteuber & Lowe, 2008).

1.1.2.2 Classification of biosensors based on bioelement

Enzymatic Biosensors

Enzymes are employed in enzymatic biosensor applications as bioelement which catalyzes the electroactive substances production on the transducer of the biosensor to determine the concentration of the interested analyte. Their usage in biosensor applications is quite widespread because of the high sensitivity and specificity properties of the enzyme molecules. Enzyme immobilization on the transducer of the biosensor is an important step in the fabrication of the enzymatic biosensors. Because enzymes have high biocatalytic activity and specificity, enzymatic biosensors are commonly being developed with the various electrochemical transducers. Enzyme electrodes among electrochemical transducers are commonly used to produce enzymatic biosensors to detect monosaccharides, amino acids, proteins, hormones, organic acids (lactic acid), urea, and alcohol (J. Wang, 2001).

Enzyme electrodes are produced with a thin layer of the immobilized enzyme on its working electrode. Enzymes catalyze electroactive component production for the detection. The detection of electroactive material decreasing or increasing is commonly determined by an amperometric transducer, in which the produced current is measured in response to an applied, constant voltage (Nguyen et al., 2019). Electrode design and electrolyte solution parameters affect the activity of immobilized enzymes. Enzyme stability and shelf life determine the lifespan of the enzymatic biosensor. Advantages of the use of enzyme electrodes can be summarized as they are, inexpensive, simple fabrication, reusable, fast response, and regenerable. However, the immobilized enzyme on the electrode has to be replaced periodically since it gradually loses activity. This disadvantages shows that stability parameters of these type biosensors are weak according to other parameters (Ronkainen et al., 2010).

Glucose oxidase enzyme is mostly used in well-known glucose biosensors. In the first glucose biosensors, which were explained in detail at the beginning of the introduction, the amperometric determination was performed using the GOD enzyme (J. Wang, 2001).

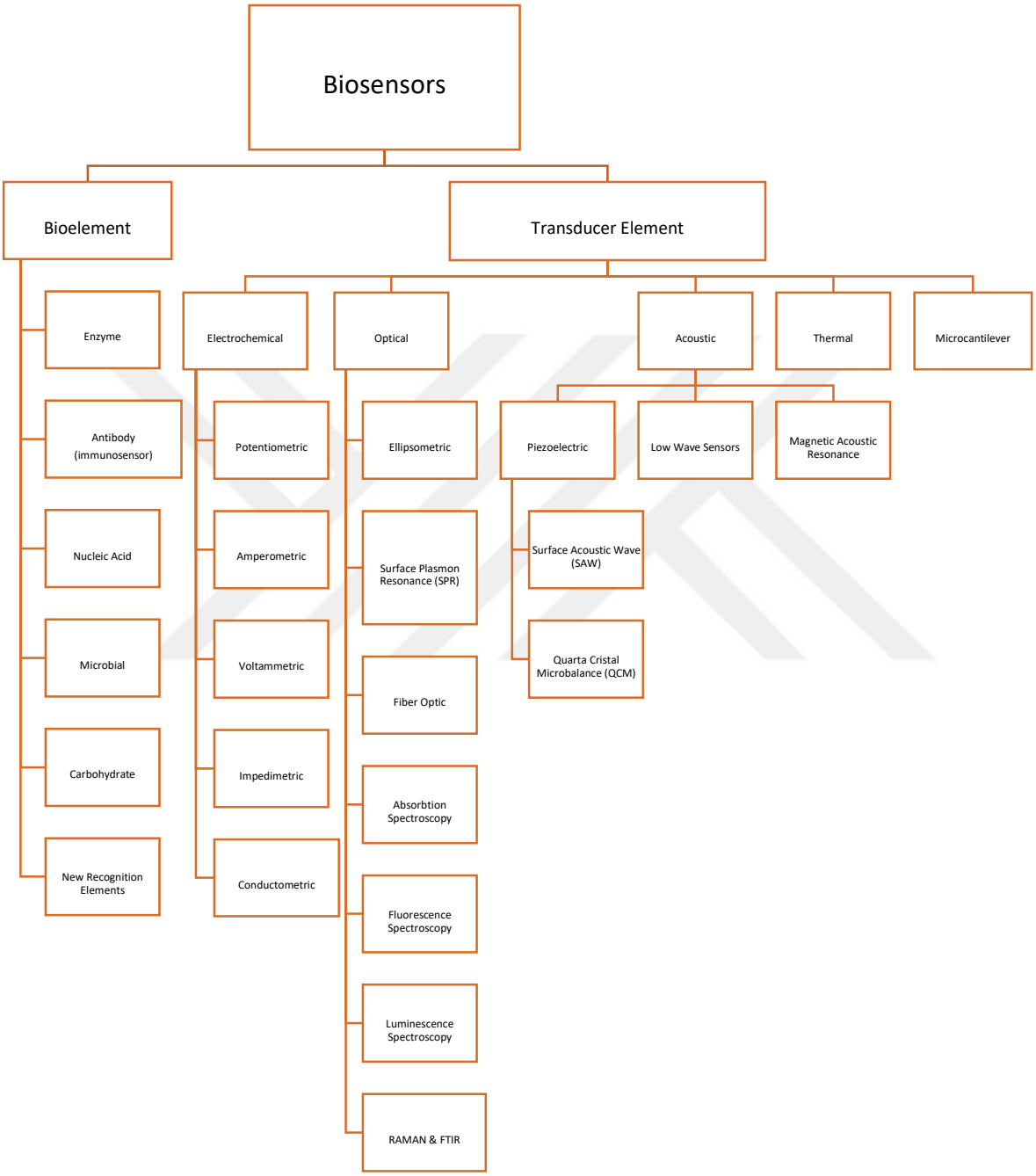


Figure 1. 5 : Schematic of biosensor classification **(Mungroo & Neethirajan, 2014)**.

Immunosensors

Immunosensors are biosensors that have fabricated with antibody immobilization on the transducer. Bioelements of these biosensors are antibodies. These biosensors are developed with prominent properties of an antibody (Ab) that are specificity and affinity to an antigen (Ag). These significant properties provide construction of the highly selective biosensor. Ab-Ag interaction generates physical or chemical changes on a transducer which converts this change to quantifiable signals. Abs recognize and bind to Ags or haptens by its specific binding regions (complementary regions). Immunosensors had been constructed by different types of transducers which are electrochemical, optical, thermal, acoustic, and microcantilever. Most of the developed immunosensors are based on optical and electrochemical transducer (W. Wang et al., 2007).

Biosensors based on nucleic acids

Biosensors based on nucleic acids are used for the detection of specific DNA sequences. Bioelements of these biosensors are single-stranded oligonucleotides that allow hybridization of its specific strand or DNA binding proteins (aptamer) which are the analyte of interest (Fu et al., 2019). In these biosensor applications, nucleic acid chain hybridization is generally observed by optical or electrochemical transducers. Nucleic acids are now turning into of larger significance as the biorecognition agent in biosensor functions ever considering that there is an increased study on the DNA molecule and artificially production of replicated DNA affinity probes. DNA hybridization biosensors are also known affinity sensors because of the high selectivity of DNA probes as bioelement. DNA affinity probes are usually used in clinical diagnostics to detect mutated DNA sequence and so to diagnose genetic diseases, viral infections, or other infectious diseases which damages DNA, for the elucidation of mechanisms of drug interaction in the organism and forensic medicine. DNA probes can be also used in determining microorganism's contamination because a gene of microorganism responsible for a specific property can determine by DNA biosensors (Bartlett, 2008).

Microbial Biosensors

Microbial biosensors include microbial organisms such as algae, bacteria, viruses as bioelement in biosensor applications. Microbial biosensors are constructed by the

immobilization of microorganisms on the transducer of biosensors. The changes in the vital activity of the microorganism like respiration, inhibition of some specific enzyme affected by the target analyte, on transducer surface are converted to an electronic signal by an electrochemical transducer (Su et al., 2011). These biosensors are employed in brewing, food manufacturing, waste-water treatment, energy production, and pharmaceutical synthesis (Ronkainen et al., 2010). To increase sensitivity, selectivity, and to develop versatile usage of microbial biosensors, genetically modified microorganisms are also used and studied (Rainina et al., 1996).

Carbohydrate Biosensors

Carbohydrate biosensors are fabricated with carbohydrate bioelements. The main reason for the study on carbohydrate biosensors is that there are plenty of biological reactions related to carbohydrates. Carbohydrates make easier immobilization of bioelements on the transducer and enhance biocompatibility (Jelinek & Kolusheva, 2004). Glucopolymers and synthetic saccharide derivatives are used as a thin-film layer on the transducer surface and this provides to enhance the sensitivity of biosensors (Eissa & Cameron, 2013). Effectively immobilized carbohydrate on transducer enhances both productivities of reaction with analyte on transducer and signal amplification of biosensors.

New recognition elements

New recognition elements have recently been found to overcome the deficiencies of conventional bioelements. Some examples of new recognition elements; aptamers, molecularly imprinted polymers (MIPs), proteins (antigens, lectins), lipids, carbohydrates, peptide nucleic acids (PNAs), engineered proteins. Biosensor recognition elements are isolated from living systems. However, many biosensor recognition elements now reachable and they have been synthesized in the laboratory.

1.1.3 The biosensor in Microfluidic System

The definition of microfluidics is a term that generally refers to the behavior of the fluids, manipulation, and precise control at the sub-millimeter scale individually or in combination. Typically, micro includes, among other things, small volumes (µL, nL, pL, fL), low energy consumption, small size, or effects of the micro field. Microfluidic systems first discovered in the early 1980s and were used in the development of inkjet printing, micro propulsion, microthermal technologies, DNA chips, and lab-on-a-chip

(LOC) technologies. The relevant field is multidisciplinary, mainly consisting of engineering aspects and intersecting areas of chemistry, biotechnology, and physics involving small volumes of liquids or practical devices to the design of these systems (Nikoleli et al., 2018).

In general, the purpose of biosensors is to obtain analytical information of some analytes in samples without pretreatment and is designed for this purpose. It is integrated with microfluidic systems to improve the deficiencies of conventional biosensors and to be applied in a wider area of applications. For example, chemical synthesis and automatic sequential separation depending on the detection part of the miniaturized systems is an important advantage for bioassays. When researchers are working on new drug discovery, the drug discovery process is difficult, expensive, and long since the amount of product used is more. Innovative bioassay technology should be used to minimize the sample volume to improve this process. One of these technology examples, LOC is popular in pharmacology, biotechnology, and, pharmacology and it is one of the main motivations to integrate micron or submicron scale biosensors into microfluidic systems.

Two important applications of microfluidic technology are medical diagnostics and bio-sensing. Microfluidics tries to defeat difficulties or challenges in conventional assays in medical diagnosis (Rivet et al., 2011). The combination of biosensors and microfluidic chips increases the analytical capability to extend the scope of possible applications. Microfluidic-based biosensors have progressed extensively in different fields over the last few decades such as disease, drug screens through biomarkers, cell-based assays, molecular separation, and cell sorting, pre-treatment, detection, and sample preparation. Multiple detections, low cost, miniature biosensors that are easy to use in many clinical and biochemical diagnostic applications, capable of analyzing small amounts of samples that require good operational stability and rapid response times (Zhang & Tadigadapa, 2004).

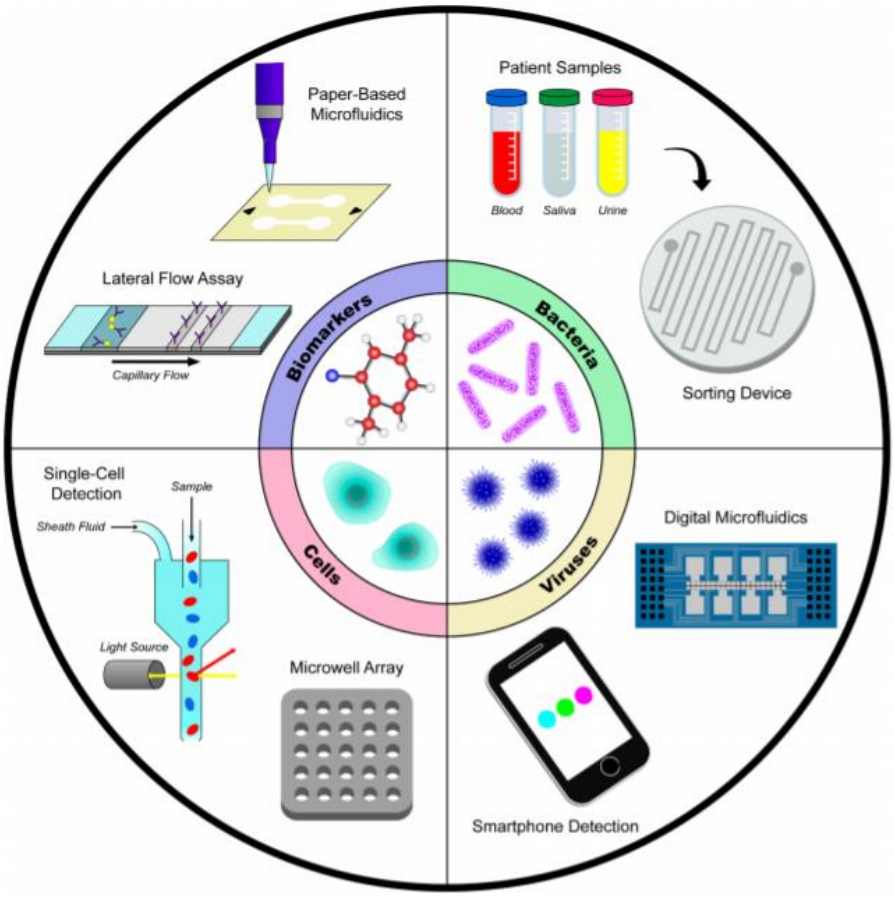


Figure 1. 6: Fields of the microfluidic chip system **(Campbell et al., 2018)**.

