DEVELOPMENT OF A PORTABLE SYSTEM FOR THE DETECTION OF THE MICROORGANISMS IN MEAT PRODUCT

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DEVELOPMENT OF A PORTABLE SYSTEM FOR THE DETECTION OF THE MICROORGANISMS IN MEAT PRODUCT

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Submitted by **Çağatay DURMAZ**

Approval of the Graduate School of Natural and Applied Sciences, Çankaya University.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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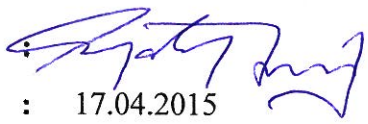
Signature	:	
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ABSTRACT

DEVELOPMENT OF A PORTABLE SYSTEM FOR THE DETECTION OF THE MICROORGANISMS IN MEAT PRODUCT

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This thesis is related to a part of the Ministry of Science, Industry and Technology (MOSIT)'s Industry Thesis (SANTEZ) Program. The specific project number given by MOSIT to the project is 00973.STZ.2011-2. In the thesis, as a part of a Project group, I participated in all the activities of design and development of a new portable device that can detect and identify the food poisoning in a very short time, accurately, and reliably. The research Project Group consisted of the Project Coordinator Prof. Dr. Taner ALTUNOK, who was responsible for the management of the Project and technically all the aspects of the Project, Engin KIRAN, from IDC Company for electronic design and software, Onur ÖÇALAN and Melike AYTÜRK, from IDC company, for software development, and Gamze YAVAŞ and Cansu ÖZDEMİR, from IDC, are the biology experts. My main responsibility was to support the electronic and software design. I developed the image processor software used to identify the bacteria that cause the food poisoning in the images taken by a camera.

The device, Biosensor System, was designed, developed and tested by the Project Group successfully. Thus, the SANTEZ Project was successfully completed and was tested and accepted by the MOSIT. The Biosensor System developed within the scope of the SANTEZ Project has extra capabilities to the devices available on the market.

The image processing method we developed takes the image of a well where a chemical reaction had taken place by introducing an antigen into the food samples before. When the chemical reaction takes place between the food under test and the antigen in the well, some photons are emitted through the chemiluminescence mechanism. The irradiation intensities of the pixels in the image of the well were measured by an electron-multiplying charge-coupled device (EMCCD) camera. Our method defines the area of the wells identified by a mask, and then calculates the average intensity of all the intensity values belonging to pixels in the well image. A detection of the harmful microorganisms is made if the average intensity pertaining to the well exceeds a limit. A decision indicating a positive detection and identification of the harmful microorganism is made if this average intensity exceeds a threshold value determined.

Keywords: Campylobacter Jejuni, Escherichia Coli O157:H7 (E.Coli), Salmonella Enteritidis, Listeria Monocytogenes, Biosensor, Food Poisoning, Pathogen.

ÖZ

ET ÜRÜNLERİNDE GIDA ZEHİRLENMELERİNE YOL AÇAN ZARARLI MİKROORGANİZMALARIN TESPİTİ AMACI İLE TAŞINABİLİR SİSTEMİN GELİŞTİRİLMESİ

DURMAZ, Çağatay
Yüksek Lisans, Elektronik ve Haberleşme Mühendisliği Anabilim Dalı
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Bu tez çalışması, T.C. Bilim, Sanayi ve Teknoloji Bakanlığı (Bakanlık)nın Sanayi Tezi (SANTEZ) Programı'nın bir parçasını oluşturmaktadır. Bu projeye Bakanlık tarafından 00973.STZ.2011-2 proje numarası verilmiştir. Bu tez çalışması çerçevesinde proje grubunun bir parçası olarak ben de gıda zehirlenmesini çok kısa bir sürede, doğru ve güvenilir bir şekilde saptayıp tanımlayabilen taşınabilir yeni bir cihazın bütün tasarım ve geliştirme faaliyetlerinde yer aldım. Araştırmayı yürüten Proje Grubu'nun içerisinde Proje'nin yönetiminden ve bütün teknik hususlarından sorumlu Proje Koordinatörü Prof. Dr. Taner ALTUNOK, elektronik tasarım ve yazılım ile ilgili IDC Şirketi'nden Engin KIRAN, yazılım geliştirme konusunda IDC Şirketi'nden Onur ÖÇALAN ve Melike AYTÜRK ve biyoloji uzmanı Gamze YAVAŞ ile Cansu ÖZDEMİR yer almaktaydı. Benim ise bu çalışmadaki esas sorumluluğum elektronik ve yazılım tasarımına destek sağlamaktı. Bu bağlamda ben

de gıda zehirlenmesine sebep olan bakterileri fotoğraf makinesi ile çekilen görüntülerden tespit etmek amacıyla kullanılan görüntü işleme yazılımı geliştirdim. Biyosensör Sistemi adını verdiğimiz bu cihaz, Proje Grubu tarafından başarılı bir şekilde tasarlandı, geliştirildi ve test edildi. Böylelikle, SANTEZ Projesi de aynı başarıyla Bakanlık tarafından test edildi ve kabul edildi. Ortaya çıkardığımız bu Biyosensör Sistemi'nin piyasadaki mevcut cihazlarla kıyaslandığında onlara göre gelişmiş ilave yetenekleri bulunmaktadır.

SANTEZ Projesi kapsamında geliştirmiş olduğumuz bu görüntü işleme yöntemi, içine daha önceden antijen verilmiş gıda örneklerinin yarattığı bir kimyasal tepkimenin içerisinde oluştuğu bir kuyunun görüntülerini çekmektedir. Test edilen gıda ile kuyunun içerisindeki antijen arasında kimyasal tepkime oluştuğunda, kimyasal ışıldama mekanizması ile bazı fotonlar yayılmaktadır. Kuyu imgesinin içerisindeki piksellerin ışın yayma yoğunlukları, bir elektron çoğaltıcı yük-bağlaşık aygıtı (EMCCD) kamerası ile ölçülmüştür. Geliştirdiğimiz yöntem öncelikle bir maskeyle kuyuların alanını tanımlanmakta ve daha sonra kuyu imgesi içerisindeki piksellere ait bütün yoğunluk değerlerinin ortalama yoğunluğunu almaktadır. Kuyuya ait ortalama yoğunluk bir sınırı aşarsa, zararlı mikroorganizmaların olduğu tespit edilmektedir. Eğer bu ortalama yoğunluk belirlenen bir sınır değeri aşarsa, zararlı organizmanın pozitif tespiti yapılmakta ve tanımlamasını belirten bir karar verilmektedir.

Anahtar kelimeler: Campylobacter Jejuni, Escherichia Coli O157:H7 (E.coli), Salmonellaenteritidis, Listeria Monocytogenes, Biyosensor, Gıda Zehirlenmesi, Patojen

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SPR	Surface Plasmon Resonance
PCR	Polymerase Chain Reaction
QCM	Quartz Crystal Microbalance
ELISA	Enzyme Linked Immuno Sorbent Assay
CCD	Charge Coupled Device
EMCCD	Electron Multiplying Charge Coupled Device
ECL	Enhanced Chemiluminescence
SNR	Signal to Noise Ratio
NM	Nanometer
HRP	Horse Radish Peroxides
GPS	Global Positioning System
DAQ	Data Acquisition
RF	Radio Frequency
UV	Ultra Violet
LED	Light Emitting Diode
RGB	Red Green Blue
sELISA	Sandwich Enzyme Linked Immuno Sorbent Assay
EM	Electron Multiplying
SBC	Singe Board Computer
ISM	Industrial, Scientific and Medical
LCD	Liquid Crystal Display
HW	Hardware
MMI	Men Machine Interface
I/O	Input/Output
RS-232	Recommended Standard 232

CHAPTER 1

INTRODUCTION

The design of the sensors developed for the identification of microorganisms which threaten human health and cause certain contagious diseases, food poisonings is highly important. Several physical and chemical changes occur in the structure, appearance, color, taste and smell of a rotten food. While on the one hand such changes make the food inedible and, therefore, cause economic losses, on the other hand they make the food dangerous and risky to health. Most of these changes resulting from food degradation occur due to the toxins produced by the bacteria, numerical increase in the bacteria, metabolic waste produced by increasing metabolic activity and biodegradation. Some bacteria can reproduce quickly into very high numbers when proper conditions of humidity, nutrition, temperature and time are present. The higher the number of bacteria, the higher the risk of infection and illness to the people. [1, 2]

Today, the increasing number of poisonings due to microorganism contamination in certain nutritional sources, makes the accurate identification of toxic and microbial contaminants as soon as possible a must. There are relevant reference laboratories in charge of identifying such contaminants; however the transportation of the sample to the laboratory (complying with the criteria of taking the sample under preserving sterility and conservation) is of great importance and very difficult in some cases. When sampling is not made in compliance with the test criteria, wrong test results are inevitably obtained due to microbiological or chemical contamination other than the target organism. Furthermore, rapid and early identification of such a case and taking necessary precautions as soon possible are crucial for reducing the number of people exposed to food poisoning. Biology-based identification systems are more advantageous compared to other systems in terms of their high sensitivity, practicality, quick response and being suitable to portable automation. For these

reasons, they are appropriate in the early and accurate identification of contaminants that cause food poisoning. Nowadays in the biomedical applications, where the automation and mechanization are constantly increasing, the effective and practical use of the tools and equipment is important. [3]

In order to enable the accurate and rapid identification of the microbial contaminants in the food sector, the validated method is to associate such bacteria to the common food poisoning in meat products as *Campylobacter jejuni*, *Escherichia coli O157:H7 (E.coli)*, *Salmonella enteritidis*, *Listeria monocytogenes*. [4] Another important criterion in identification of food poisoning is that the device that makes this identification should be portable and effective and reliable enough to make the detection reliably and quite rapidly. This device can also be moved to the location desired easily. In this thesis such a device is developed and a prototype is built. To design, develop and build a prototype of this device requires a multidisciplinary team. As a SANTEZ Project, such a team was formed with experts from the related areas of expertise such as mechanical design, electronic design and development, software design and development and biological and chemical analysis and applications.

Biosensors are those systems that analyze the biological parameters and turn them into proper electrical signals. [5] Biosensors are basically made up of three parts. As shown in Figure 1 they are; bioreceptors which can recognize relevant biological substances, transducers which turn the signals resulting from the interaction of bioreceptors with the biological substance into electrical signals, and electronic parts. [6]

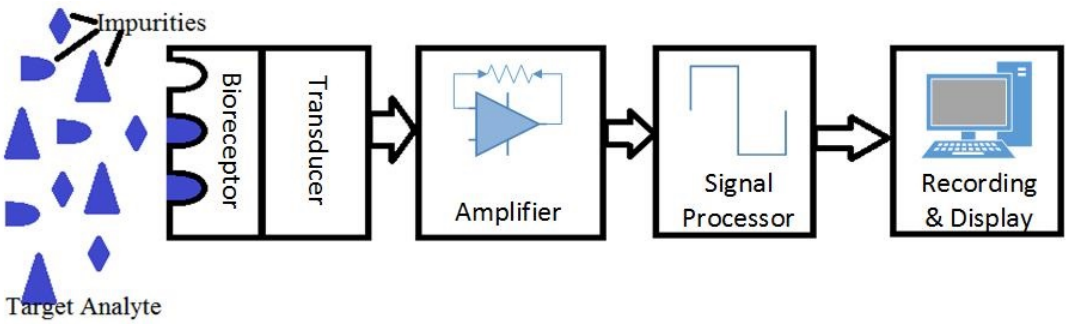


Figure 1: Biosensor System [7]

Sensors are systems whose features such as material properties (its ingredients, or its characteristic material property electrical conductivity) change due to physical alteration (pressure, heat, force, radiation, kinematic motions etc.). The electrical/electronic mechanisms which convert this alteration resulting from physical and environmental changes into a measurable signal are called transducers. [8, 9]

The transducers convert any environmental or physical alteration into an electrical signal in compliance with specified rules; however, during the conversion process, how accurate does the measured electrical signal convert the actual physical or environmental changes originating from either the sensor itself or the electrical/electronic set up of the transducer is dependent on certain criteria. The definitions of the concepts that determine the main characteristics of a transducer are given below. [10]

a. Sensivity: For example, the minimum change of temperature that a mercury thermometer can show in its scale (if its scale is between 0°C and 100°C) is the sensitivity of that thermometer.[10]

b. Resolution: In the above-given example of a mercury thermometer, the minimum measurable amount of change along the scale of the thermometer is resolution. For example, between 9°C and 10°C the minimum unit a naked eye can see is 1°C and it does not change between 80° and 81°C.[10]

c. Linearity: The condition when the rate of electrical change all along the measurable range of physical change in the transducer remains constant. For example, in the example given above, if the mercury in the thermometer increases by 1 mm as the temperature rises from 10 to 11, the 1 mm change should be the same when the temperatures rises from 50 to 51, 80 to 81 (the thermometer exemplified above is a sensor whereas a system which converts the heat measurement into electrical an electrical signal is a transducer).[10]

d. Hysteresis: It is the difference of the measurement with the direction of change (increase or decline) of a physical quantity measured by a transducer or the changes

in the electrical signals produced based upon the absolute value of a physical value. For example; while a thermometer shows a temperature increase from 40 to 50 with an 8 units difference in its scale, the same thermometer shows a decrease from 50 to 40 different than 8 units.[10]

e. Precision: It is important that a transducer converts any value of a physical quantity it measures to an electrical signal of same gratitude every time, throughout its life cycle. In other words, it is desirable for the repetition of a measurement that the same transducer measures a physical quantity at the same value every time; this is called repeatability. Likewise, if two or more transducers of the same model, from the same producer measure the same quantity at once at the same value, it indicates that the transducers were produced with the same characteristical values. In other words, it means if one transducer is broken, it can be replaced with a new one without any change in the measurement results. This is called reproducibility. A transducer that has these two characteristics is a measurement-sensitive, precise transducer.[10]

f. Accuracy: In the thermometer example given above, if we suppose that 9 different people measure a room temperature at 23°C with 3 different thermometers, the average of that 9 people may be 20°C. Likewise, 9 different people with 3 equal thermometers their average can be calculated as 22.5°C. When a preference is to be made between the two thermometers, measuring the actual 23° room temperatures 20° and 22,5°C respectively, the latter would be chosen for sure. In other words, the temperatures which measure actual 23° room temperature as 22.5°C are more reliable.[10]

g. Response Time: It is the time that is spent between the moment a transducer is exposed to an environmental effect until it converts the that effect it measures into a meaningful electrical signal. For example, if we suppose that two thermometers are brought into a room with a 23°C temperature; the first one that reaches to 23°C in the scale and remains steady has a response time shorter than the other. This is not to be confused with latency. While the concept of response time is the time between the moment when the sensor/transducer is exposed to the physical state it measures and the moment when the measurement is completed; latency always occurs when the

sensor/transducer shows the magnitude of the physical change it is exposed after a certain time.[10]

h. Digitization error: In today's technology many of the sensors/transducers produce the measurement results numerically. In such type of transducers, there are such interfaces as microcontrollers that convert the analog change in the sensor into a digital value, analog to digital converters (ADC) etc. During this conversion, some errors which do not originate from the sensor but the digitization process may affect the result. These errors are called digitization errors.[10]

i. Dynamic error: Especially the sensors which measures physical quantities such as heat and radiation tend to produce errors that originate from their own structures due to the nature of the quantities they measure. For example, the heat capacity of the mercury in a mercury thermometer is a factor that affects the measurement of that thermometer. It is inevitable that an ideal thermometer keeps the physical quantity it measure inside itself. Likewise in the optical sensors, the energies of the light photons that reach the sensor should somehow be removed from the environment after being detected by the sensor. For instance, the temporary blindness emerging after direct exposure to sun is caused by the dynamic error in the human eye. In other words, a sensor/transducer should keep measuring the physical quantity despite the sudden changes in the physical quantity along the scale. As in the sun example, the human eye which is suddenly exposed to too much sunlight is unsuccessful to do this. However, the new generation cameras are able to react to these sudden and drastic changes rapidly and continue measuring.[10]

j. Signal to Noise Ratio (SNR): It is the ratio of the signal that corresponds to the physical quantity measured by a sensor, to the noise signal that sensor generates.[10]

k. Quantum efficiency: The number of photons that corresponds to the minimum sensitivity measured by an optical sensor. In other words, it is the ratio of the minimum number of photons that an optical sensor can measure within the wavelength it operates, to the total number of photons received by the optical surface of the sensor.[10]

Biosensors are generally assessed in terms of 8 criteria [11,12]:

1- Sensitivity : Reaction of the biosensors to the material to be examined.

2- Selectivity : Operation of the biosensor oriented to the substance to be examined.

3- Range : Measurement range of the biosensor.

4- Resolving time : Test duration of the biosensor.

5- Accuracy : The accuracy in the test results of the biosensor.

6- Minimum Range : The minimum limit that a biosensor can detect.

7- Life : Life cycle of the biosensor.

8- Reliability : A measurement criterion that takes into account the changes in the operation of the biosensor.

The technologies utilized for diagnostics are classified as follows [13]:

- Antibody-based diagnostics,
- Aptamer-based diagnostics,
- Peptide Nucleic Acid (PNA) and Deoxyribo Nucleic Acid (DNA) based diagnostics,
- Molecular-based diagnostics,
- Surface Plasmon Resonance (SPR)-based diagnostics,
- Polymerase Chain Reaction (PCR)-based diagnostics.

The systems that convert the biological reaction into electrical signals are grouped under four main titles in the biosensor technologies [14]:

- Mass-sensitive converters,
- Thermal converters,
- Electro-chemical converters,
- Optical converters.

Emitting of light by substance which has absorbed light or other electromagnetic radiation is called fluorescence. Fluorescence is a form of luminescence that the emitted light of which has longer wavelength but shorter light energy.[15]

In some chemical reactions energy occurs. Result of these reactions energy forms are heat, light etc. Light based reactions are named as chemical luminescence, or chemiluminescence. [16]

According to the above-given converter types, when the Piezoelectric Quartz Crystal Microbalance (QCM) from the biosensor systems, SPR from the optical converters category, the chemical luminescence and fluorescence technologies are examined, it is observed that some of them are capable of identifying only one pathogen while some of them are not able to identify the desired number of pathogens at once. It is concluded that most of the devices that use these biosensors available on the use in the market are either too heavy or have large-sized that cannot be carried by one person alone, with very long detection and identification periods. [17, 18]

The Enzyme-Linked Immuno Sorbent Assay (ELISA) systems, which became common very rapidly and very popular in many countries, are investigated among these devices as well. For this reason, the scientific discipline that is used for the detection of harmful microorganisms in meat products is the sandwich ELISA. As can be seen in Figure 2, simply, the biological identification by sandwich ELISA method is realized as follows: the enzyme which is attached to the second antibody marked by the enzyme, reacts with the chemiluminescence substrates and generates photon, and then this photon is perceived by the relevant detector, thus, the microorganism detection is realized. It is obligatory for these devices, which investigate a mere biomolecular structure, to give rapid and reliable results. [17, 19]

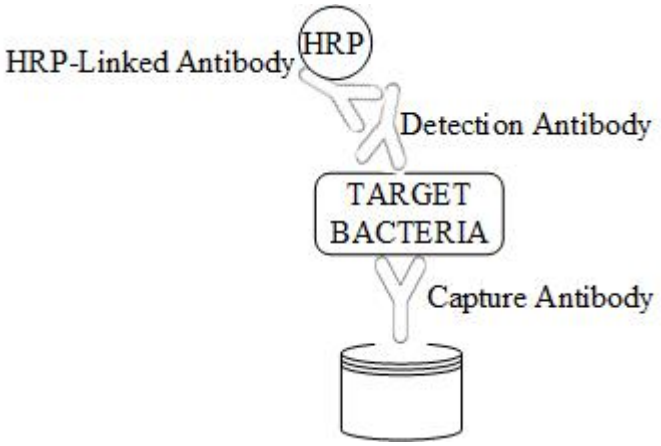


Figure 2: Sandwich ELISA method [20]

In the literature search, 36 devices that function according to the luminescence scientific discipline were found. Most of these devices are the systems using photomultiplier and a minority of them is the devices using a standard charge-coupled device (CCD) camera. Some of them using the Electron Magnifying Charge Coupled Device (EMCCD) camera technology are processing with tubes and another type with microplates. However, those systems are found to be designed for clinical applications, heavy and not possible to be carried by single personnel and without automation integration.[21]

Antibody-based detection technology is used in the system which is aimed to be obtained at the end of this research. The detection of the harmful microorganisms, which are few in number, is aimed to be carried out with the help of ELISA system and EMCCD camera technology. In Table 1 different types of ELISA methods and their advantages and disadvantages are listed.

Table 1: ELISA types, advantages and disadvantages [22]

FLUORESCENCE SANDWICH ELISA	LUMINESCENCE SANDWICH ELISA	COLORIMETRIC SANDWICH ELISA
DISADVANTAGES : • Fluorescence radiation strength is low. • Duration of radiation is short (≈10 ms). • Fluorescence reaction triggering radiation is required. ADVANTAGES : • Technologically common. • Relatively cheaper.	DISADVANTAGES : • Limited in common use. • Requires automation. • Relatively more expensive. ADVANTAGES : • Luminescence radiation strength is high. • Luminescence radiation duration is long (≈120ms). • Triggering	DISADVANTAGES : • Repeatability of the test is limited. • Duration of test is long. • Requires reaction inhibition process. • Low sensitivity. ADVANTAGES : • Technologically common. • Relatively cheaper.

	radiation is not necessary. • High sensitivity (105 times higher than the fluorescence.)	