In addition to these advantages, another important part of microfluidic systems is the use of point-of-care (POC). In POC applications, many microfluidic systems with sensing modules increase the efficiency of the assays and reduce cross-contamination. Besides, microfluidics has the ability to minimize expensive reagent consumption, analyze small sample volumes, reduce processing time, and automate sample preparation. Overall, these advantages can potentially reduce the cost of the assays and provide faster diagnosis. These advantages are exemplified by meaningful results in studies of the biosensor applications (Rivet et al., 2011).

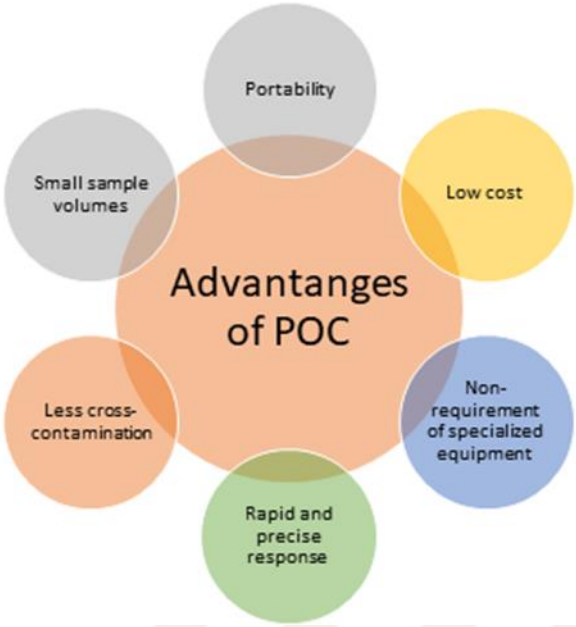


Figure 1. 7: Advantage of microfluidic point of care applications.

Srinivasan *et al.* studied biological lab-on-chip to detect the concentration of human body metabolites. An optical absorbance measurement in the microfluidic system is used to detect glucose, lactate, glutamate, and pyruvate with a colorimetric enzyme-kinetic assay that gave a response less than 40 seconds and in the linear range of 25mg/dl to 300mg/dl, with less than 5% deviation. This study shows that is an example showing that microfluidic systems are faster than conventional methods (Srinivasan et al., 2003).

In traditional biotechnological applications, cell incubation, DNA-RNA isolation, and modification are completed in a very long time. Such conventional methods are time-consuming, generally requiring more than 24 h. This long process was completed within 15 minutes using the microfluidic system. Natalya V. Zaytseva et. al. fabricated the biosensor module includes of microfluidic channels and obtained a fluorescent detection that could be used for the fast identification of pathogenic viruses and organisms with their messenger, genomic RNA or ribosomal. In this study, the designed microfluidic biosensor is a system consisting of microchannels that analyze in only 15 minutes including incubation time. This design can be integrated with a LOC system that will combine detection sample preparation steps on one chip. This study shows that microfluidic sensor systems prevent waste of time and give fast results (V. Zaytseva et al., 2005).

One of the biggest problems in a conventional system is that sensor sensitivity and functionality are not sufficient in the presence of other species in complex solutions and sensor selectivity is reduced. There is a need for low-cost, miniature systems that can accurate identification and rapid detection. Boehm *et al.* developed a simple and rapid method to detect bacteria in a microfluidic measuring chamber with surface derivatization of the sensing chamber using monoclonal antibody by impedimetric detection. They show that this biosensor could detect $9x10^5$ CFU mL^{-1} E. coli in the solution with successive perfusions in solution and this approach would be useful for detecting low bacterial concentrations. (Boehm et al., 2007)

1..3.1. Bead derivatized affinity-based biosensor in the microfluidic chip

Integrated affinity sensors inside of the microfluidic structures show great interest in life environmental and science analytical science applications. They are generally placed in the base of a fluidic flow channel on which an analyte solution is passed. The analyte detection on the sensor depends on the event of a recognition-binding, most typically, DNA hybridization or antibody-antigen, for which the recognition molecules for the analyte are attached to the surface of the sensor. The analyte-recognition molecule complex is detected on the sensor (Friedrich et al., 2008).

Affinity-based microfluidic structures attract great attention to life sciences and environmental analytical science applications. In the microdevices, sensitivity is always the key parameter, especially when analytes are found to be at overly low concentrations in certain projects. An apparent approach to obtain sensitivity high enough to overcome this obstacle is the use of microbeads functionalized by biomolecules with the affinity between template DNA and dNTP (as developed by companies such as Ion Torrent and Roche (Figure 1.7)). Indeed, numerous research groups have proven that microfluidic chips harboring such microbeads had afforded very sensitive results with a limit of detection reduced to zeptomolar, and attomolar levels. Also, the evaluation of binding parameters for biomolecular recognition between one immobilized molecule and another in solution is provided by microbeads derivatized microfluidic system (Friedrich et al., 2008).

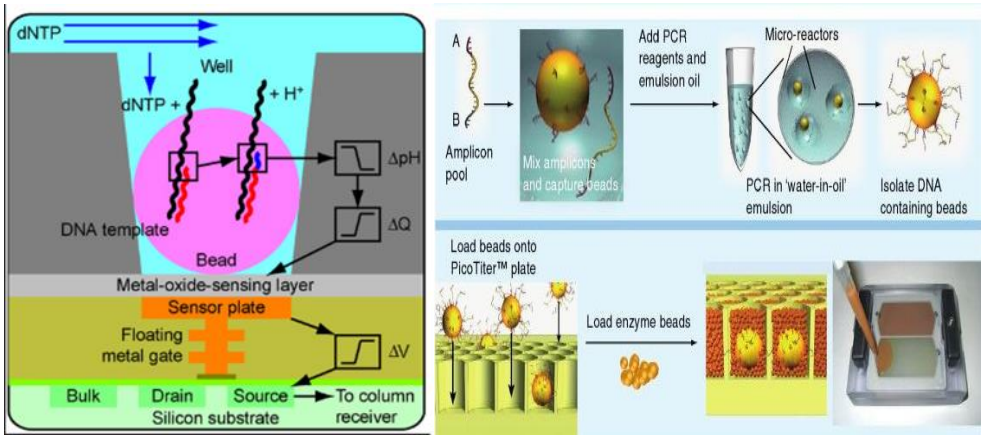


Figure 1. 8: a.) In Ion Torrent bead derivatized microsystem b) Roche 454 bead derivatized microsystem (Voelkerding et al., 2009).

Hilton *et al.* investigated that the affinity-based biosensor allowed the detection of micromolar quantities of cocaine. The device includes a microchamber integrated with an on-chip packed with aptamer-functionalized microbeads which act as a sensing surface. Traditional methods include chromatography, immunoassays, and hypothetical testing to detect cocaine. Unfortunately, traditional methods are expensive, requiring a lot of time, non-specificity, complex equipment, and trained staff. This study shows that aptamer-based detection on a microfluidic platform has the potential to provide rapid, highly specific detection of cocaine and low-cost in practical applications (Hilton et al., 2011).

Recently, researchers' interest in the medical field is the integration of bead-based immunoaffinity assays into microfluidic chips. It is becoming more and more important that the experiments have a fast and reliable quantitative analysis. Even though the use of beads is not restricted to biological applications, cellular measurements like antibody-mediated agglutination, cell counting and, diagnostics utilizing cultured cells attract exceptional interest as they could profit hugely from beads. Microbeads offer a few points of interest over conventional methods, planar technologies like a stage to immobilize biomolecules which are the accessibility of a library of pre-functionalized (pre-coated with biomolecules) bead types from numerous vendors, enormous surface areas to help responses (increasing sensitivity), and the ability to be assembled into arrays that test for different analytes at the same time (Thompson & Bau, 2010).

Many studies have also shown the power of bead-based immunoassays to improve sensitivity detection and reduce assay times. Pereira *et al.* developed antigen-coated

magnetic microbeads with chip-immobilized enhanced fast and precise measurement of human serum immunoglobulin G antibodies to bacteria (Pereira et al., 2010). Another example of reducing assay time for the bead is that Choi *et al*. have developed a microfluidic integrated system that can detect biomarkers using magnetic beads that are used as both biomolecule carriers and immobilization surfaces. Thanks to this system, fast and low volume immunoassays were carried out using microbeads. After five repeated experiments, the sample volume loss was found to be less than 50 µl and the total time required was less than 20 minutes for immunoassay, including sample incubation. Rapid and low volume biochemical analysis was successfully performed by the advanced biofilter and immunosensor integrated into the microfluidic system(Choi et al., 2002)

Baselt *et al.* developed a method for detecting and characterizing many individual forces using magnetic microbeads that bind antibody-antigen, ligand-receptor pairs, or DNA–DNA together. The Bead Sequence Counter (BARC) attaches magnetic microbeads to a solid substrate, thanks to these interaction forces. The removal of the beads into the substrate based on the binding affinities occurs by the application of controlled magnetic forces. In this study, the bead particles were counted with a micromechanical force transducer that measures the total magnetic force utilized by the particle. They developed this method for the use of a high-sensitivity immunosensor for in situ detection of bacteria, toxins, and viruses (Baselt et al., 1998).

Sato *et al.* developed an immunosorbent test system integrated into a glass microchip to reduce assay time and examine with simpler operation. The beads were then placed in a microchannel, and then the human immunoglobulin adsorbed onto the bead surface was reacted with the colloidal gold conjugated antibody and detected. The value of the liquid micro space on the molecular behavior contributed significantly to the reduction of assay time. The integration reduced the time necessary for the antibody-antigen reaction by 1/90, so the total analysis time was less than 24h to less than 1 h. Besides, laborious operations required for traditional immunosorbent assays can be replaced by simple operations (*Integration of an Immunosorbent Assay System: Analysis of Secretory Human Immunoglobulin A on Polystyrene Beads in a Microchip | Analytical Chemistry*, n.d.).

1.2 Project-2: The Biochemistry of Learning

1.2.1 How does the brain function?

Neurons and glial cells are two major cells form the nervous system. Neurons are specialized cells and electric signals of the body are transmitted and received by them. As shown in Figure 1.8, dendrites, a cell body (called the soma), and an axon are three main parts of neurons. Neurons have a single axon and are the output of the neuron. Different neurons are connected by axons and the actual connection gets occurred by axon terminals which placed at the end of the axons. Signals in axon terminals from different neurons are collected by several dendrites six times. Synapses refer to the communication point of neurons and they cover dendrites from different neurons and the collected signals from these synapses are combined by neurons. Until signals reach the synapse, dendrites travel to the cell body after received by dendrites. The target neuron gets a message from another one at a synapse or gap. The transmission of the signal to another one is caused by firing of an action potential in one neuron at the synapse (the presynaptic or sending) therefore own action potential of postsynaptic neuron gets fired by itself whether more or less likely.

Most synapses are chemical and chemical messengers are used during communication. Other synapses that ions flow directly between cells are electrical. Even though functions of those synapses seem too independent and unrelated, there is close interaction between these two modalities during both development and adult brain. Also, there is a considerable difference between glial cells and nerve cells. Even though synaptic contacts are defined and signaling abilities are maintained by the help of supportive functions of glia, there is no direct effect of glia on synaptic interactions. Also, synaptic transmission and plasticity are regulated by astrocytes which is a type of glia. Also, it responds to neural activity. Therefore, processing, transfer, and storage of information have an active role in astrocytes. Even though there is a paradigm that suggests the exclusive functioning of the brain from neuronal network activity, pieces of evidence show that neuron-glia networks allow the functioning of the nervous system. In other words, the physiological consequences of the bidirectional communication between astrocytes and neurons and knowledge about properties are presented at cellular and molecular levels.

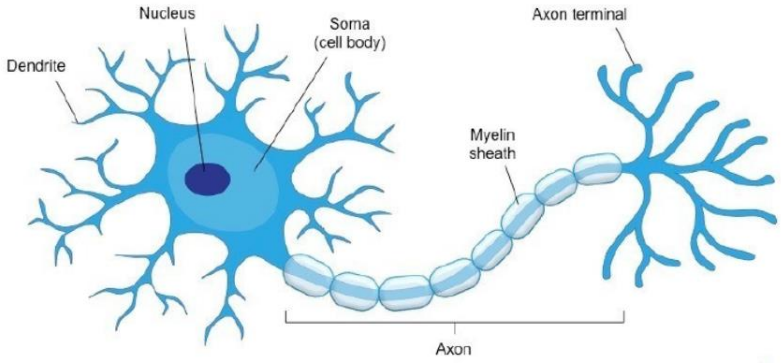


Figure 1. 9: Nerve Cell Structure **(*Ch. 3 – Neurons & Synapses - Psyc251 Cognitive Neurosciences*, n.d.)**

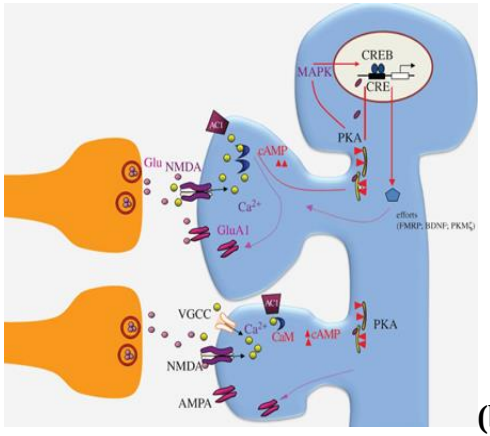
1.2.2 Neuroscience and artificial neural networks

Biology allows neural networks to get understood in the scientific sense. There are about 10 billion neurons (nerve cells) and 6000 times as many synapses (connections) in the human brain (Tierney & Nelson, 2009). Neurons and synapses are a particular part of the body because they process and assess information taken by humans. While compared with the silicon logic gate, the neuron is slower in itself but sufficient. Therefore, the brain operates the complex, non-linear, and parallel computer ((PDF) Stock Index Forecasting Using PSO Based Selective Neural Network Ensemble., n.d.).