As a result, among the methods that are listed in Table 1, Luminescence Sandwich ELISA (sELISA) is selected to be used for our enhanced biosensor system. Besides, it is measured by a radiation cooled EMCCD camera using Enhanced Chemiluminescence (ECL) reaction. The main reason for using cooled camera is to reduce SNR (Signal to Noise Ratio). The quantum efficiency of the camera used changes between 65% and 90% depending on the wavelengths of radiation generated during the process. The wavelength of the radiation generated from the chemiluminescence mechanism is about 428 nm. The ECL used in the sELISA process is a thousand times or more sensitive than the standard chromatic emission assay or fluorescence assay methods. During the conducted tests, p-idefenol (p-IP), which joins the reaction as a catalyst and enables the control of the reaction velocity, was added to the process and, thus, reaction was optimized and the efficiency was increased ten folds in comparison to the ordinary chromatic assay processes, reaching a total of approximately 10.000. It also means that the method optimized within the context of this research, produces ten-thousand times more photons compared to the standard fluorescence assay systems and its reaction period is five-thousand times longer. This comparison is shown in Fig. 3.

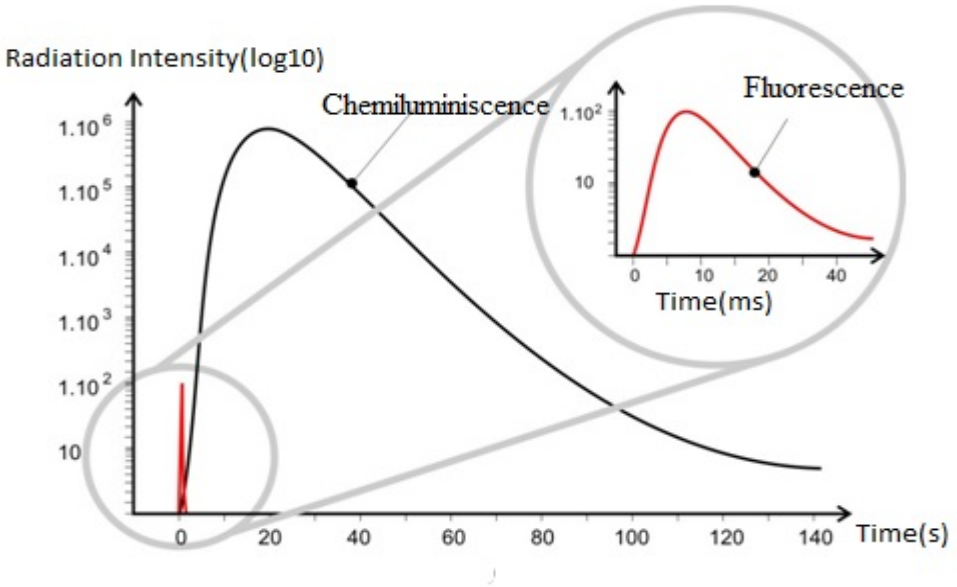


Figure 3: Comparison of the radiant intensities generated by Enhanced Chemiluminiscence (ECL) process and standard fluorescence assay method. The difference is obvious considering that in both graphics the area under the curves reflect the numbers of photons produced by the reactions.[21]

As a result of the literature search explained above, it is concluded that the most reliable technology, the very subject of this study, with a portable weight and dimensions, lowest power consumption, shortest identification period and able to give the most sensitive possible and repeatable results would be a combination of the above-mentioned scientific discipline, technology and methods. In this context, it is concluded that, the use of antibodies, which are the smallest and lightest sensors that exist in the nature as biosensor, would be appropriate.

By enabling the simultaneous measurement of many reaction wells by the EMCCD camera, we would be able to reduce the test duration as much as possible.

In this project, the perception system, called a Biosensor System is developed, which perceives the pathogens, working with the electrochemical luminescence (ECL) sandwich ELISA (sELISA) system method. The Biosensor System developed, detects and identifies the obtained radiant intensity by using a light-sensitive Electron Multiplying Charge Coupled Device (EMCCD). The system detects the presence of harmful pathogens, such as harmful microorganisms in the meat products, and

identifies their types. It is a portable system that can be carried by a person to the field and the results of its detections and identifications are reliable.

The developed Biosensor System contains an automation system which carries out some of the activities during the sELISA process. This automation system automatically carries out the sample transfer, well washing, enzyme and buffer transfer steps of the sELISA process, and the luminescence radiation obtained at the end of the process is recorded by an EMCCD camera and the result is obtained after its analysis by an embedded software developed.

The system designed and developed in this project is able to detect and identify the harmful microorganisms simultaneously in many reaction wells. The device possesses the capability of measuring of more than one pathogens, more sensitive, more time-saving, less power consuming, and lighter compared to the other systems available in the market.

As a researcher in the project group, my responsibility was to develop the image processing in the Data Processing Software Module (DATPROG). The image processing software module of the Biosensor System is responsible for the calculation of the Luminescence radiant level by processing the images received from the EMCCD camera driver. This software module runs on the Single Board Computer (SBC). This embedded analysis software evaluates the gray intensity levels in the wells (we also call them pits) on the image taken by the EMCCD camera, and makes the detection and declaration whether in any of the particular well on the image contains harmful microorganisms. This image processing software is defined in detail in Chapter 5.

CHAPTER 2

METHOD

In this chapter we provide a literature review about the methods to detect and identify the harmful microorganisms. We provide information about the multi-well calorimetric assay technique. We introduce the innovative aspects of the Biosensor System developed in comparison with the existing systems on the market. We also explain some design features that make these innovations a reality.

2.1. ELISA Process and General Analysis Algorithm

2.1.1. Single-Well Assay Method

Single Well Assay Method is one of the methods commonly used in medical application centers in Turkey and abroad. It is a method which is generally used when the searched agent is a single one; however it is a method with high levels of time and money consumption in the applications when more than one agent is searched. In the single well assay premises built by the immobilized monoclonal antibody-specific plates technique, the measurement sensitivity is increased generally by using more than one similar references on a single plate. Yet the fluorescence and absorbance assays mechanisms used in the single well assay method are also composed of photomultiplier optical sensor systems designed for single well assays. Those systems, which carry out single assays for each well despite their high photon sensitivity, make only one measurement of the glow luminescence value per well. [23]

In the chemiluminiscence systems with an average glow luminescence period of 180 seconds, as much photons as possible should be counted in order to reach an accurate and sensitive result. This way, it is possible to detect all the components involved in the reaction. In the single well assays, waiting for this amount of time per agent

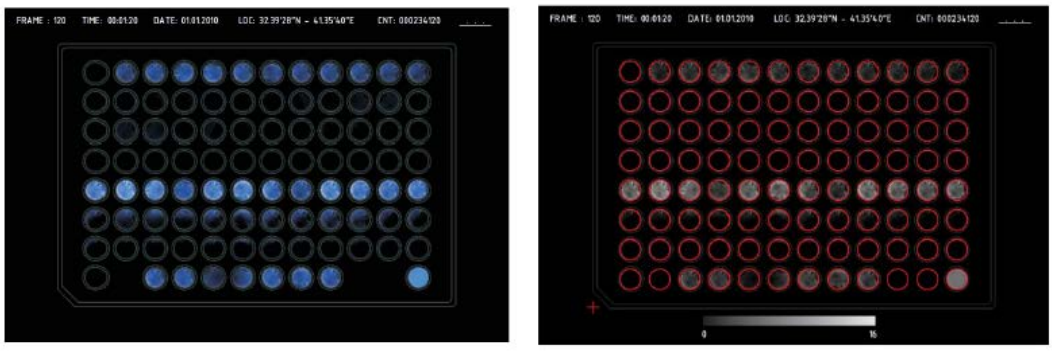
renders the completion of the test of a sample within the period designated in the requirements impossible, and the test period would be much longer. Therefore, single well assay method is not applied in this thesis.

2.1.2. Multi-well Colorimetric Assay

The high-sensitive CCD technology developed particularly for use in astronomy, has the ability to detect the photons with a quantum efficiency reaching up to 80%. With an EMCCD camera, the sensitivity of detection of photons can be improved up to a thousand times without a photomultiplier. In this technology, along with sensor cooling applications, background noise and colorimetric noise problems are also minimized. Being cooled down to -20°C, the developed micro lens CCD sensor is able to detect photons sensitively at the required wavelength. With the electron magnifying added to the system, the desired amount of gains are attained without increasing the background noise.

Therefore we decided that the biosensor device to be developed would include a camera having the EMCCD technology, also using the high-sensitive photos of the Polistren plates, on which the monoclonal antibody could be fixed before. For each agent required, sample tests are realized by using the polistren plates on which the designated wells are engraved. This way it is possible to simultaneously test each sample with the particular agent required.[24]

Photographs (images) of the prepared sample were taken throughout a posing period which is specified during the chemiluminescence process with the EMCCD camera. In the images certain periods are used. Having these properties, the biosensor system to be developed would be superior to the similar systems. Many similar systems obtain the results either by using lower-sensitivity sensors or by cumulative intensity calculation method during all the posing process or by making a single measurement at a moment “t” when the fluorescence or chemiluminescence intensity is at its maximum. In Fig. 4, we provided the images generated through the data processing procedure based on standard ELISA plates.



(a) **(b)**

Figure 4: a) The MicroDisc image that was obtained from the biosensor device developed demonstrates the EMCCD camera image and label. **b)** Image masks corresponding to each well make it possible to determine pixel intensity values and estimate average intensity values for each well.

2.2. Innovative Design Features

We aimed to make the innovations listed below in developing the Biosensor Device:

- To achieve the detection and identification of one or all agents by MicroDisc and chemiluminescence methods in a single test procedure.
- By using the cooled EMCCD camera, to attain a better signal to noise ratios (SNRs) than the other devices available,
- Using multi-well Colorimetric Assay Method to increase the sensitivity.
- To provide data analysis and processing capability, and to better make use of commonality of the software available on the market, using a capable on board computer.

2.3. Biosensor System Design

We decided to use an EMCCD camera in the design of the biosensor device. The biotechnology, chemiluminescence radiation is perceived by the monoclonal antibody method and the solution is reached through the pre-reference and calibration data.

The computer-aided design (CAD) illustration of the biosensor device's design is given in Figs. 5 and 6.

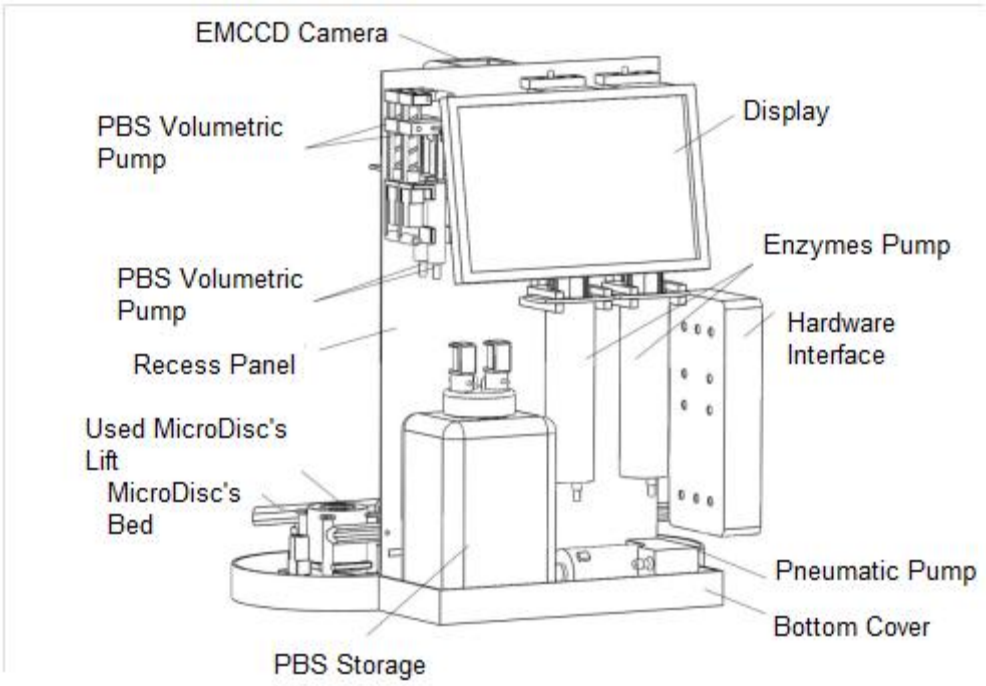


Figure 5: CAD illustration of the Biosensor System

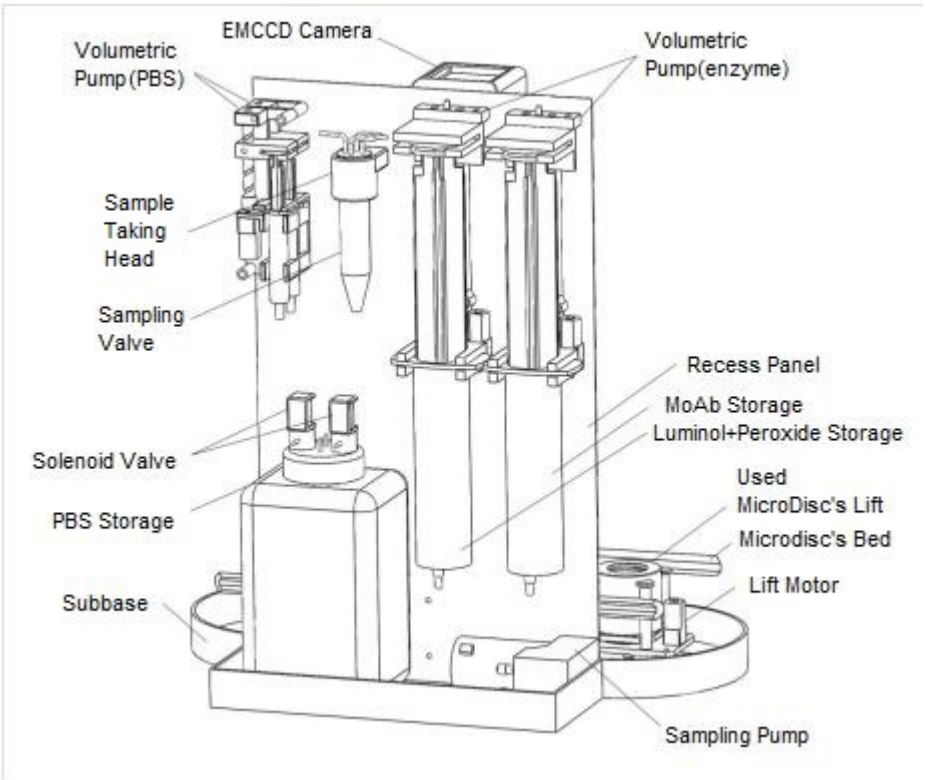


Figure 6: Another view from the Biosensor System's CAD illustration

2.4. The Desıgn of the Sample Collectıon System

The sample collection system in Fig. 7 is conjoint with the washing system and is designed in a multi-tasking way to carry out both functions. The essential reason behind this design is that it can decontaminate itself in long-term use. The PBS buffer storage, which is used in both sample collection and washing phases in the system, is also used for the washing of all liquid transfer units constituting the system.

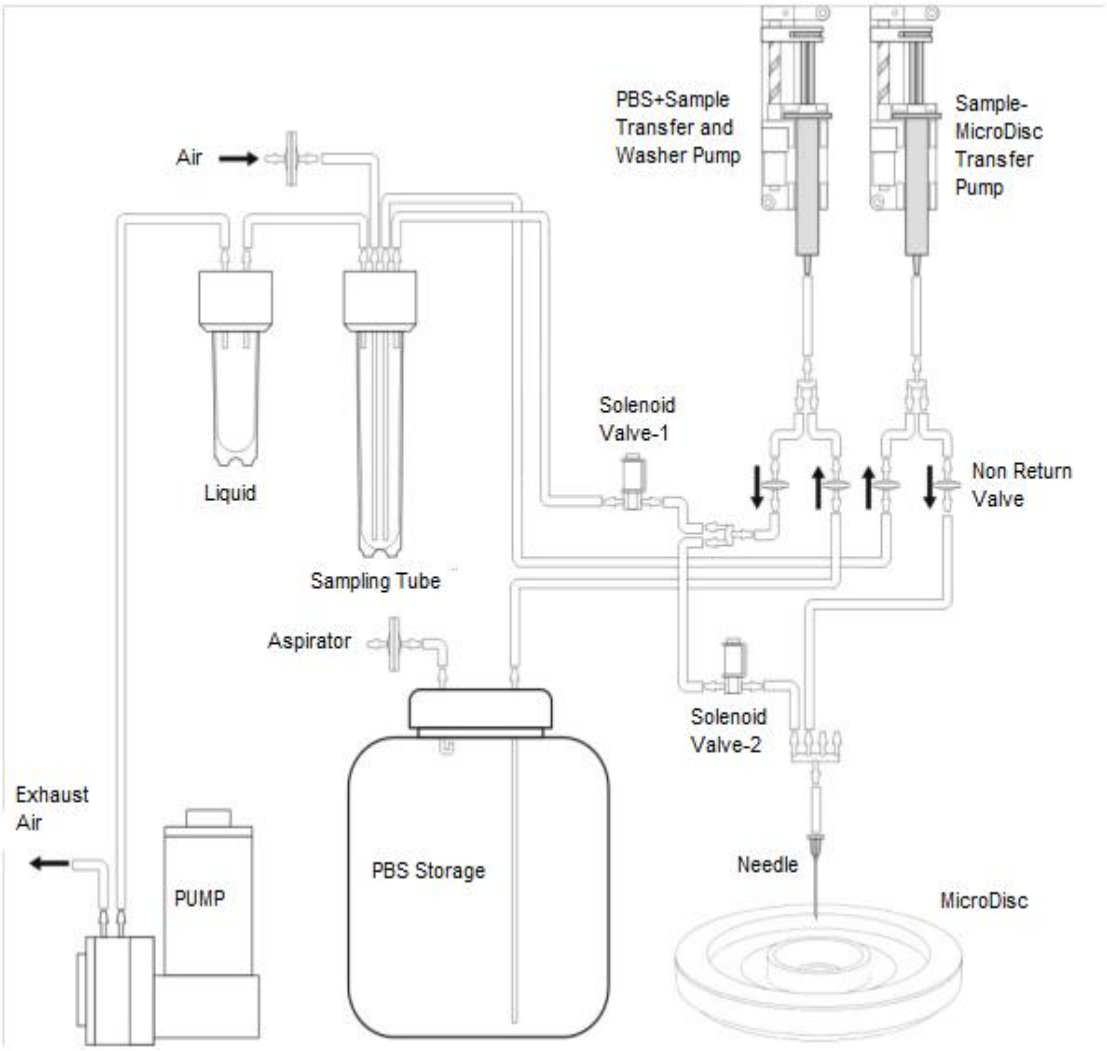


Figure 7: Automatic washing system design scheme

In the design of the “sample transfer and washing” unit illustrated in Fig.7, standard products, that can be changed or auto-claved, when necessary, to enable decontamination, which is a monitored and important criterion in the design are used.

This way, decontamination and the prevention of samples' melding into each other in consecutive use is aimed.

All the selected products (timber pipe, elbow pipe, volumetric syringe, sample collection vial, one way valve, sample transfer syringe, etc.) are autoclavable and made of transparent material through which UV light can permeate. Thus, in consecutive tests, the system cleanses on its own the parts that carry the risk of bacterial contamination. Furthermore, by adding an UV light source (an UV LED) near the transparent timber pipe, one way valve etc. (to the endolymph transfer system part), and passing the light through the collected sample, each of these transparent units will be subjected to an UV light sterilization procedure after each test.

2.5. Control System Hardware Units

The device developed in this thesis will mainly be based on a Single Board Computer (SBC). The SBC generally has the interrelated subunits of electronic, mechanical, pneumatic and hydraulic control. The control and automation hardware of the Biosensor System as well as the data processing hardware are specified.

In the system, there is a Single Board Computer (SBC), which controls the main procedures, internal communication, user interface and information exchange with the outer environment, and a Data Acquisition (DAQ) system linked to it. All the control or drive of the mechanical actuators or the environmental measurement sensors such as position, temperature will be realized by the DAQ hardware and processed by the SBC.

Using an SBC provides a commonality with the existing software programs and tools. This way we can use the commercial tools and programs available on the market and also we can guarantee the updating and maintenance of all the software that will be used in the Biosensor System.

2.6. Registry Structure

They are the driver interface software of all the peripheral hardware used in the Biosensor System, as connected to the SBC. All of the driver software modules run on the SBC. These driver software are given below. Driver software will be provided together with the hardware that will be used as finished product as well, and communication protocol will be prepared between some units.

- EMCCD Camera: The driver software of the EMCCD camera.
- ISM Modem: The driver software of the Industrial, Scientific and Medical Modem (ISM) (900 MHz and 2.4 GHz).
- SCREEN: The driver software of the Liquid Crystal Display (LCD) used in the system.
- Touch Board: The driver software of the touch board where the user makes all the controls in the system.
- GPS: The driver software of the GPS.
- DAQ: The software part of the DAQ used in the system, which runs on the SBC. This unit was also realized within the scope of the Project. An as example is given below regarding the driver interface between DAQ and SBC. 32-bit in total is taken as the basis of the example protocol register structure.

Table 2: The communication structure between the SBC and DAQ is exemplified.