The relationship between the stimulus and the individual/ensemble, neuronal responses, and electrical activity of neurons are main areas Neuroscience and artificial neural networks field focus on. There is a theory that suggests that networks of neurons represent sensory and information in the brain. According to theory, digital and analog information could be encoded by neurons (Thorpe, 1990). In the process of learning, nerve cells, the basic computing units of any nervous system, are thought to exhibit digital and analog properties.

1.2.3 How do we remember?

The processes within the brain can be defined as both analog and digital. That is to say, whether neurons fire or not is accepted as digital data whereas biochemical events in the neuron cell can be considered as analog data. Thus, the communication of the two nerve cells is neither digital nor analog, instead; they should be considered as a combination of both.


(a)


(b)

Figure 1. 10: Digital part of memory (a) and Analog part of memory (b).

1.2.3.1. Digital Part

In meaning, digits represent digital. Whether they are binary digits or not, they have to be digits. Neurons have well-known binary "all" or "none" 1/0 spike response behavior. There are "all" or "none" action potentials that provide propagation of an electrical signal in most neurons. When a certain threshold is exceeded, the input stimulus is integrated and fired by these neurons. It is a form of information transmission and enhanced by voltage-gated ion channels. Also, it provides signal transmission over long distances to be rapid and robust. Action potential drives burst of neurotransmitter release as the output of these neurons. There are two states as on state regarding persistent firing and off states without firing. Neurons could be in one of them and brief stimulus triggers transition between on and off states.

Rosenblatt introduced a simple neural network model in the late 1950s which is more complex than the model of McCulloch and Pitts. The model has a classification goal and different problems about classification get solved depend on the number of combined nerve cells. Evidence shows that correct classification needs classes to divide into a planer (Nygren, 2004, Minsky & Papert, 1969).

The responses of the biological nervous system elements in the artificial nervous system are given below.

Table 1.2: The responses of biological nervous system elements in the artificial nervous system (Koç, 2004).

1.1.2.1 Biological Nervous System	Artificial Nervous System
Neuron	Processor Element
Dendrite	Aggregate Function (g(x))
Cell Body	Transfer Function
Axons	Artifical Neuron Output (f(x))
Synapses	Weights (wi)

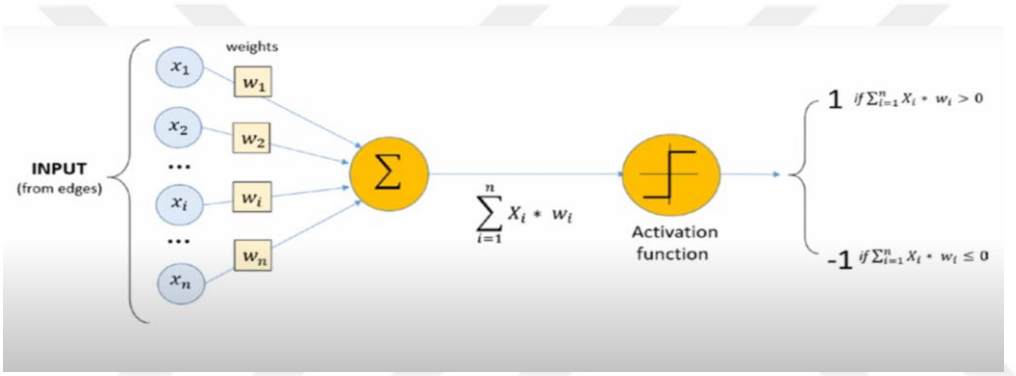


Figure 1. 11: Simple neural network (Elmas,2003).

According to the principle of the model, taking more than one input allows nerve cells to produce output. There is one or zero logical value as the output of the neural network and it is calculated by threshold value function. Signals xi transferred into neurons by synapses wi represent that connection. Specific input xi is represented as important or not by interpretation of wi. The sum of weighted inputs wi*xi is taken inside the neuron. The output function is emitted by neuron while sum x is greater than an externally applied threshold.

It may be divided into 2 parts. In Figure 1.10, considering the summation function g (x), activation function f (x); the first part, g takes an input(dendrite) performs aggregation and based on the aggregated value the second part, f makes a decision.

$$g(x_1, x_2, x_3, \ldots, x_n) = g(x) = \sum_{i=1}^{n} xi \quad (1.3)$$

$$y = f(g(x)) = 1 \quad \text{if} \quad g(x) \geq \theta \quad (1.4)$$

$$= 0 \quad \text{if} \quad g(x) < \theta$$

We can see that g(x) is just doing a sum of the inputs- a simple aggregation. And theta here is called thresholding parameter. In the McCulloch-Pitts neuron model; After the input values are multiplied by the weight values, the g (x) function is obtained. The total value obtained; the output value is assigned "1" if it is greater than the threshold value, and "0" if the output value is less than or equal to the threshold value. The firing of a neuron (transmitting a signal from the cell body to its axon and eventually synapse on another neuron or end-organ) is either completely or nothing. A neuron can just fire so it cannot fire a little or a lot. Since this is a binary on/off phenomenon, it appears to have digitized neuronal function. The obtained value of 1 means the ignition of two neurons, that is, the communication, while the value of 0 means that the two neurons cannot communicate.

1.2.3.2. Analog Part

Chemical messengers are released during chemical transmission ant it is called neurotransmitters for the analog part. There are many synaptic vesicles filled with neurotransmitter molecules that are membrane-bound spheres in the axon terminal of sending cells. Synaptic cleft refers to the gap between the axon terminal of the presynaptic neuron and the membrane of the postsynaptic cell. The membrane is depolarized by action potential while action potential reaches axon terminal and voltage-gated Na^{+}channels are opened. The voltage-gated Ca^{+2} channels are opened by depolarized presynaptic membrane after Na^{+} ions enter cell. The concentration of Ca^{+2} is higher at outside the neuron compared with inside and it rushes into the cell. Synaptic vesicles are allowed by Ca^{+2} to fuse with axon terminal membrane and Ca^{+2} releases neurotransmitters into the synaptic cleft. After diffusion of neurotransmitter across the synaptic cleft, receptor proteins are bonded on the postsynaptic membrane. There are few channels control translation of neurotransmitters into the electric signal at the membrane of the postsynaptic neuron.

Synaptic vesicles store neurotransmitters and after entering of Ca^{+2} to axon terminal as a response to an action potential, neurotransmitters are released and affect to receptors on the membrane of the postsynaptic cell. More than 100 different agents have the role of neurotransmitters. There is tremendous diversity in chemical signaling between neurons which are allowed by numbers of transmitters. The panoply of transmitters could be divided into 2 categories based on their size. Neuropeptides

contain 3 to 36 amino acids and they are large transmitter molecules. There are individual amino acids called small-molecule neurotransmitters such as glutamate and GABA smaller than neuropeptides. The acetylcholine, serotonin, and histamine are transmitted by individual amino acids. Chemical properties and postsynaptic actions of small-molecule ones are similar therefore biogenic amines (dopamine, norepinephrine, epinephrine, serotonin, and histamine) could not be discussed at total. The particulars of synthesis, packaging, release, and removal are different for each neurotransmitter. On the most common transmitters of the central nervous system is amino acid neurotransmitters with three categories as glycine, glutamate, and GABA. The majority of excitatory and inhibitory neurotransmission in the nervous system are provided by them. NMDA receptors (NMDARs) are localized with other ionotropic glutamate receptors [kainate receptors and AMPA receptors] and with metabotropic glutamate receptors at glutamatergic synapses. The process of neuronal development, synaptic plasticity, excitotoxicity, pain perception, learning, and memory require glutamate receptors. Quisqualate activates three ionotropic receptors and one metabotropic receptors. There are NMDA receptors (named for N-Methyl-D-aspartate), AMPA receptors (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate), Kainate receptors and Receptors named based on molecules they bind (IntechOpen - Open Science Open Minds | IntechOpen, n.d.).

The Biochemistry of Memory: Long Term Potentiation

Regarding on how neurotransmitters function in the process of memory and learning, the most widely accepted model is long-term potentiation, which is the result of synaptic plasticity. Currently, it is widely accepted that the process of learning is the outcome of enhanced communication between neurons via neurotransmitters. In this context, the memory hypothesis and synaptic plasticity state that “activity-dependent synaptic plasticity is induced at proper synapses during memory formation, and the information underlying the type of memory mediated by the brain area where plasticity is observed is both necessary and sufficient for storage”. Memory is believed to happen in the human brain as a result of two different types of synaptic plasticity: short-term plasticity and long-term potentiation.

The cortex is a place where the learning process starts. Sensory signals are transmitted from cortex to hippocampus. For the establishment of long-term memory, the signal should be strong or repeated. After memory established, the signal is transmitted back

to the cortex for storage. Even though old memories are affected, the formation of new memories is impaired by lesions in the hippocampus. There is “use it or lose it” rule followed by synaptic plasticity. Synaptic plasticity refers to changes in synaptic connections and changes in time cause frequently used synapses to get stronger while rarely used ones are eliminated. Synaptic plasticity has an important role during the process of learning and memory retention. Synaptic connections get stronger over time by high-frequency signals or repeated stimulations. The best action of LTP occurs at the glutamate synapse of the hippocampus but LTP can study at most excitatory synapses too. The stimulation of glutamatergic neurons allows action potential to reach its axon and release glutamate into the synaptic cleft. Then, glutamate connects its receptors on the post-synaptic neuron.

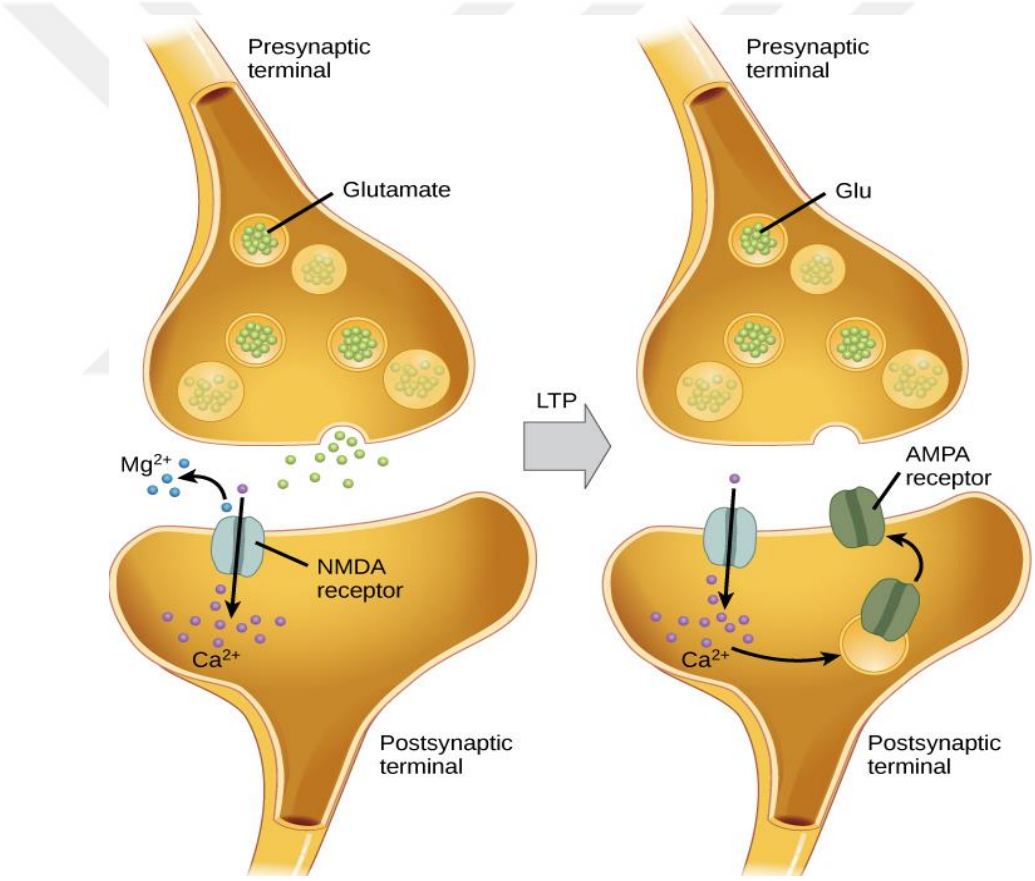


Figure 1. 12: The Mechanism of the Long Term Potentiation (Introduction - Biology - OpenStax CNX, n.d.)

AMPA and NMDA receptors are the two main glutamate receptors often co-existed in a synapse. The connection with glutamate is activated by them and they are called ion channels. While weak signal stimulates pre-synaptic neurons, glutamate is released but it is possible for the only small amount of it. Even though receptors and glutamate are

connected, weak stimulation activates only AMPA. The slight depolarization of the postsynaptic membrane is the result of Sodium influx through the AMPA channel. Magnesium ions block pore of NMDA channel therefore channel remains closed. On the other hand, the release of a large amount of glutamate exists when there is the strong or repeating signal which stimulates pre-synaptic neuron. It provides de-polarization to be greater because the AMPA receptor is open for a long time and transmit more sodium into the cell. Magnesium is expelled from the NMDA channel by activation of an increased influx of positive ions and both sodium and calcium (mediator of LTP induction) are admitted into the cell.

LTP has two phases as early phase and late phase. The early phase is the basis of short-term memory. Several protein kinases are activated by signaling pathways and in the early phase, signaling pathways are initiated by calcium. Synaptic communication is enhanced by these kinases in two ways. AMPA conductance to sodium gets increased by phosphorylating the existing AMPA receptors and AMPA receptors are transmitted from intracellular stores to the post-synaptic membrane. In the late phase which is related to long-term memory, the connection between two neurons exists with making new proteins and activation of gene expression. The new dendritic spines and synaptic connections grow with AMPA receptors newly synthesized and other proteins.

The relationship between α-syn and LTP

The alpha-synuclein(α-syn) is another protein that exists in the human brain abundantly and other body tissues like heart, muscle, and gut have an essential role to sustain LTP in the brain. The chemicals between neurons - neurotransmission are released by synaptic vesicles therefore α-syn tends to concentrate tips of the nerve cells to associate with synaptic vesicles. It has a major role in Parkinson’s Disease.

The clumps of misfolded proteins α-syn with neurons are distinctive pathology for Parkinson. The α-syn is a characteristic component of Lewy bodies as the most common type of disease. Small repeated units which are called oligomers or longer fibrils are formed by α-syn. The oligomers or longer fibrils are known as toxic for neurons therefore has a key role for Parkinson's disease. Even though cell’s different types of protein degrading machinery clears unwanted proteins normally, by misfolded α-syn overwhelms these systems, therefore, neurons are killed in some shreds of evidence.

There is no problem in 20%of people over 70 in motor or memory function even they have Lewy bodies in the brain. İt is a mystery about α-syn and the link between α-syn and Parkinson. Furthermore, it is found that half of the people died because of Alzheimer's has Lewy bodies in their bodies. It shows that α-syn lesions are not specific to Parkinson's disease. It brings some questions about whether Lewy bodies cause disease or Lewy bodies are only disposed of an excess of protein attempted by neurons.

Parkinson's has also been linked to problems with mitochondria. Mitochondria provide cells with the energy to perform vital functions and they are highly dynamic and can fuse or break up into smaller versions in response to the cell's energy demands. They can also be transported to areas of a cell that need them the most. However, in Parkinson's these processes can be impaired and mitochondria are unable o sustain proper neural function. As they become old or damaged, mitochondria are removed and replaced. This recycling is thought to be disrupted in Parkinson's leading to the accumulation of damaged or worn-out mitochondria. Another idea is that the cells' glia, surrounding neurons, may play a role in Parkinson's. As dopamine neurons are lost, one particular type of glial cell, called microglia, is thought to take up the resulting cellular debris, triggering an immune response. Once activated they release inflammatory cytokines that activate neighboring microglia and another type of glial cell called astrocytes. Chemical released by activated microglia and astrocytes have been shown to injure neurons. And one emerging idea is that this happens through the transmission of misfolded α-syn. The hippocampal, controlled by endogenous dopamine, represents the main experimental model for prolonged enhancement, learning, and synaptic changes underlying memory. Hippocampal long-term potentiation is transformed in both a neurotoxin and transgenic model of Parkinson's disease and this plastic alteration is connected with an impaired dopaminergic transmission. (Costa et al., 2012)

Figure 1.12 shows implicated pathways for α-syn toxicity. The left side represents healthy cellular pathways whereas the illustration of perturbed pathways in Parkinson's disease is shown in right. The protein and other debris in the cell are impaired by autophagic and lysosomal clearance under normal situations. In PD, pathways are blocked therefore more protein is aggregated due to the accumulation of aggregated protein. The endoplasmic reticulum (ER) function is maintained in healthy

cells however PD ER stress causes calcium efflux into the cytoplasm. The healthy cells' mitochondrial function and oxidative balance are preserved in the healthy cell but reactive stress gets increased because electron transport chain (ETC) and mitochondria function are compromised in PD therefore oxidative stress exists. Also, synaptic dysfunction is caused by the interaction of α-syn and synaptobrevin-2 (Fields et al., 2019).

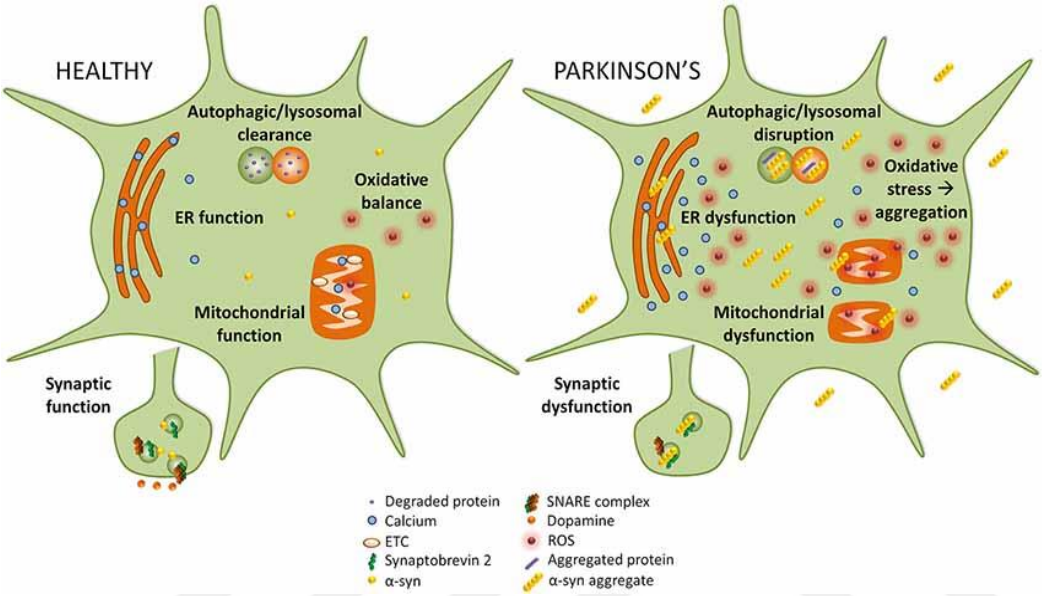


Figure 1. 13: Implicated pathways for α-syn toxicity **(Fields et al., 2019).**

Many factors affect α-syn aggregation, some of which are oxidative stress, post-translational changes and, raised metal ions such as Ca^{+2}. Recently, cell culture models have been used to examine that transient increases of intracellular Ca^{+2} induce cytoplasmic α-syn aggregates (Rcom-H'cheo-Gauthier et al., 2014).

Lautenschläger et al. discovered that calcium can provide the interaction between the small membrane structures inside the nerve endings that are important for neuronal signaling in the brain and α-syn, the protein associated with Parkinson's disease. High levels of calcium or α-syn have been shown to be the initiating chain reaction leading to the death of brain cells. (C-Terminal Calcium Binding of α-Synuclein Modulates Synaptic Vesicle Interaction. - PubMed - NCBI, n.d.). When the level of calcium increases due to neuron signaling in the nerve cell, α-syn binds to the synaptic vesicles from multiple points and causes the vesicles to come together. From this comes the following conclusion: The normal role of α-syn is to ensure that information helps chemical transmission between nerve cells. The interaction of α-syn with synaptic

vesicles is influenced by calcium. The α-syn is thought to be almost like a calcium sensor. In the presence of calcium, it changes its structure and interaction with the environment, which is very important for its normal function.

There is a very delicate balance between the calcium and α-syn in the cell, and when there is too much of any, the balance deteriorates and accumulation begins, leading to Parkinson's disease. Such an imbalance may be caused by genetic doubling (gene duplication) of α-syn, slowing the excretion of excess protein due to age, increasing calcium levels in neurons sensitive to Parkinson's disease, or lack of calcium retention capacity in neurons. Understanding the role of α-syn in physiological and pathological processes can help develop new treatment methods for Parkinson's disease. (C-Terminal Calcium Binding of α-Synuclein Modulates Synaptic Vesicle Interaction. - PubMed - NCBI, n.d.)

The relationship of a similar mechanism associated with intraneuronal calcium imbalance with Alzheimer's disease was described in the study of Garaschuk et al. In the study of Garaschuk, it was understood that there was an abnormal increase in amyloid protein such as α-syn proteins accumulation in the brains of mouse models with Alzheimer's disease, and irregularity in the pre-synaptic side calcium storage. This releases a larger amount of neurotransmitters into the cerebral cortex, thereby creating hyperactivity in nerve cells (Alzheimer’s Research—Intracellular Calcium Store Malfunction Leads to Brain Hyperactivity, n.d.).

1.2.4. Modeling of Learning in Microfluidic System

This thesis aims to make a biological computer by understanding what is in the analog part of learning. The digital and analog parts of learning and memory are modeled by the chemical interaction between two yeast cells within the microfluidic channel design, taking advantage of the advantages of microfluidic systems as a hybrid system. The dimensions of the microfluidic system have been optimized with the simulation program, making it suitable for chemical interaction between yeast cells.

The popularity of microfluidic platforms is increasing for molecular and cellular biology. The physiologic and pharmacologic responses to the single-cell level are studied by use of them. The environment surrounding individual cells is controlled precisely by microfluidic platforms which is a considerable advantage for those platforms while compared with conventional in vitro techniques or mini culture

systems. The deposit of single cells in dedicated areas by microfluidic architecture provides control of spatial distribution and its' interaction with other cells. Furthermore, microfluidic channels control the quantity of added reagents and factors distributed to these isolated cells in microfluidic platforms. The vivo-like microenvironment provides experiments to be performed at the physiologically relevant time and length-scales therefore microfluidic cell cultures prepare that environment. Although there are some problems in vitro techniques such as uncontrollable conventional mixing and undesired extraneous factors, they could be avoided by microfluidic platforms. Furthermore, microfluidic experiments might put forth new details about cellular physiology. The dynamic perfusion and the extracellular chemical microenvironment are precisely controlled by microfluidics because there is flow in the laminar regime in microfluidic channels. In addition, the expenses about media, hormones, and growth factors are lesser compared with those of conventional culture flasks therefore microfluidic experiments are advantageous with cost-saving benefits. Furthermore, experiments could be miniaturized therefore they provide microfluidic devices to process tens or experiments in very small are which is another advantage (Gross et al., 2007), (Guo et al., 2013).

Controlling cells in microfluidic systems encourages cell-cell communication circumstances. Cell-cell communication has an important place in directing cellular functions. The intercellular exchange of information allows the regulation of cell activity and tracing any biochemical pathway of cell activity. There is a lot of proof of impaired intercellular communication in diseases which are autoimmune disorders, diabetes, cancer, and nervous system disorders. Therefore, it is necessary to examine the underlying mechanism of intercellular communication to diagnose and treat diseases. Furthermore, intercellular communication signals can be isolated, measured, and quantified in situ for further analysis (Guo et al., 2013).

Investigation of communication of different types of intercellular was provided by microfluidic systems developed. The study of nerve cell interactions and the determination of signal quantification is important for illuminating nerve diseases. Neuron cells, unlike other cells, never divide and are in most projects not replaceable after their loss. Difficulties of obtaining extracellular circumstance of nerve cells are directed to yeast cells as a model cell for nerve cells.

1.2.5. New substrate for synapses and neurons

The budding yeast Saccharomyces cerevisiae was chosen to understand analog events during learning in the brain. In the mid-thirties of the 20th century, budding yeast Saccharomyces cerevisiae is known as an experimental organism. Thenceforth, its potential has been used as a model organism in many areas of biological experiments. Many researchers have been used Saccharomyces cerevisiae as a common model organism to study cellular processes in evolutionarily species, covering humans. Yeast has recently been used to investigate neurodegenerative disorders such as Parkinson's and Alzheimer's Diseases, although it is deficient for the physiological complexity of the mammalian nervous system.

Here, yeast Saccharomyces cerevisiae is considered the choice of organic substrate for the neuron, based on the considerable genetic resemblance of yeast to mammalian cells. Although a unicellular organism such as yeast may be limited in its ability to explain neuron interactions under all conditions, it is nonetheless a particularly adept substrate that shares with humans a significant fraction of the functional pathways controlling key aspects of eukaryotic cell biology and many key signaling pathways. Thus, yeast Saccharomyces cerevisiae dubbed the “unicellular neuron” will be used in paving the way for the development of mathematical models configuring the dynamics in the brain and to propose new substrate and rules for computation of intelligent systems (Scope and Limitations of Yeast as a Model Organism for Studying Human Tissue-Specific Pathways | BMC Systems Biology | Full Text, n.d.).