	Start Bit	R/W	Process ID								Command				Result				Error	Reserve								CRC				Stop Bit
Bit	1	1	8								4				4				1	8								4				1
Value	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

The system developed records or sends the test results by using the result format as given below in Table 3.

Table 3: Test Result Format

SN	DATA	VALUE	RULE
01	TEST NO	00..99	2N
02	MALFUNCTION CODE	00..09	2N
03	BATTERY	00..99	2N
04	DATE	GGAAYY	6N
05	HOUR	SSDD	4N
06	LATITUDE	DDMMSS(K/G)	8AN
07	LONGITUDE	DDMMSS(D/B)	9AN
08	ALTITUDE	0000…9999	4N
09	INTERNAL TEMPERATURE (IT)	000…100	3N
10	AMBIENT TEMPERATURE (AT)	000…100	3N
11	Escherichia coli O157:H7	000-101…199	3N
12	Salmonella enteritidis	000-101…199	3N
13	Listeria monocytogenes	000-101…199	3N
14	Campylobacter jejuni	000-101…199	3N
15	CHECK SUM		4N

N:Numeric;

A:Alphanumeric

DATE: DDMMYY – Day:Month:Year

HOUR: HHMM – Hour:Minute

LATITUDE: DDMMSS(N/S) – Degree:Minute:Second:North/South

LONGITUDE: DDMMSS(E/W) - Degree:Minute:Second:East/West

ALTITUDE: 4 digits in terms of meters

INTERNAL TEMPERATURE (IT): 3 digits in terms of ºC

AMBIENT TEMPERATURE (AT):3 digits in terms of ºC

CHECKSUM: 4N – This value is the sum of all 4 BVALUES (Bacteria values). Theoretically, this value may change between all the BHM's giving negative results (000) or giving positive and 99 ACPLA value (2587).

MALFUNCTION CODE: 9 different codes are possible. If the malfunction code is 00 there is no malfunction. In other cases, the result may not be evaluated depending

on the malfunction code.

Malfunction codes are listed below:

00: No malfunction.

01: Ambient temperature is out of range (AT< +4^{o} C or AT>+35^{o} C).

02: IT cannot be balanced.

03: Not enough memory.

04: Hardware Driver error.

05: Automation error.

06: GPS data cannot be obtained.

07: IT or AT cannot be measured.

08: Not enough consumables.

09: CHECKSUM error.

The device either keeps the tests with this format or sends them to the center through wireless modem. The data package is composed of consecutive characters and configured by the sort-out with the protocol given above.

Table 4: A Sample Data Package

SN	**DATA**	**VALUE**	**EXPLANATION**
01	TEST NO	04	TEST NO:4
02	MALFUNCTION CODE	00	Malfunction Code: 00 (No Malfunction)
03	BATTERY	95	Battery Charge Level (%)
04	DATE	120413	12 April 2013
05	HOUR	1742	Hour 17:42
06	LATITUDE	3993472K	39 Degrees 93’47.2” North
07	LONGITUDE	03251350D	32 Degrees 51’35” East
08	ALTITUDE	0942	942 Meters
09	INTERNAL TEMPERATURE (IT)	35	35 ºC
10	AMBIENT TEMPERATURE	29	29 ºC

	(AT)		
11	Escherichia coli O157:H7	185	Positive (1) – 85 ACPLA
12	Salmonella enteritidis	000	Negative
13	Listeria monocytogenes	000	Negative
14	Campylobacter jejuni	000	Negative
15	CHECKSUM		0185
The sample data package will be conveyed as follows. …/040095120413174239934 72K03251350D0942352918500000000000185/…			

CHAPTER 3

BIOSENSOR SYSTEM'S COMPONENTS

In this chapter, we describe the subsystems of the Biosensor System. We provide some details about the EMCCD camera and the reasons why we have chosen this camera.

3.1. The EMCCD Camera

Cameras with CCD-sensors are produced with the objective of capturing images in dim light. A surface is made of the light-sensitive diodes lined up on it. These diodes convert the light falling upon them into electric signals. The greater the intensity of light, i.e. the number of photons, the greater the charge accumulated, and the voltage generated by the respective diode. While a normal CCD camera reads the image, there is always a noise generated with or interfering with the signal. Therefore, the optical image information formed by the data is deteriorated with this noise. As seen in Fig.8, there are two registers in the EMCCD sensor : a shift register, and a data register. The shift register is integrated to the data register.

In the CCD when a light falls on a cell, which is a tiny semiconductor diode, its photons generate electrons and holes if the frequencies of the photons are good enough to give the semiconductor sufficient energy to generate the electron-hole pairs. Then the electrons generated are accumulated in the depletion region capacitance of the diode. The higher the number of electrons, the higher the voltage level appearing across this capacitance. Some other mechanisms such as generation of electron-hole pairs due to thermal effects, or photons generating heat in the semiconductor instead of generating electron-hole pairs, or losing some of the generated electron-hole pairs before they are accumulated in the capacitance can cause noise, which is superimposed on the voltage, the signal that is due to the effect

of the light. The noise superimposed on the signal that deteriorates the data (the signal) produced by the optical data in a normal CCD will remain the same, because both the signal and noise will constitute the composite signal. In an EMCCD the signal is multiplied by using techniques to eliminate the common noise. This way, the EMCCD sensors can operate in very dimly lit conditions. A photograph of the EMCCD camera used is given in Fig. 9. The datasheet of the product used is given in [25].

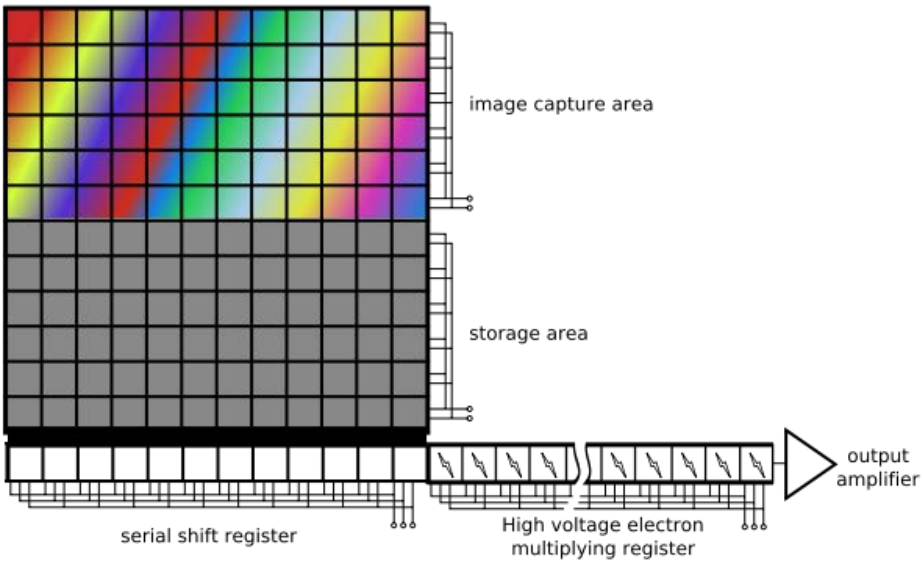


Figure 8: Working principle of the EMCCD camera [26]

Figure 9: The EMCCD camera used in the Project [27]

3.2. The DAQ System

Embedded automation software modules are embedded software modules that run on the DAQ (data acquisition) system. The DAQ's embedded software modules provide functionalities such as, controlling of all the motor drivers, driving the Peltier temperature controller, and reading the sensors and built-in testing of the system. Embedded software modules are described as:

- Hardware driver: Operates all the motor drivers and Peltier temperature controller.
- Sensors: Responsible for the reading of all the temperature and position sensors in the automation system.
- Solenoids: Responsible for the operation of the solenoids which control all the liquid flow.
- Control: In the manual or automatic test modules of the Biosensor System, they are responsible for the time-dependent proceeding of the process series that should be used in built-in test or calibration procedures.
- Settings: Responsible for the update of the settings updated or changed by the users via the SBC.
- Update: Responsible for the alteration and the first installation of the automation processes via the SBC.
- Built-In Test (BIT) : When the biosensor device is initiated or when requested by the user, it is responsible for testing the electro mechanics of the entire system.

The DAQ used in the system is obtained as a ready-to-use product. Its datasheet is provided in [28].

3.3. The GPS System

It is composed of the electronic hardware and software units that calculate the global coordinates where the antenna is located. The GPS is obtained a ready-to-use product in this Project. Its datasheet can be found in [29].

3.4. The ISM Modem

The ISM is a modem that uses the full duplex RS232 interface with the frequency at 900 MHz at the speed at 9600 baud. The modems used in the system are designed in such a way to enable the communication between two identical modems by making the tunings with the keys on the hardware. Modems are obtained as ready-to-use products. Their datasheets are given in [30].

3.5. The RS232 Serial Communication System

It is the serial communication standard used to transfer the digital data to the short-ranged units. The objective of using the RS-232 communication interface in this thesis is to enable the communication of the system components of the RF modem, the GPS and the DAQ with the single chip CPU in the onboard computer. Standard details are given in [31].

CHAPTER 4

THE INTEGRATED SYSTEM

In this chapter we give the information about the hardware and software modules of the Biosensor System. We describe how these modules are communicating with one another and how they are integrated.

4.1 Integrated Hardware

The electronic hardware structure in Fig. 10 is composed of the integration of the three main units. First of these is the CPU on which software modules such as analysis, and image processing run. The other one is the DAQ unit that implements a pre-determined specific automation process in accordance with the instructions given by the CPU. These software modules are embedded software, on which the automation processes are coded, responsible for the execution of electromechanical commands and for the inspection whether those commands are executed or not. The third unit is the HMI (Human Machine Interface) which enables the connection between the whole system and the humans (users) and is consisted of units such as communication and screen.

When the system is started it completes the starting processes of self-check, heat adjustment, obtaining location information, obtaining information pertaining to the consumable materials, and later the system moves from the HMI to command prompt. With the command received, first the command of the EMCCD camera is given. The camera is prepared for test. Later, the SBC gives the DAQ the command of cleaning the liquid transfer channels. With the command given by SBC to DAQ, the process algorithm, embedded into the SBC, carries all the process flows necessary for the realization of the relevant command. During this process SBC does not operate, but waits for the delivery of the information whether the process is completed or not. In this process, DAQ gives the command for the volumetric pump

to take in the necessary amount of liquid to realize the cleaning procedure and realize the procedure for the solenoid valves to open and close in the appropriate order. While the DAQ implements all these automation commands, it inspects the feedbacks received from the position sensor integrated into the system. The SBC informs the DAQ when the command it gave DAQ for the cleaning is completed.