Alpha-syn has a particularly important role in Parkinson's disease, and since Parkison's disease is associated with memory and learning, the a-syn metabolism provides direct information about learning. To investigate the basic molecular effects of α-syn in the context of a living cell, human α-syn was expressed in yeast and found to induce dose-dependent cytotoxicity. It was found that GFP-α-syn fusions were initially associated with the plasma membrane and then accumulated in cytoplasmic inclusions reminiscent of protein aggregates, using reporter GFP and galactose (GAL) as an inducible promoter. Subsequently, many different studies followed that used this yeast model for high-throughput analyzes that proved to provide information about the molecular mechanisms involved in α-syn toxicity. Microfluidic technology based on transparent elastomeric materials which are polydimethylsiloxane (PDMS) has enabled the rapid development of prototypes of devices suitable for live-cell imaging

and cell culture. Neuron cells, unlike other cells, never divide and are in most projects not replaceable after their loss (Ismailov et al., 2004).

A hybrid platform is needed to observe both electrical and chemical phenomena in brain activity studies. Due to the above-mentioned advantages, microfluidic platforms are an ideal system for creating the experimental environment of the hybrid system. With help from microfluidics as an experimental setup, this thesis proposes to model the mechanism of LTP. The biological pathway to achieve this goal begins with the release of overexpressed α-syn proteins to the extracellular medium and its binding to N- Methyl-D-aspartic acid receptors (NMDAR). This binding event triggers LTP/LTD by changing the ratio of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/NMDA receptors[Ref]. The figure illustrates how this pathway will be studied in this thesis (to trigger this pathway, yeast cells will be challenged with chemicals to overexpress α-syn).

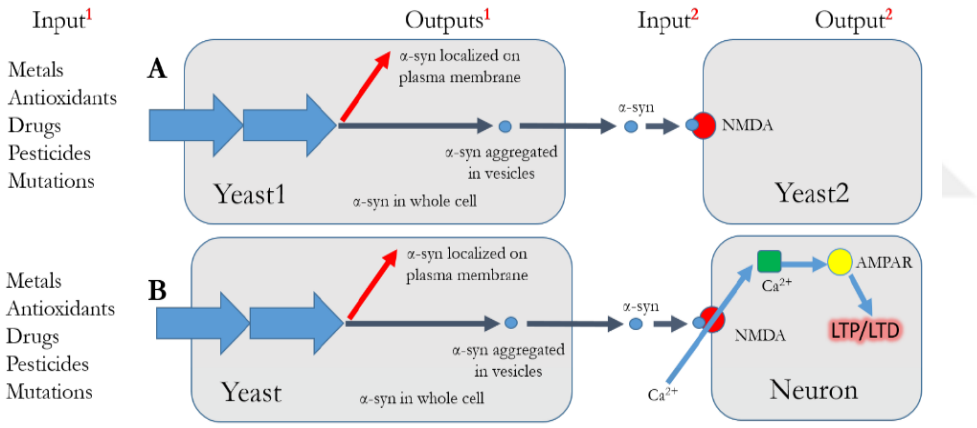


Figure 1. 14: The communication between Yeast1/Yeast2 and Yeast/Neuron populations through a pathway that partly mimics LTP

2.OBJECTIVE OF THE THESIS

2.1 Project-1: Design and Simulation of a Microfluidic Biochip for Optic Detection with Derivatized Microbeads

This thesis aims to design a bead derivatized sensor in a microfluidic chip to detect biomarkers. Many parameters in the design need to be optimized for the best detection of biomarkers. Bead sizes, shape, and dimensions of the array and flow rates were optimized and flow simulation was provided in the microfluidic chip. These simulations should be computational to obtain efficient results in the experimental stage. In this study, the microfluidic device has been designed with different substrate patterns in the continuous flow of PBS, and microbeads were examined. Functionalized microbeads by biomolecules used to enhance affinity to biomarkers for high sensitivity. Microchannel was patterned with square pyramid well array, conic well array, triangle pyramid array, and the each microbeads were placed into the each microwell; PS beads were simulated with different flow rates. Initially, PBS was utilized to simulate blood serum, and PS nanoparticles simulated functionalized and fluorescent-tagged nanoparticles allowing the detection of biomarkers by fluorescent microscopy. All simulations were performed with the COMSOL 5.2 Multiphysics Programme. Optimized parameters for microfluidic devices obtained from the simulations will be implemented for the fabrication of polymethyl methacrylate (PMMA) channels on the glass substrate, which will pave the way to the design, and fabrication of chips for expeditious and easy detection of disease biomarkers.

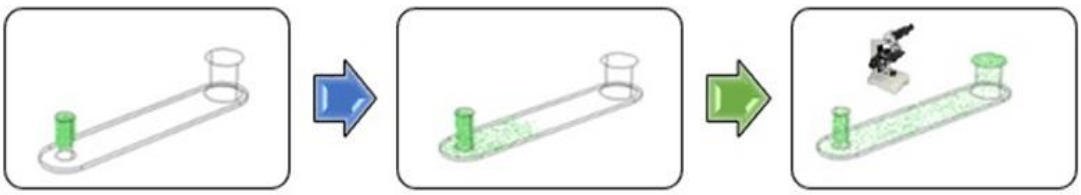


Figure 2. 1: Schematic illustration of affinity-based microbead in the microfluidic chip.

2.2 Project-2: The Biochemistry of Learning

Microfluidic systems are the most suitable systems for modeling on nerve cells before working in an experimental environment. The purpose of this thesis is to create a biosynthetic neuron-on-a-chip to reproduce the key signaling pathways of neuronal function. The digital part and the analog part must be examined together to examine the events that occur between the two nerve cells. Therefore, the communication of two nerve cells in the microfluidic system and the transfer of α-syn protein, which is effective in long term potentiation, from one neuron to another neuron is modeled. It refers to nerve cells in the computer environment by yeast cells. A channel that enables the communication of two yeast cells was designed and these yeast cells were placed in the traps located at the entrances of the channels. The activating agent was sent to produce α-syn of yeast cells in the A channel. A-syn protein, which is synthesized from yeast cells in the A channel, has passed through the channel and attached to the NDMA receptor in the other yeast cell in the B channel. Then, LTP was provided by activating the α-syn protein bound to the NDMA receptor in a balanced manner with Ca^{+} ions. Irregularity in the ratio of protein Ca^{+} and α-syn prevents the formation of long term potentiation and causes Parkinson's disease. In this thesis, the most suitable platform for communication between two yeast cells and the passage of α-syn proteins through channels is optimized and designed. Our simulation provides preliminary information for experimental nerve cell studies.

3. METHOD, DESIGN, AND SIMULATION OF BEAD DERIVATIZED MICROFLUIDIC BIOCHIP

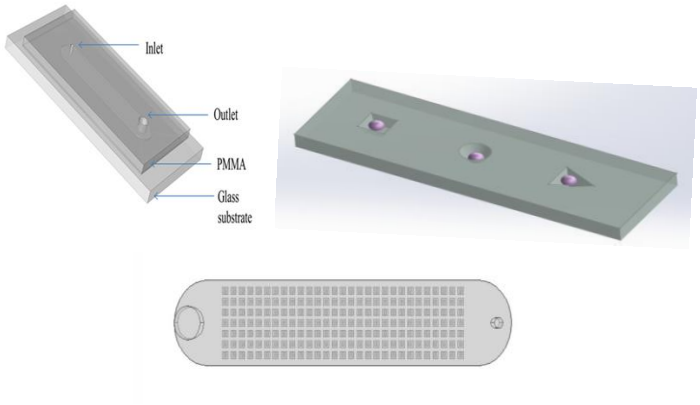
3.2. Project-1: Design and Simulation of a Microfluidic Biochip for Optic Detection with Derivatized Microbeads

Microfluidic systems use and/or transfer fluids with various mechanisms in devices with inner sizes in the order of 1μm. Because of the small size, the physical changes in the macro dimensions differ in the micro-dimension; as the diffusion distance decreases in microsystems, the reaction yield increases, the reaction time decreases, so the faster response is obtained (*COMSOL5 for Engineers (Multiphysics Modeling): Tabatabaian, Mehrzad: 9781942270423: Amazon.Com: Books*, n.d.). COMSOL Multiphysics provides various advantages in terms of microsystem simulation and modeling with its integrated modeling and stimulating environment. In this study, the microfluidic device has been designed with appropriate inlet and outlet size and then change of physical parameters in the continuous flow of phosphate buffer saline (PBS) and polystyrene (PS) nanoparticles were examined. Initially, PBS was utilized to simulate blood serum, and PS nanoparticles simulated functionalized and fluorescently tagged nanoparticles allowing the detection of biomarkers by fluorescent microscopy. All simulations were performed with COMSOL 5.2 Multiphysics Software. Microchannel was patterned with pyramid well array and conic well array and each microbeads were placed as into each microwell; PBS flow in the microchannel was simulated with different flow rates. Optimized parameters for microfluidic devices will be implemented for the fabrication of PMMAchannels on the glass substrate, which will pave the way to the design, and fabrication of chips for expeditious and easy detection of biomarkers.

The microchannel, particle, and fluid properties required for the designed biochip are summarized in the table below. Besides, COMSOL modules used in the simulation are also included in table 3.1.

Table 3. 1: Microchannel Properties.

Microchannel Properties (Geometry, patterns, and material)	
Inlet radius	0.25 mm
Outlet radius	0.762 mm
Channel length	16.2 mm
Channel height	0.45 mm
Channel width	4 mm
Substrate material	Glass
Substrate pattern	No pattern, conic and pyramid patterns
Pattern array number	29x7
Fluid Properties	
Reynolds number	$39x10^{-4}$
Fluid type	Laminar flow
Material	Water
Density	1000 kg/m^3
Flow rates	25, 50, 100, 150, 200, 300, 400, 500, 600 µl/min
COMSOL	
COMSOL Physics	Laminar flow(SPF) Particle tracing for fluid flow()
Mesh Properties	
Minimum element size	225080



(a) (b) (c)

Figure 3. 1: a) The basic form of microchannel. b) The array format of microwell. c.) The different microwell patterns, respectively square pyramid microwell pattern, conic microwell pattern and, triangle pyramid pattern.

The sensing array of biochip was designed three geometric shapes, different patterns for microbeads illustrated in Figure 3.1. The sensing array was constructed as a 29x7 microwell array. The fact that the wells formed as array on the microchip increases the recognizing surface. Therefore, many wells were created on the microfluidic chip. Different designs were tried to observe the effect of the shapes of these wells on the flow rate. The thickness and width selections for the chip design are taken from the reference article.

There are some points to consider when choosing materials for microfluidic chip design. Materials were selected for future biochips as a glass substrate, PMMAchannel.

Original materials which are silicon and glass are the first to be used for microfluidic applications. After that, new materials like polymer substrates, composites, or paper were used with new technological advances that happened and time passed. The commonly used polymer to product and prototyping of microfluidic chips are polydimethylsiloxane, called PMMAor dimethicone (Rewatkar et al., 2018). For these reasons, PMMAhas been chosen to make microfluidic chips: PMMAmaterial is transparent at optical frequencies (240 nM – 1100 nM), making it easier for the observation of contents in micro-channels visually or through a microscope. It is considered as bio-compatible and low autofluorescence. It is deformable, which permits the integration of microfluidic valves utilizing the deformation of PMMAmicrochannels, the simple association of leak-proof fluidic connections, and

its utilization to detect very extremely low forces like biomechanics interactions from cells. PMMAmaterial is inexpensive compared to previously used silicone materials. Glass has some benefits which are excellent optical transparency, well-defined surface chemistries, superior high-pressure resistance, chemically inert, biocompatibility allows efficient coatings in microfluidics applications ("Microfluidics Applications," n.d.). Capillary electrophoresis (CE) is important application of glass microfluidic chips. This method is more suitable compared to the Standard CE method because it is easier to conduct parallel analysis and also offer valveless injection using the electroosmotic flow directly, which can separate analytes in seconds. Other applications are on-chip reactions, solvent extraction, droplet formation, and on-site manufacturing. The purpose of the COMSOL Multiphysics program is designed to solve and model "multiple physics" problems. Combining and analyzing different physical events expressed as partial differential equations (PDE) in a single model is done through COMSOL. The COMSOL Multiphysics program analyzes equations using "physics interfaces". These physics interfaces are the choice of pre-built physical equation packages and associated boundary conditions. COMSOL Multiphysics interfaces solve constituent partial differential equations (PDEs) by using the finite element method in the boundary condition of the geometry. In this study, Physics of microfluidic chip interfaces includes Laminar Flow for incompressible fluids which is modeled by Navier–Stokes equations.

Flow in a microfluidic channel is described by the steady-state Navier–Stokes equation (D. Li, 2015):

$$\rho[+ u.\ \nabla u] = -\nabla p + \mu\nabla 2u \qquad (3.1)$$

Where ρ is pressure, μ is dynamic viscosity, u is the fluid velocity, p is applied pressure, and t is time.

Reynolds number is an important parameter for microfluidic flow. Reynolds number is considerably reduced in microsystems. The inertial force terms in Navier-Stokes equations are neglected in the flows where the Reynolds number is very small. In our microchannel, Reynolds number was calculated as $39x10^{-4}$ according to formula 2 (D. Li, 2015).

Newtonian Fluid Reynolds Number (Re) Formula;

$$Re = \frac{pVD}{\mu} \tag{3.2}$$

μ – fluid dynamic viscosity in $kg/(m.s)$

ρ – fluid density in kg/m^3

V – fluid velocity in m/s

D – pipe diameter in m

Such single-phase flows are called Stokes flow. When we click on the box of "Neglect inertial term (Stokes flow)" on laminar flow settings in COMSOL, we directly change the COMSOL program physics from laminar flow to creeping flow. Particle tracing for fluid flow module has been chosen as a model for particle trajectories, and bounce boundary condition applied to the walls except for the inlets and outlets. 200 particles are released from the upper inlet of the microchannel, random initial position according to the surface plane has been given an initial velocity collected from the flow model. The outlet has the freeze boundary condition to visualize the particles at the outlet.