The SBC which receives the information on the cleaning procedure delivers the next command of transferring a non-used microdisc to the camera optical axis to the DAQ. The DAQ accomplishes the command of transferring the microdisc and gives the information to the SBC that the command is accomplished. After such consecutive commands between the SBC and the DAQ are carried out appropriately to the procedure, the SBC gives the command to the previously prepared EMCCD camera to take the shoot necessary for the analysis and later puts the image through the image processing and analysis software procedures which are running by themselves. In addition to the test results obtained at the end of the analysis procedure, the analysis software assigns also labels such as location, time, date etc. to the relevant result. These test results are sent via the HMI to the screen and the modem and the test procedure are completed. In Fig. 11, the flow chart is given and the route to initiate the system is specified. In .Fig. 12, the 3D solid model of the design is given.

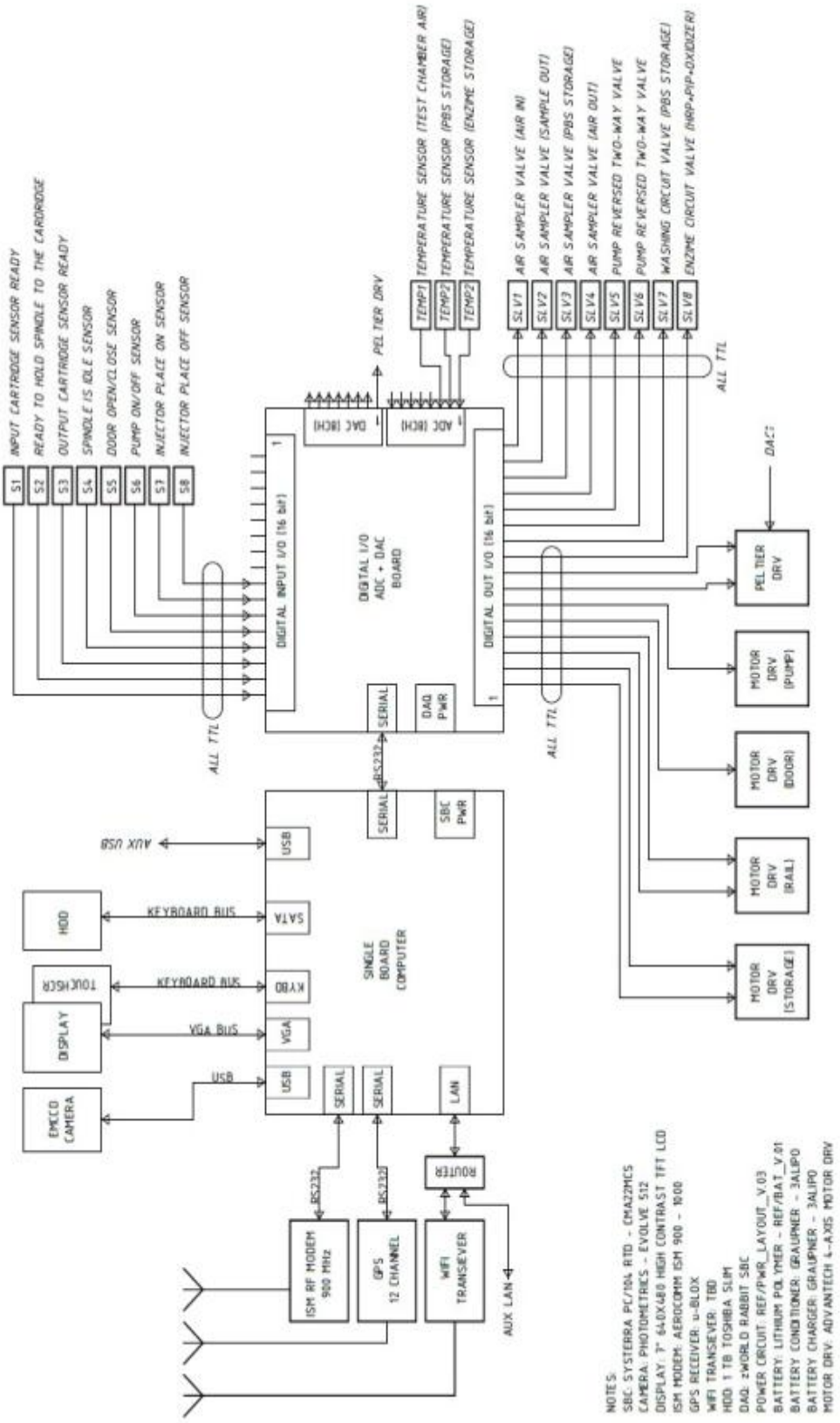


Figure 10: Hardware and connections of the Biosensor System

Start the test.

PASSWORD: User password or confirmation maintenance user control.

SELFTEST: DAQ, SBC, Battery, Temperature etc. hardware units will be tested. If there is a fault, it is displayed or warning LED panel lighting.

TEMPERATURE CHECK: Testing the medium temperature between the -4 and +35 °C. (Until the proper temperature device will estrange or warm.)

TAKING THE SAMPLE: Checking the sample door is truly closed.

PROCESSING THE SAMPLE: Automatically taken sample has been passed chemiluminescence steps.

MEASURING THE SAMPLE: The emission of light is captured by EMCCD camera from the processing sample.

ANALYSE: The capture which has been taken by EMCCD camera will apply image processing and calculate the result.

RESULT: Result are shown in display.

If there is not any other test won't be done, go to finish.

Ending the test.

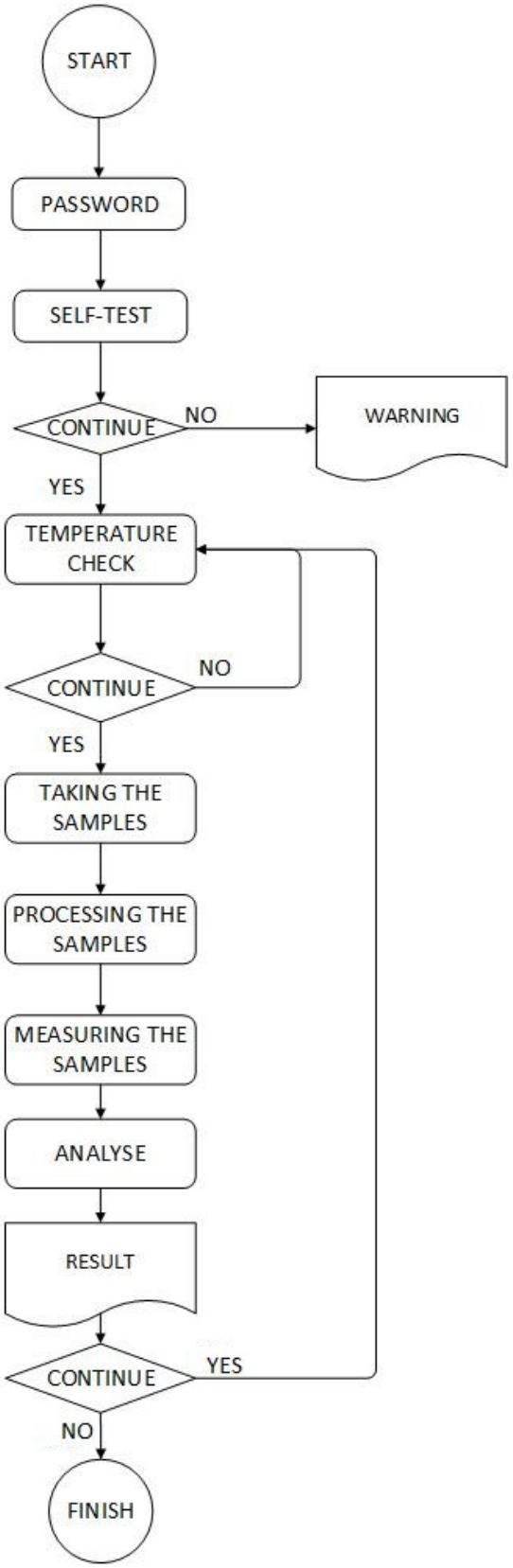


Figure 11: General algorithm structure

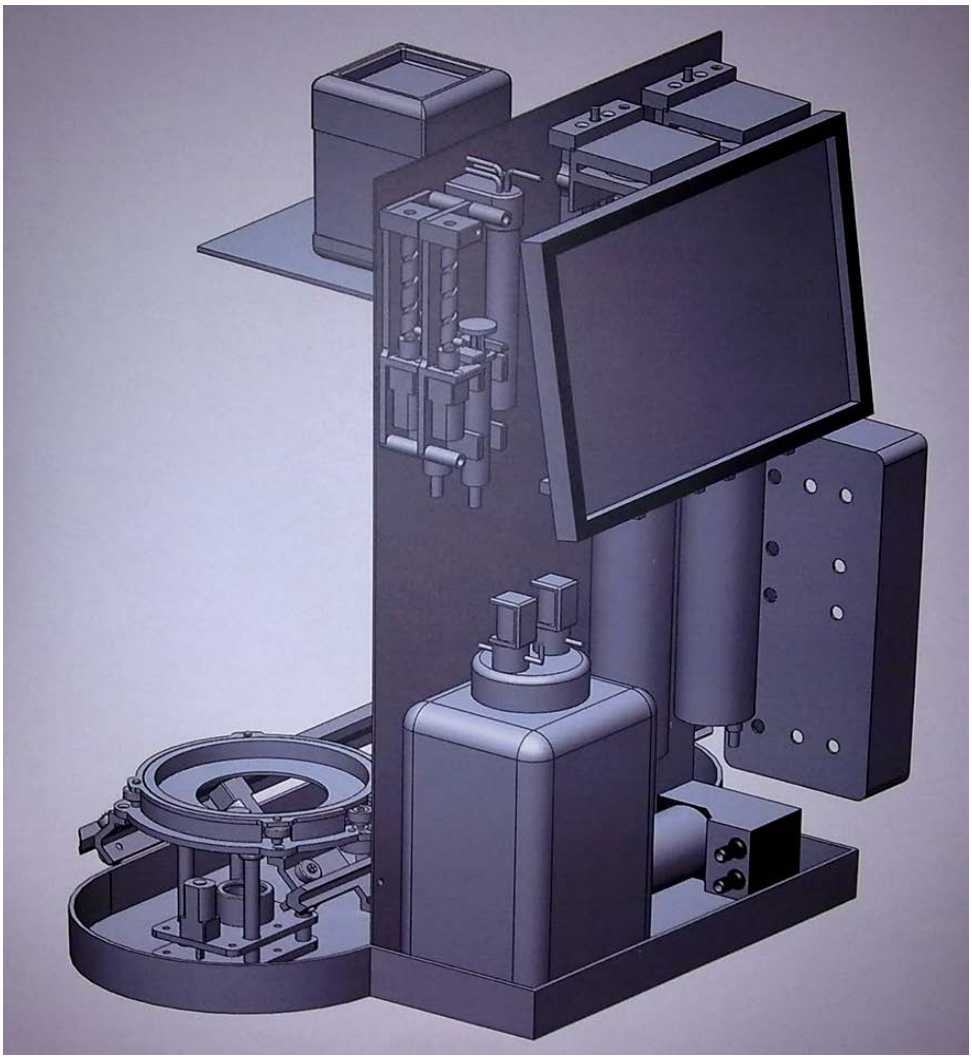


Figure 12: Solid model of the Biosensor System

4.2. The GUI / MVIS / COMM software modules

Hardware interfaces of the control system are designated in Fig.11. While the systems as prototypes are tested one by one, systems are established and their characteristics as power, I/O, control, memory, antenna and protocol are tested. The system components' software interfaces are tested and protocol tests are conducted. Some of the external hardware interfaces of the device and the required connectors, antennas etc. are procured. The DAQ that is used in the control system has the capacity to run all the servo motor, sensors or actuators, except for the peltier unit, without a separate driver. The features of the control software modules constituting the system are given below.