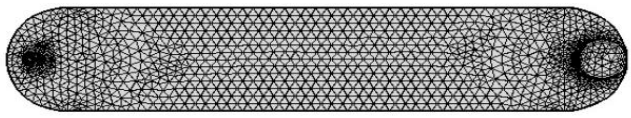


Figure 3. 2: The mesh of the model.

3.2. Project-2: The Biochemistry of Learning

In the microfluidic chip design, two chambers were designed, in which two liquids containing and without α-syn protein would flow. Then, traps were placed to allow the yeast cells sent to A and B chambers to hold. Microchannels were designed between the chambers A and B for the passage of the α-syn protein, which enables the interaction of yeast cells. α-syn proteins will be produced by yeast cells in microchamber A. The transition of α-syn proteins in chamber A into yeast cells in microchamber B by diffusion in different channel sizes is simulated. The dimensions of the communication channels are sized to prevent yeast cells from passing between the chambers. As seen in Figure 3.4, the cells are placed in traps and are in a size that cannot pass through the channels.

In simulations with the Comsol program, the interaction of two yeast cells through the channels was observed. Simulations were made by changing many parameters as a variable factor. As seen in Figure 3.3, these parameters are as follows; the number of channels along with the microfluidic chip, the width of chamber A and B, the width of the communication channel, the distance between communication channels, the length of yeast cells chamber, the length of yeast cells communication channel, the inlet-outlet radius of chamber A and B were determined.

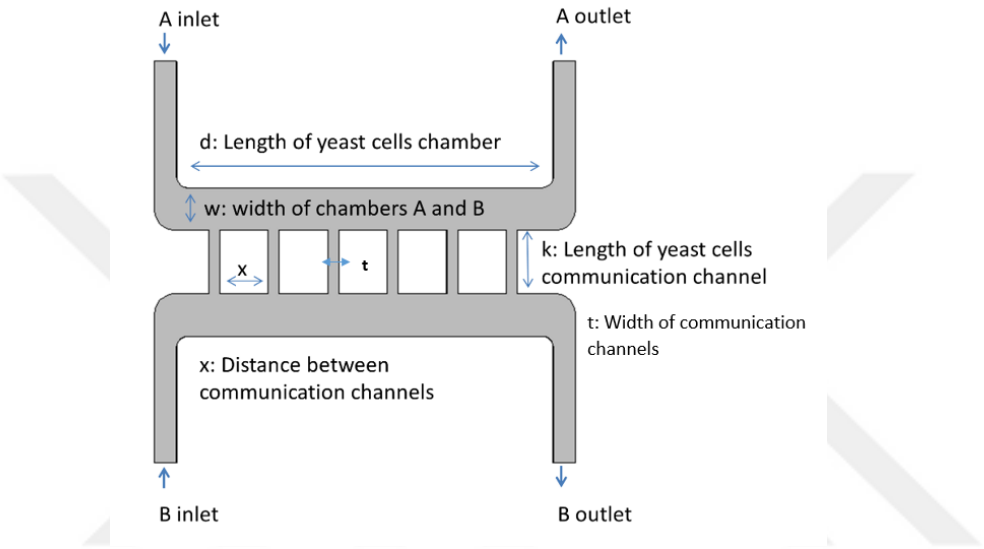


Figure 3. 3: Diagram of the microchamber for yeast cell communication.

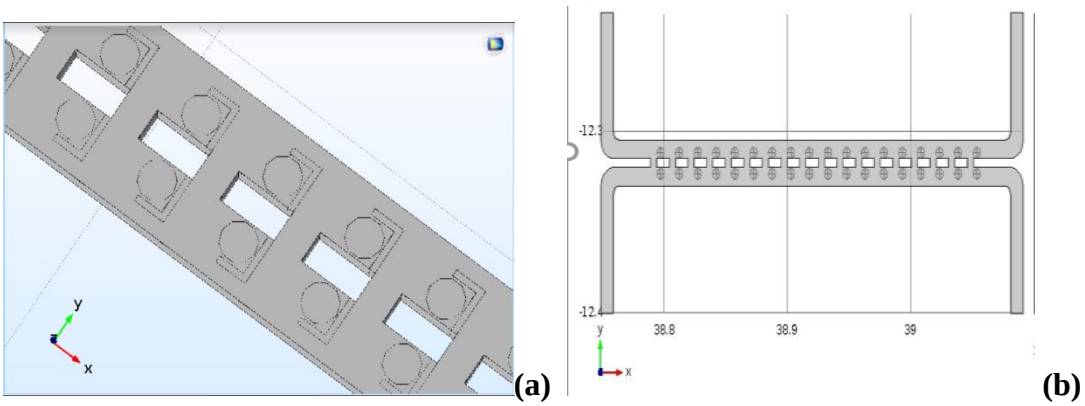


Figure 3. 4: Close-up view of yeast cells placed on traps(a) and Remote view of yeast cells placed on traps (b).

As seen in Figure 3.5, the red liquid represents α-syn proteins released from yeast cells. The blue liquid represents body fluid that does not contain α-syn protein. After activating agents in the red liquid activate the yeast cell in chamber A, the yeast cells

produce the α-syn protein. The α-syn protein diffuses through the microchannel into the yeast cell in chamber B. Thus, communication occurs between two yeast cells.

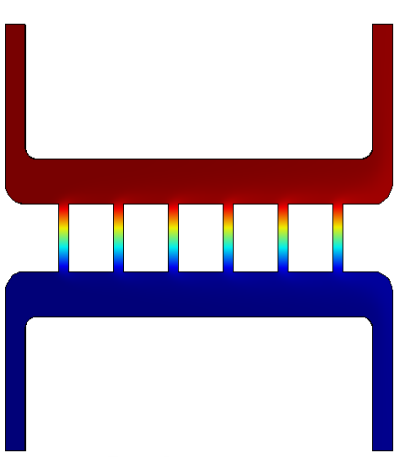


Figure 3. 5: The red color indicates PBS containing the substance that activates the α-syn protein production of yeast cells and the blue color represents PBS without activator.

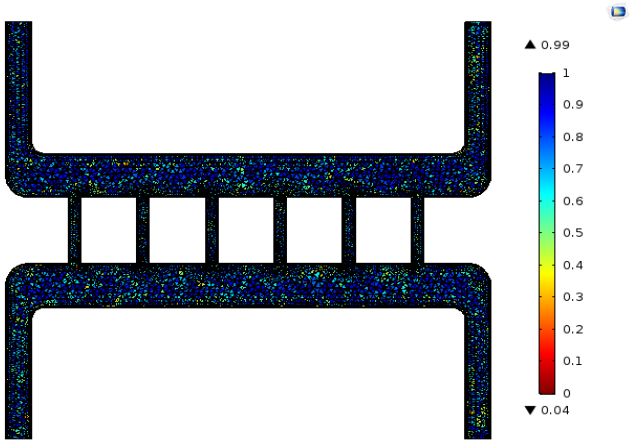


Figure 3. 6: Mesh properties of the microchannel.

The mesh can be seen for the model in Figure 3.6, which is a normal mesh, and mesh properties which consist of tetrahedral and triangular elements. The average element quality is higher than 0.1 which is an acceptable level.

These simulations are used to solve and analyze the fluid flow in the H cell. The flow rate at the inlet is roughly 0.1 mm/s in conformity with the specifications. This means a low Reynolds number, well inside the area of laminar flow:

$$Re = \frac{d\rho u}{\eta} = \frac{1.10^{-5}.1.10^{3}.1.10^{-4}}{1.10^{-3}} \tag{3.3}$$

The channel dimensions, are given in Table 3.1, a value typical for microchannels, and Reynolds number of 0.001 for a water solution are given Equation 2.1. Therefore, it is easy to obtain a numerical solution of continuity equations and full momentum balance for incompressible flow with a logical number of elements. The equations to be solved are the steady-state Navier-Stokes equations given in equation 2.2. (*COMSOL Multiphysics® Software - Understand, Predict, and Optimize*, n.d.)

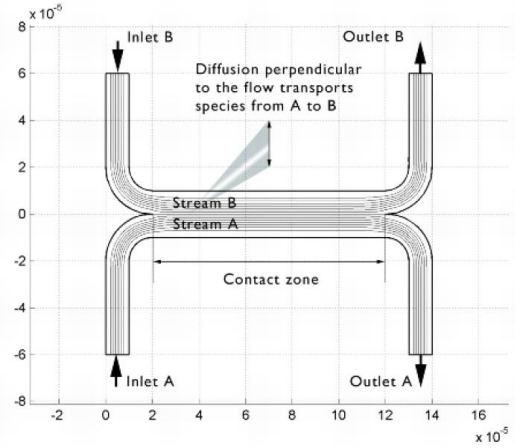


Figure 3. 7: Diagram of the H-microcell.

$$\rho(u.\nabla)u = \nabla.[-p\mathrm{I} + \eta(\nabla u + (\nabla u)^{T})] \tag{3.4}$$

$$\nabla.u = 0$$

In this formula, ρ shows density (kg/m3), p equals pressure (Pa), η denotes viscosity (Pa·s), and u is the velocity (m/s).

H-cell separation includes species at relatively low concentrations instead of the solvent. This means it's safe to use Fick's law here. Since dissolved molecules only interact with water molecules, and Fick's law is used to identify diffusive transport in the cell. The convection and diffusion application module in the Comsol library is used for the mass balance equation of the solute.

$$-\nabla.(D\nabla c + cu) = 0 \tag{3.5}$$

In this formula, c represents the concentration (mol/m^3) and D shows the diffusion coefficient (m^2/s). Using the parametric solvent, the formula in Equation 2.3 is used to simulate the mixing of different types for two different diffusion D values (1.10^{-10} and $1.\ 10^{-11}\ m^2/s$).

It is necessary to solve two versions while analyzing modeling in H cells.

It is assumed that the density and viscosity of the fluid are not affected by the change in the solute concentration in the first version. This means that the Navier-Stokes equations must be solved first and then it is possible to solve the mass balance equation.

It is necessary to add a concentration-dependent correction term to the viscosity quadratically in the second version.

$$\eta = \eta_0(1 + \alpha c^2) \tag{3.6}$$

α is a constant dimension (SI unit: m6/mol2) in Equation 2.4. Generally, in solutions of larger molecules, the effect of concentration on the viscosity of this type is observed. In this project, the flow and mass transport equations have to be solved simultaneously.

Then, starting from the Navier Stokes equations, the boundary conditions of geometry are determined. Geometric surfaces related to boundary conditions are given in Figure 3.8.

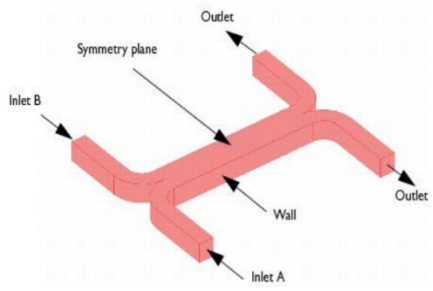


Figure 3. 8: Model domain boundaries.

For the Laminar Flow interface:

• Pressure conditions are valid at the inlet and outlet of the H channel with the disappearance of viscous stress. The pressure conditions at the inlets and outlets are as follows; the pressure at the outlets is set to zero so that the pressure at the inlets shows the pressure drop on the cell. These outlet and inlet conditions are compatible with the

H cell being part of a fixed-width channel system that confirms the improved flow assumption.

• Non-slip conditions indicate that the velocity is zero at the walls.

• Symmetry boundary conditions are used at the symmetry plane to set the velocity component to zero in the normal direction of the surface.

For the Transport of Diluted Species:

• Concentration conditions are used to adjust the concentration at the inlet of the channel. At inlets A and B the concentrations are 1 mol/m3 and 0 mol/m3, respectively.

• Convective flux condition is applied according to the outflow boundary condition and it is stated that the diffusive deliver perpendicular to the normal boundary is neglectable at the outlets. This condition will thus eliminate concentration gradients in the flow direction.

• The No Flux condition is used to model the symmetry plane and cell walls. This equation indicates that the flux of species perpendicular to the boundary equals zero.

4. RESULT AND DISCUSSION

4.1.Project-1: Design and Simulation of a Microfluidic Biochip for Optic Detection with Derivatized Microbeads

The beads should be placed in the wells to measure the labeled bead derivatized biosensors under a fluorescent microscope. Appropriate flow and suitable time were determined to place the beads in the wells. After the appropriate time was determined, it was planned to place the beads easily into the wells by centrifugation. Providing a flow of 50 µl /min for 60 seconds caused the beads to be distributed homogeneously on the array.

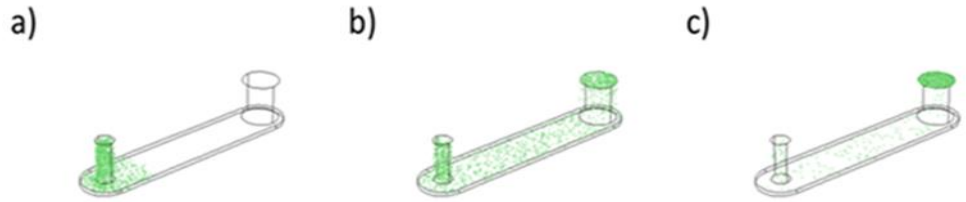


Figure 4. 1: Trajectory of microspheres at a) 10s, b) 60s c) 225s with 50 µl/min flow rate.

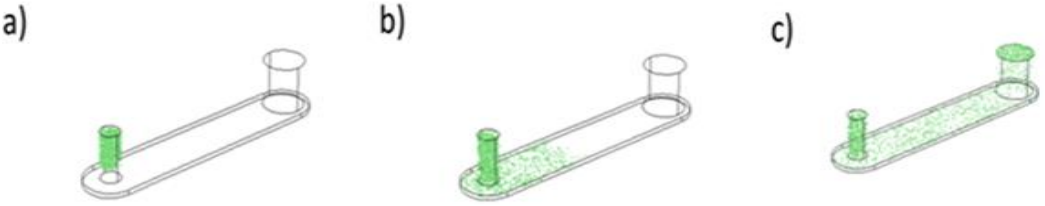


Figure 4. 2: Trajectory of microspheres at a) 10s, b) the 60s, c) 225s with a 25 µl/min flow rate.

The simulation was performed with a flow rate of 25 µl / min and a flow rate of 50 µl / min. It was concluded that after 3 minutes at a flow rate of 25 µl, centrifugation was required to stop the flow and place the beads in the wells. When a 50 µl / min flow rate was chosen, it was determined that the beads should be placed after 1 minute and the beads should be placed. It has been discussed that the time elapsed decreases as the

flow rate increases, and optimized values for the chip design were obtained in the future.

As the liquid flows through the microchannel, it is affected by the inner surface of the channel wall and the flow velocity of the liquid at the edge point becomes 0 due to the no-slip condition. Microchannel was patterned with pyramid well array and conic well array (Figure a) and the each microbeads were placed into the each microwell; water flow in the microchannel was simulated with different flow rates (25, 50, 100, 150, 200, 300, 400, 500, 600 µl / min). It was observed that there was no obvious change in the ratio between the surface average velocity of the inlet and surface average velocity of the outlet of the microchannel. But the ratio between the surface average velocity of inlet and surface average velocity of the cross-section in the middle of the microchannel changed according to different microbead sizes and patterns.

Three different geometric patterned microwells designed for three different diameters of bead sizes were designed. The purpose of this design was to investigate the effect of geometric shapes and bead diameter on the flow of the sensing chip.

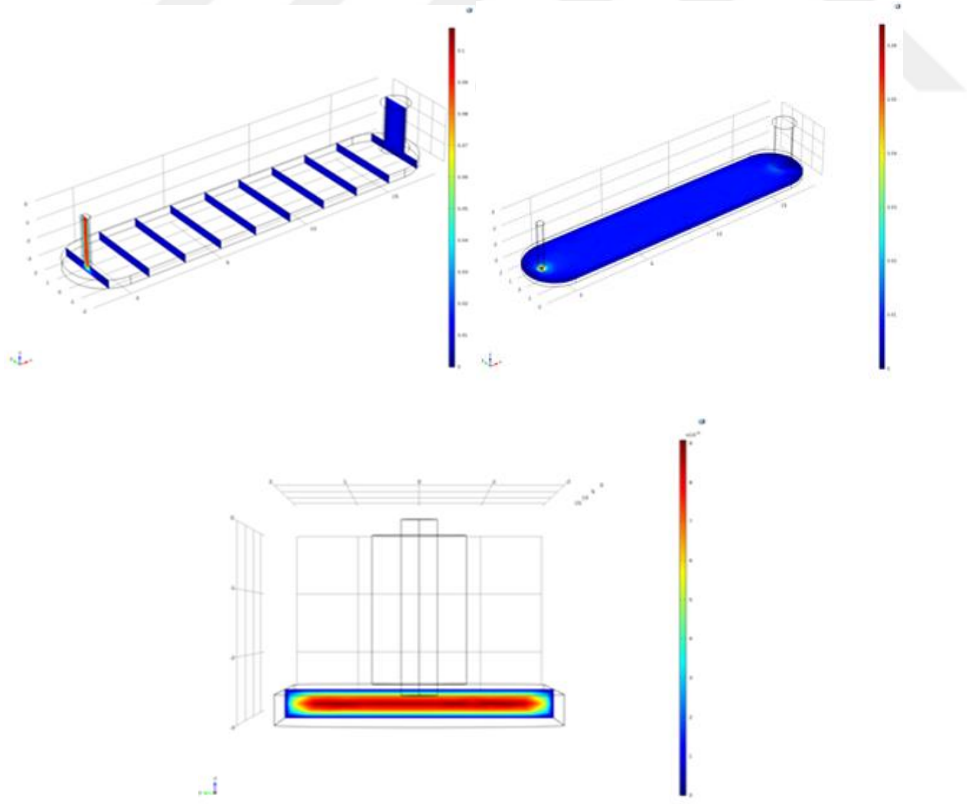


Figure 4. 3: a) Velocity profile in a microchannel with 300 µl / min flow rate b) Cross-section velocity profile xy plane and c) yz plane d) Velocity profile change according to different cross-sections of the microchannel.

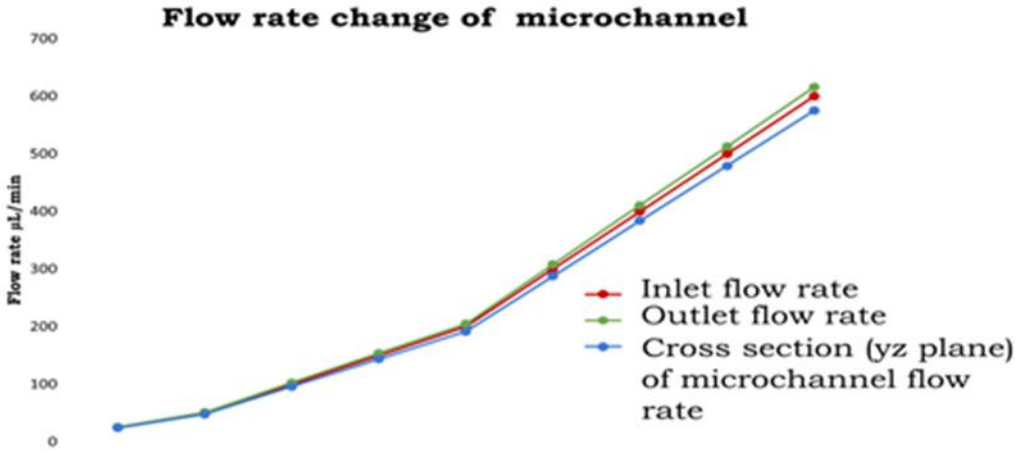


Figure 4. 4: Velocity profile change according to different cross-sections of the microchannel.

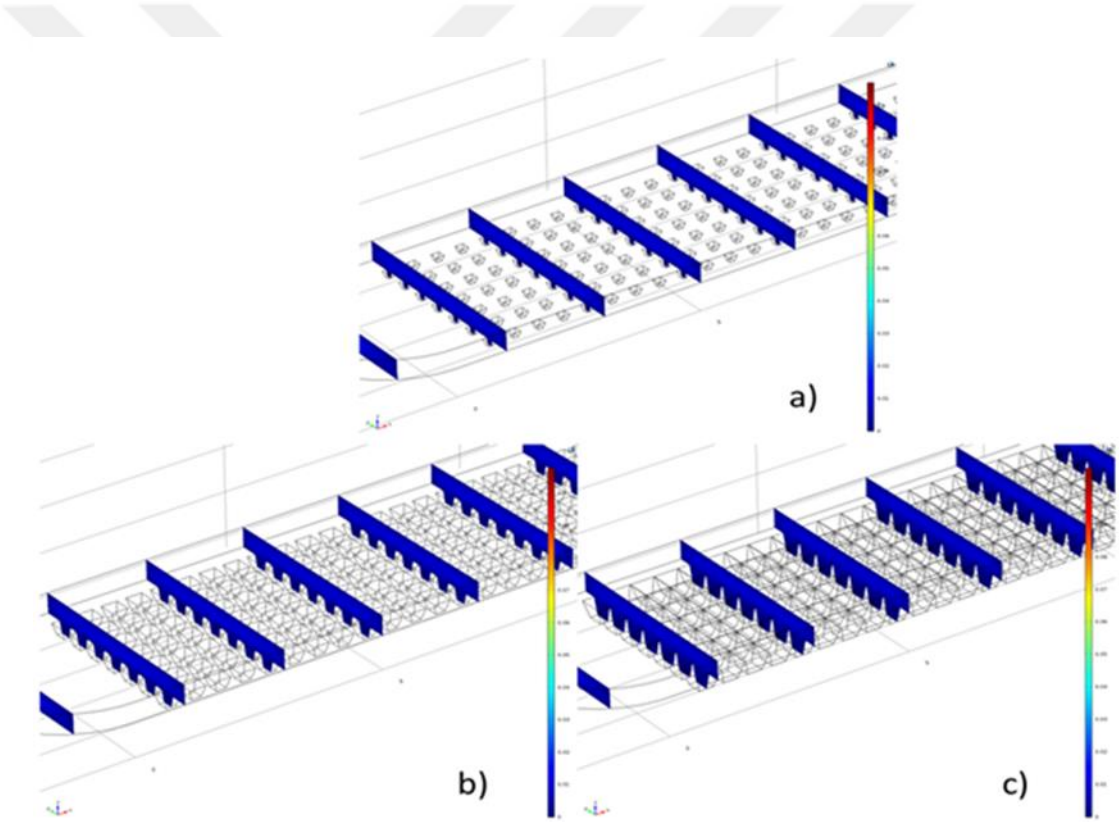


Figure 4. 5: Different sizes patterned microchannel substrate for a) r=50µm b) r=100µm, and c) r=150µm.

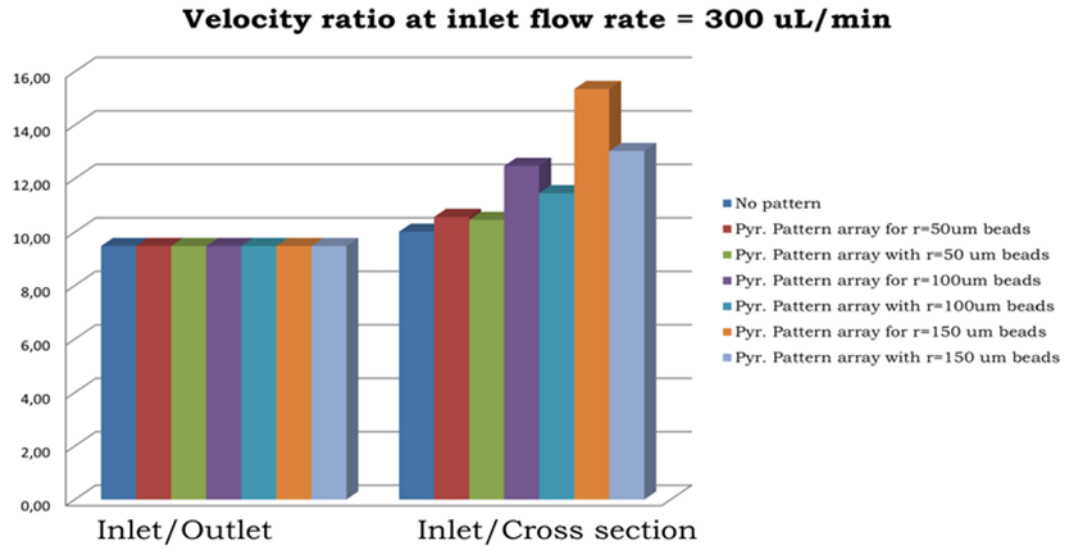


Figure 4. 6: Different sized square pyramid patterns showed different velocity ratio between inlet and cross-section of microchannel at 300 ul/min flow rate.

Flow rate at yz-plane cross-section of the different patterned microchannel (inlet flow

The values of the velocity ratio between the inlet and cross-section of different patterned microchannel at 300 ul/min flow rate are given Figure 4.6.

Array üzerindeki flowrate farklı desenlerdeki.

Square pyramid microwell pattern: 289,25 µl/min

Conic microwell pattern: 270,66 µl/min

Triangle pyramid pattern: 298,87 µl/min

The effect of patterning of the soles of beaded arrays on speed was observed. The lowest cross-section flow rate of the fluid sent from the inlet of the channel with a flow rate of 300 µl was obtained in conical design. This indicates that there will be more interaction with the surface compared to other patterned arrays. Conical design and 150um bead size are thought to provide us with optimum measurement in chip design.

It is important to determine the time during which the bead containing PBS fluid will be poured to place the beads in the wells. As a result of these simulations, the time allowing maximum bead placement to the sensing surface was defined. Providing a flow of 50 µl /min for 60 seconds caused the beads to be distributed homogeneously on the array. It was observed that it dispersed homogeneously within 3 minutes at a flow of 25 µl / min. After determining the flow rate, the optimal time must be

determined by using microinjector simulation in the experimental study to ensure the homogeneous distribution of the beads on the array. As a result of three different geometric well patterns and three different bead size simulations, it was determined that the shape of the well should be conical and the bead size should be 150 µm. The conical pattern has been shown to have the slowest speed and, accordingly, greater interaction with the surface. While the conical physical conditions meet better, the pyramid one will be easier to manufacture. Also, as shown in Figure 4.6, the size tests of microwells and beads were made in the square pyramid microwell, as much more professional computers were needed to mesh the conical shape in the simulation program. The lowest inlet/cross-section ratio flow rate of the fluid was obtained with 150 µm bead size and it indicates that there will be more interaction on the array. With the optimization values of all the parameters obtained, the interaction of the beads on the surface with the target molecules in PBS fluid was increased.