4.2.1. The Biosensor System Software :

The Biosensor System software, excluding the operating system, are designed with five main groups. They are composed of embedded software modules, some of which are running on the SBC and the other on the DAQ hardware.

4.2.2. The Data Processing Software Module (DATPROG):

The DATPROG software module of the Biosensor System is responsible for the processes given below. This software module is responsible for the calculation of the Luminescence radiant level by processing the images received from the EMCCD camera driver. This software module runs on the Single Board Computer (SBC). I developed the image processing software module (MVIS) as described in the next chapter in detail.

The features and functions of each software module are listed below in detail:

- MVIS: Image processing software module.
- MATH: The software module where the mathematical processes are realized.
- STAT: The software module where the statistical processes are realized.
- Calibration (KLIB): The software module where the test calibration processes are realized and the calibration tables are located.

4.2.3. User Interface Software Module (Graphical User Interface, GUI):

The GUI is responsible for the user's making the controls of the Biosensor System, displaying of the produced results to the user, making the settings of the Biosensor System, keeping retroactive database. This software module consists of the sub-modules given below. The user interface module will run on the SBC. It is shown in Fig. 13. Setting display is given in Fig.16.

- Start: Built-in testing of the system is carried out and system starts.
- Password is required.
- Main menu is started.

- Settings: It is the module which enables that all the automation and process details of the biosensor device are carried out by the user. It is shown in Fig. 16.
    - Test: Settings of the test processes are made.
    - EMCCD: Settings of the EMCCD camera are made.
    - Data Processing: Analysis criteria settings are made.
    - System: Driver settings for the system environment hardware are made.
- Information Display: System, environmental conditions, location, time details are displayed.
- History: Views the saved history, given in Fig. 14.
- Test: Test start command screen, given in Fig. 15.
    - Automatic Test
    - Manual Test
- Exit: Goes back to password screen.
- Shut down: Shuts down the system.

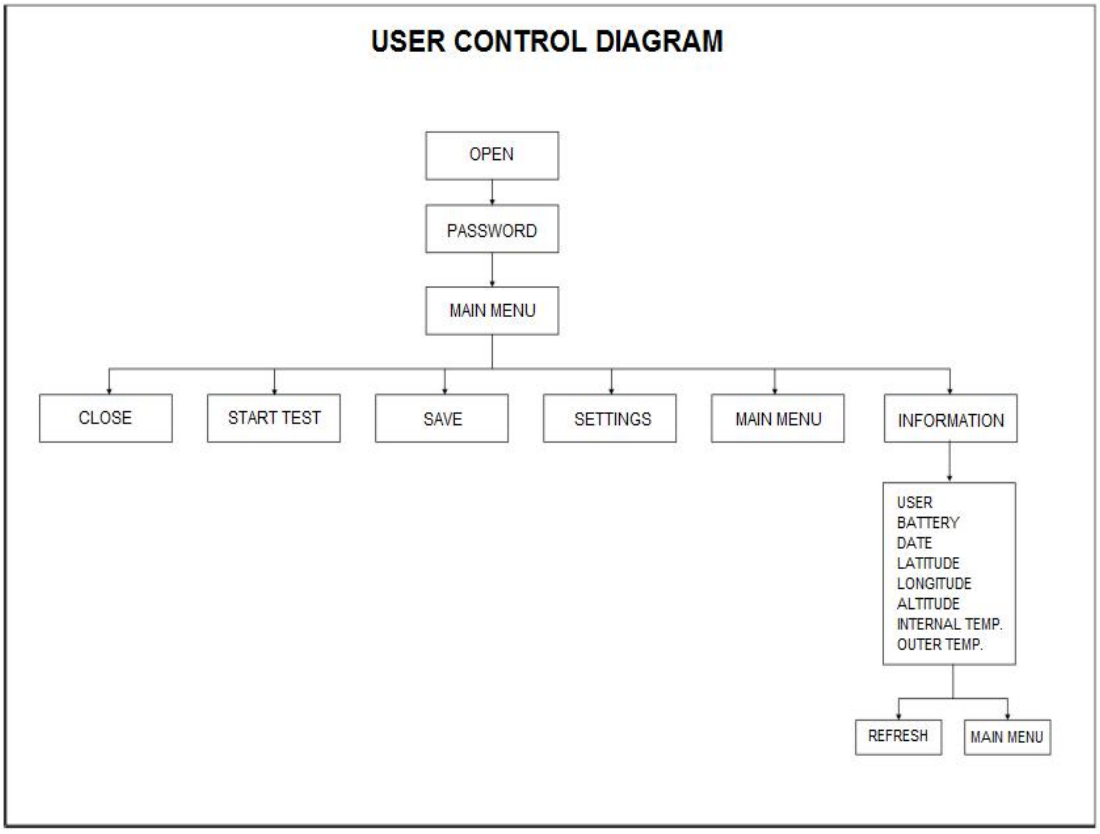


Figure 13: User interface control diagram

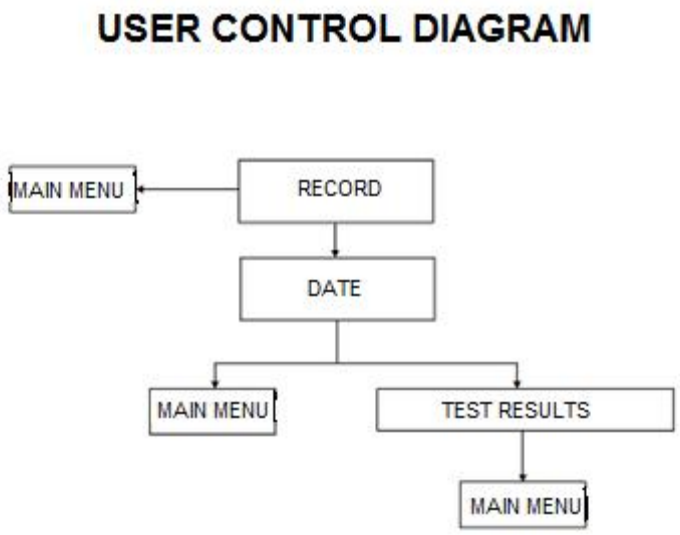


Figure 14: User history display

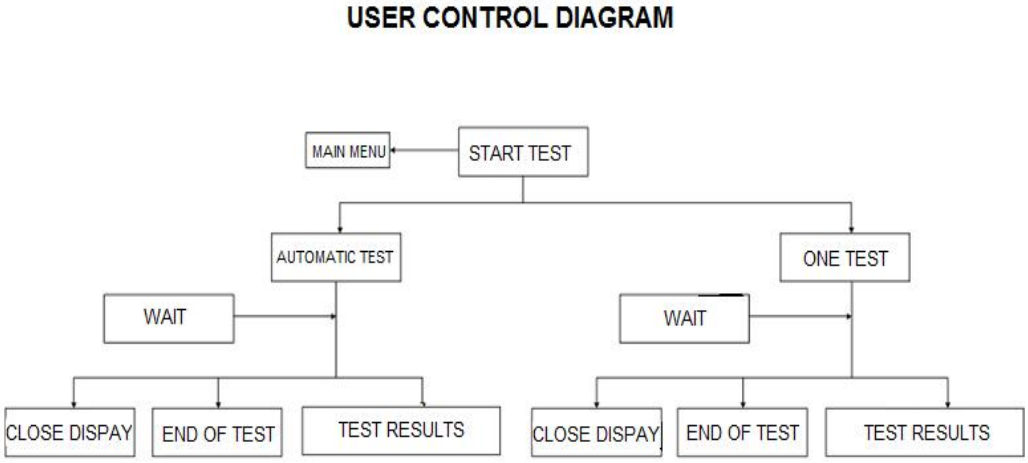


Figure 15: User test display

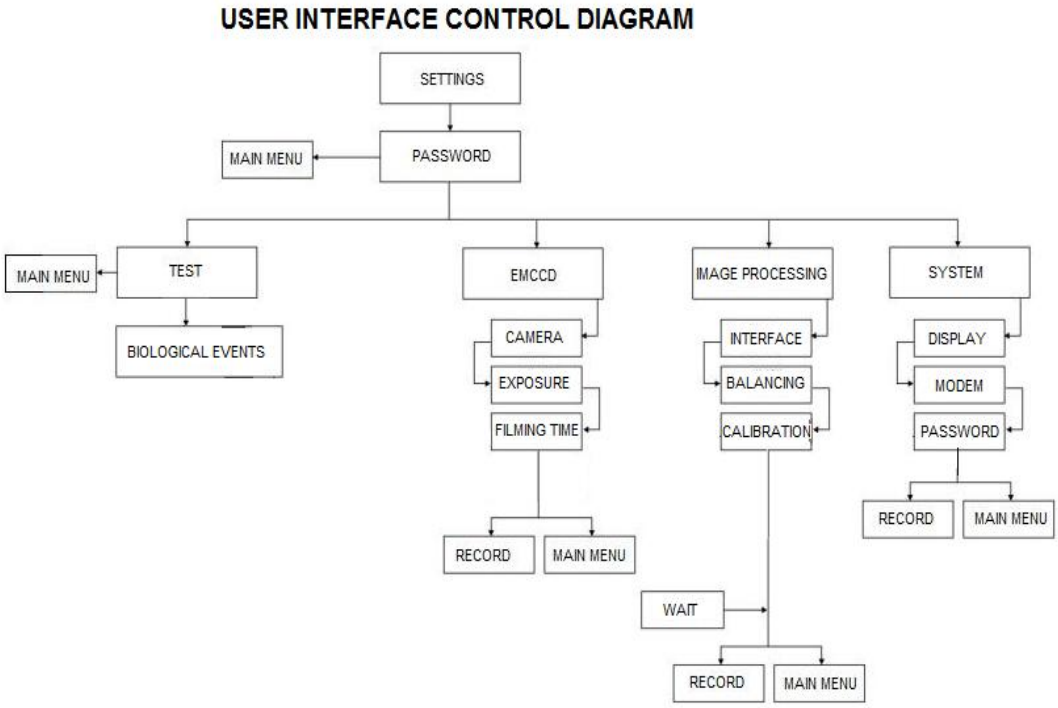


Figure 16: User settings display

4.2.4. The Communication Module (Comm):

The communication software module is responsible for the communication both with the external ambient and within the device. It is also the software module that is responsible for the communication and protocols between the SBC and all the peripheral hardware. These software modules run on the SBC. Its sub-units are given as:

- WIFI: It is responsible for the data structure and hardware operation of WIFI Ethernet port that is integrated to the system.
- SERIAL (RS232) : Responsible for the communication of data sharing through the RF Modem by a data structure developed similar to the NMEA 0183 protocol.
- GPS: Responsible for the communication between the GPS and the SBC.
- ISM RF MODEM: Responsible for the communication between the SBC and the ISM 900 MHz RF Modem which is used for remote connection to the system.
- DAQ: Responsible for the communication between the SBC and the DAQ by a data structure developed similar to the NMEA 0183 protocol.