4.2. Project-2: The Biochemistry of Learning

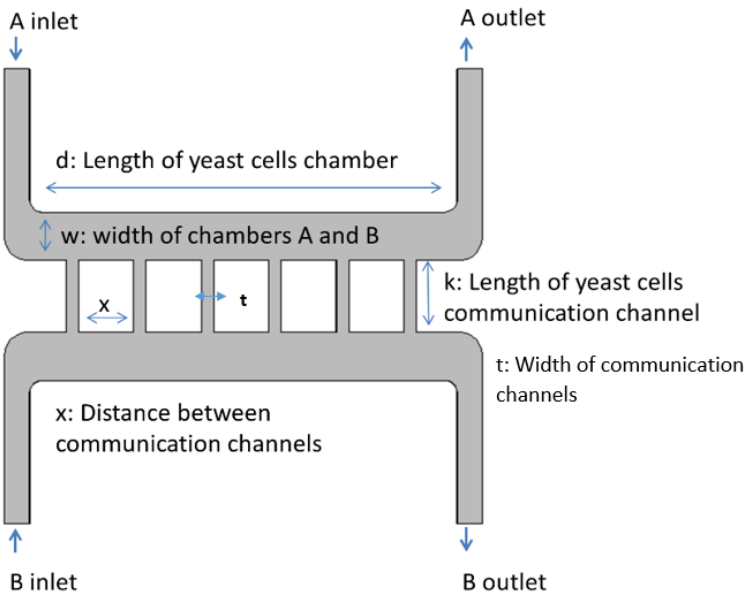


Figure 4. 7: Variable parameters of the microfluidic chip.

The parameters of the microfluidic chip have been optimized. The parameters changed in this optimization are shown in Figure 4.7.

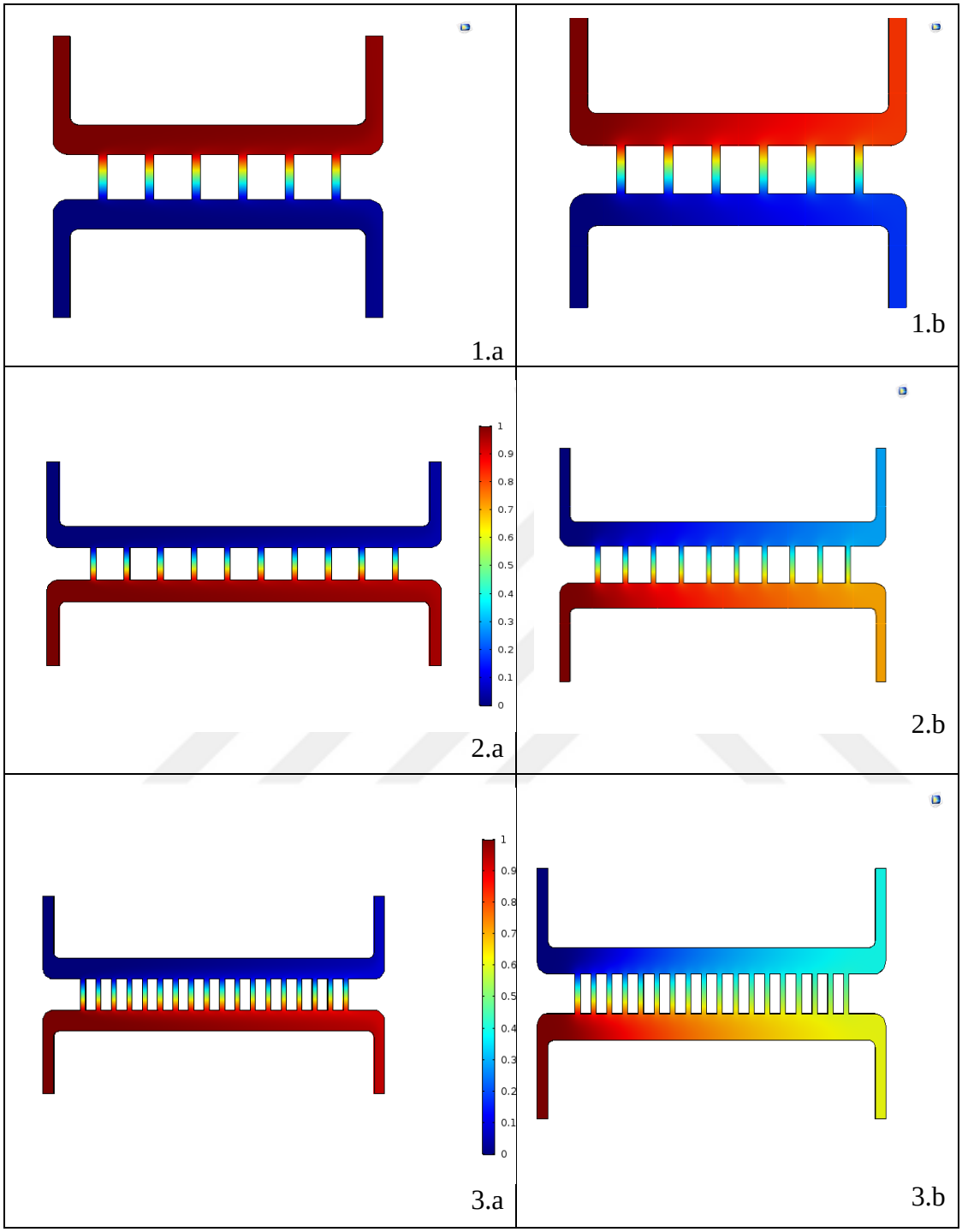


Figure 4. 8: Simulation of the microfluidic channels for two yeast communication. The parameter information of each channel is given in Table 4.1.

Table 4. 1: Parameter variables.

Figures	Ain=Aout=Bin=Bout	W(mm)	K(mm)	T(mm)	X(mm)	D(mm)	Number of channel	Diffusion coefficient
Figure 1.a	0.01	0.02	0.03	0.005	0.02	0.16	6	10^{-11}
Figure 1.b	0.01	0.02	0.03	0.005	0.02	0.16	6	10^{-10}
Figure 2.a	0.01	0.02	0.03	0.005	0.02	0.30	10	10^{-11}
Figure 2.b	0.01	0.02	0.03	0.005	0.02	0.30	10	10^{-10}
Figure 3.a	0.01	0.02	0.03	0.005	0.01	0.30	18	10^{-11}
Figure 3.b	0.01	0.02	0.03	0.005	0.01	0.30	18	10^{-10}

When the number of channels was increased, it was observed that diffusion from A chamber to B chamber was more. The greater diffusion indicates that the amount of α-syn protein passes more from chamber A to chamber B. This is an indication of the communication of two yeast cells.

According to the article Predicting protein diffusion coefficients published by Brune *et al.* The diffusion coefficient for macromolecules was between 10^{-10} to 10^{-11}. Based on this article, these two values have been tried (Brune & Kim, 1993).

There is a relationship between diffusion coefficient and molecular weight. It was shown that the diffusion coefficient should be increased for the diffusion of a substance with a large molecular weight by simulation. The diffusion coefficient was taken as 10^{-11} in Figure 1a 2a and 3a and the diffusion coefficient was taken as 10^{-10} in Figure 1b 2b and 3b. As the diffusion coefficient increased, more homogeneous mixture and accordingly better interaction was observed. The number of channels is 18 and the diffusion coefficient is 10^{-10}, which is considered as optimal values. In other simulations, these two parameters are considered constant and it is observed how other parameters change the interaction of two yeast cells.

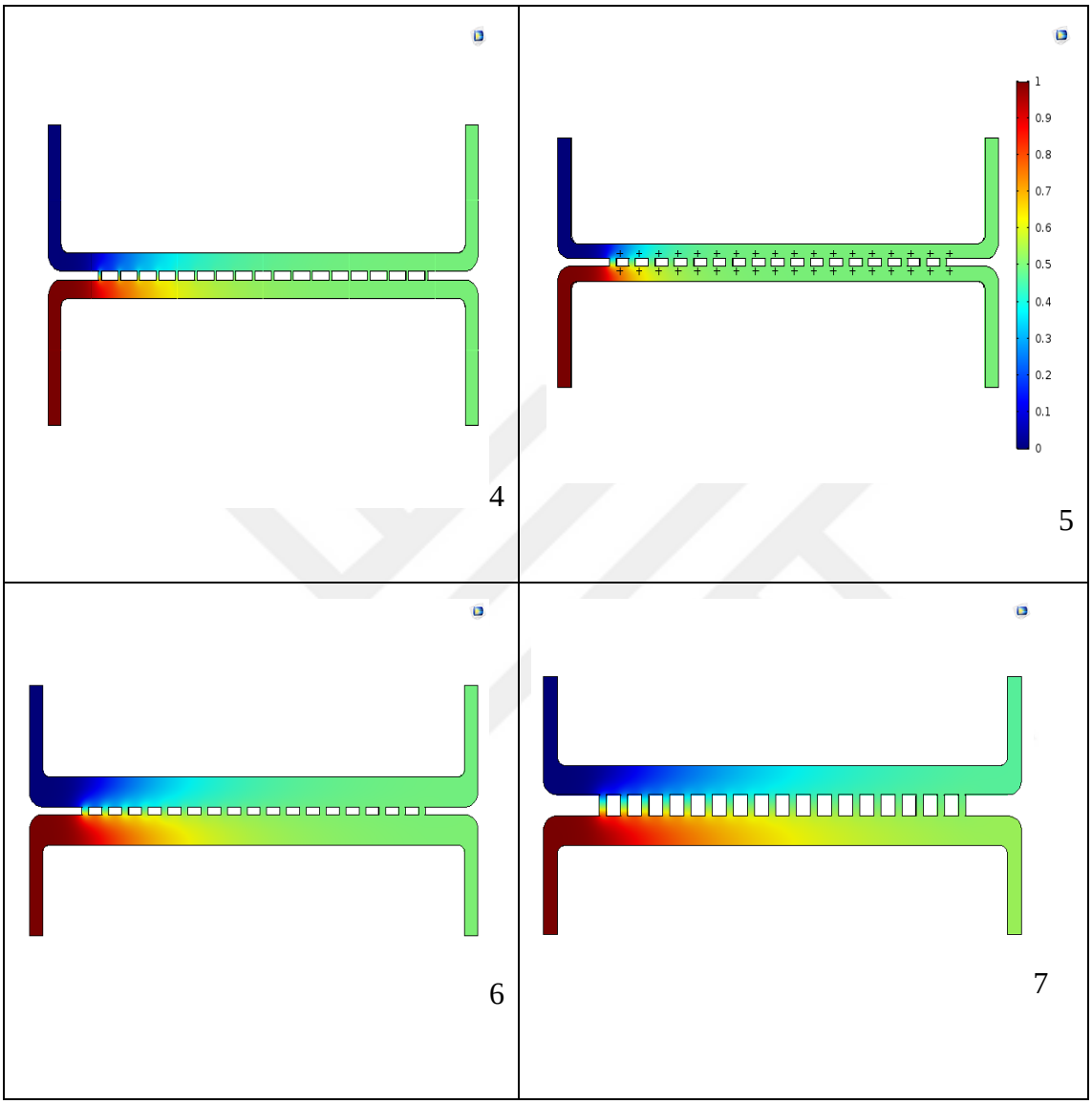


Figure 4. 9: Simulation of the microfluidic channels for two yeast communication. The parameter information of each channel is given in Table 4.2.

Table 4. 2: Parameter variables.

Figures	Ain=Aout= Bin=Bout	W(mm)	K(mm)	T(mm)	X(mm)	D(mm)	Number of channel	Diffusion coefficient
Figure 4	0.01	0.01	0.005	0.002	0.13	0.31	18	10^{-10}
Figure 5	0.01	0.01	0.005	0.005	0.01	0.31	18	10^{-10}
Figure 6	0.01	0.02	0.005	0.005	0.01	0.31	18	10^{-10}
Figure 7	0.01	0.02	0.015	0.005	0.01	0.31	18	10^{-10}

Comparing Figure 5 and Figure 6, although the number of channels is the same, the difference between chamber A and B channel width(w) affected diffusion. A smaller width of chamber A and B increased diffusion. While the communication of two yeast cells started on the 3rd communication channel in Figure 5, it was observed that the communication started on the 6th communication channel in Figure 6. It was observed that the diffusion of α-syn proteins that enable the yeast cells to communicate is more homogeneous.

When the effect of length of yeast cell communication channel (k) on diffusion was analyzed, the diffusion increased as the k number decreased. When Figure 6, Figure 7, and Figure 3 are compared, it is observed that as the distance between the chambers decreases, homogeneous distribution increases.

When the effect of the width of the communication channel (t) on diffusion was examined, it was observed that the increase of t started diffusion earlier. When Figure 5 and Figure 4 graphics were compared, it was seen that the diffusion in Figure 5 started at the beginning of the channel. Therefore, it enabled more yeast cells to interact.

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CU**RRICULUM VITAE**

Name Surname **:** Tuğçe TÜYSÜZ

Place and Date of Birth **:** İSTANBUL, 13.08.1993

E-Mail **:** tugce.tuysuz7475@gmail.com

Education **:**

2017, FMV Işık University, Engineering Faculty, Biomedical Engineering

Work Experience :

- Vygon Medical (2017- Present)
- American Turkish Business Development Council (Executive Assistant (April 2017 – 2017 June)
- **E**rtunç Özcan A.Ş. (Internship – 2016)
- Gülhane Askeri Tıp Akademisi (Internship –2016)