- Center: Responsible for sharing the produced results with the center.
- Internal Communication (INT COMM) : Responsible for the communication realized by the staff in charge through SERIAL communication for processes such as system update, update of the DAQ calibration data etc.

4.2.5. The Embedded Automation Software Module (DAQ):

The embedded software modules run on the DAQ system. It contains the embedded software units that provide the functionalities such as controlling all the motor drivers, driving of the Peltier temperature controller, reading the sensors, and built-in testing of the system. Embedded software modules are: This module is defined in Chapter III.

4.2.6. The Device Driver Software (DRV):

These are the driver interface software modules of all the peripheral hardware used in the Biosensor System, as connected to the SBC. All of the driver software modules run on the SBC. These driver software modules, which are given below will be provided together with the hardware that will be used as finished products.

- EMCCD Camera: The driver software of the EMCCD camera.
- ISM Modem: The driver software ISM Modem.
- WIFI: The driver software of the WIFI module.
- SCREEN: The driver software of the LCD used in the system.
- Touch Board: The driver software of the touch board where the user makes all the controls in the system.
- GPS: The driver software of the GPS.
- DAQ: The software part of the DAQ used in the system, which runs on the SBC.

CHAPTER 5

IMAGE PROCESSING

In this chapter, we describe the image processing method which is developed to detect and identify the wells (pits) that contain harmful microorganisms in images captured by the EMCCD camera. In the ELISA method, if the pit contains the microorganism, the enzyme which is attached to the second antibody marked by the enzyme reacts with the chemiluminescence substrates and generates photons, and then these photons are perceived by the relevant detector (EMCCD). The higher the irradiation in the particular pit the higher the probability it contains the microorganisms. We calculate the average intensity of irradiation in each pit in the image captured by EMCCD, which contains many pits in the frame. If the average value of the intensity of irradiation in a pit is higher than a threshold value, we declare the detection of the microorganisms in that pit.

In determining the average intensities of the individual pits in an image frame, we have to overcome the following problems:

- We have to determine first the exact positions of all the pits (12x8=96) in an image of size 496x658 pixels, captured by the EMCCD camera.
- After determining the border of a particular pit, we have to count the intensities of all the individual pixels and take the average of the intensities of all the pixels in the particular pit.

The decision function that we use to declare the detection of the microorganism in a particular well (pit) is

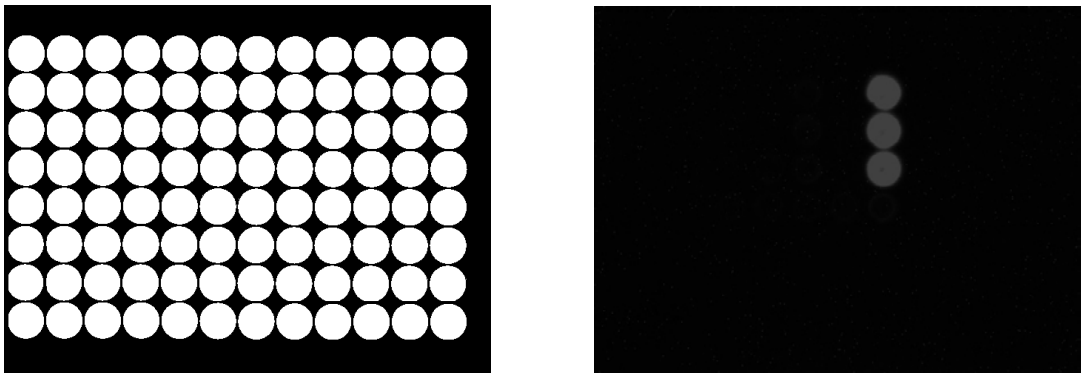
$$D(W\_n)\begin{cases}1, if\ \ I\_ave(W\_n)>T;\\ 0,\ otherwise\end{cases} \tag{5.1}$$

where W_n is the n'th well, D(W_n) is the decision regarding W_n, I_ave(W_n) is the average intensity (irradiation) value of W_n, and T is the threshold value of irradiation intensity in the image(frame).

The threshold value for T is taken as the irradiation value of the intensity, which is half of the sum of the maximum and the minimum irradiations of the 96 pits in the frame. If the average irradiation intensity of any pit (W_n) is greater than this threshold intensity then D(W_n)=1, and it is declared that the well (pit) is contaminated, i.e., the harmful microorganisms are present, otherwise it's clear. Analyzing the frames and the average irradiance values of the pits in the frame we observed that the distribution of the irradiance values of them is not a normal distribution. Therefore, we chose to use this decision criterion.

To implement this, we developed a method as follows:

- First, we decided to use a mask that has identical size of our EMCCD image, and having the same number of pits, the same size and located at the same coordinates as the pits located in the original EMCCD image. This mask has pits located in the mask image and has about 1900 pixels of white color for each pit.
- Then we compare the mask with the image. We arrange the pits of the mask such that all the pits on the mask match all the pits in the original image. To do that properly we have to associate pit number 1 on the mask to the first pit on the image. This is the most critical step.



(a) (b)

Figure 17: a) the mask to locate the pits on the image **b)** an image captured by the EMCCD camera

- After associating the first pit on the mask with the first pit on the image, we know that all the other pits shall be aligned correctly because the pits on the mask and the pits on the image are located from one another in the same manner, having the same sizes of the pits in both, as well.

These 3 steps actually solve our first problem of determining the exact positions of the pits in the picture (the locations of the center of all the pits in the original image).

To solve the second problem after determining the pits locations (pit numbers), that is, the exact contours of all the pits in the image, we have to determine all the pixels included in a pit and get their intensity values of these pixels and take the average of them to calculate the average intensity for the pit.

Our EMCCD camera has a bit-depth of 16 bits per pixel to represent intensity associated to each pixel digitally. This corresponds to a range of values between 0 and $(2^{16}-1)$ for the grayscale image in proportion with the result of the chemiluminescence activity of the associated pixel in the actual pit on the device.

After having associated the pits of the mask and the image, we can easily associate the pixels of the certain pit of the mask to the pixels of the associated pit of the original image. About 1900 pixels on each pit and their intensity values are read by comparing with the pixel arrangement of the mask, and average of all the intensities corresponding to all the pixels in the pit is calculated.

To implement all these operations explained above we developed a software. Our software uses this masking technique to calculate all the average intensities related to all the pits in the image generated by the EMCCD camera based on the chemiluminescence activities took place in the 8x12= 96 pits (wells).

In order to verify that our software is performing these operations properly, we first compared our mask with three test images artificially generated by us. We firstly used a pure white image, secondly a pure black image, and thirdly an image composed of gray pixels. These artificial images generated in the computer have a range of representing the grayscale with a resolution of 8 bits. Therefore, the

intensity values would extend between 0 and 2^8-1 for each pixel for these test images.

(a) A pure white test image (b) A pure black test image (c) A gray test image

Figure 18: Three test images

We compared these images with our mask and the results are given in Fig. 19, Fig. 20, and Fig 21.

	A	B	C	D	E	F	G	H	I	J	K	L
1	255	255	255	255	255	255	255	255	255	255	255	255
2	255	255	255	255	255	255	255	255	255	255	255	255
3	255	255	255	255	255	255	255	255	255	255	255	255
4	255	255	255	255	255	255	255	255	255	255	255	255
5	255	255	255	255	255	255	255	255	255	255	255	255
6	255	255	255	255	255	255	255	255	255	255	255	255
7	255	255	255	255	255	255	255	255	255	255	255	255
8	255	255	255	255	255	255	255	255	255	255	255	255

Figure 19: The calculated average intensity values of the result of the pure white picture (Fig.18.a).

	A	B	C	D	E	F	G	H	I	J	K	L
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0

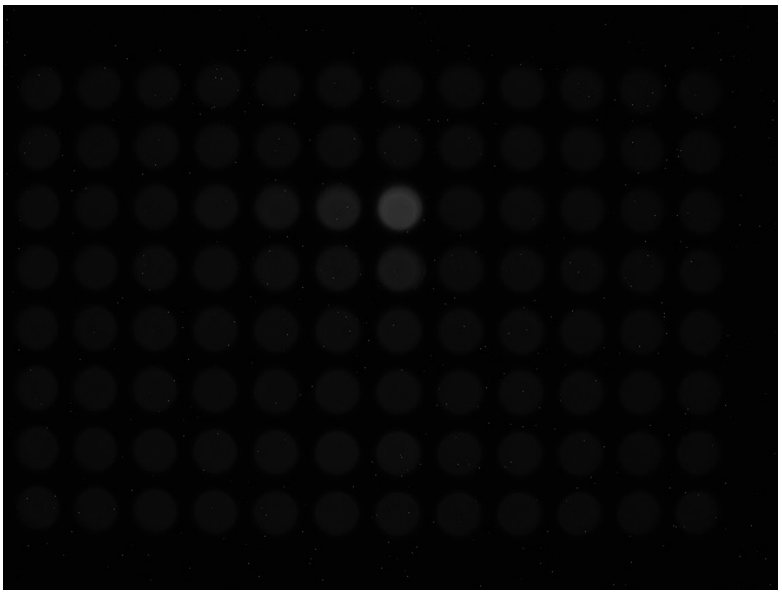
Figure 20: The calculated average intensity values of the pure black picture (Fig.18.b.).

	A	B	C	D	E	F	G	H	I	J	K	L
1	7	7	7	7	7	7	7	7	7	7	7	7
2	7	7	7	7	7	7	7	7	7	7	7	7
3	7	7	7	7	7	7	7	7	7	7	7	7
4	7	7	7	7	7	7	7	7	7	7	7	7
5	7	7	7	7	7	7	7	7	7	7	7	7
6	7	7	7	7	7	7	7	7	7	7	7	7
7	7	7	7	7	7	7	7	7	7	7	7	7
8	7	7	7	7	7	7	7	7	7	7	7	7

Figure 21: The calculated average intensity values of the gray picture (Fig.18.c)

These results verified that our associating (matching), comparing and calculating the pixels intensity values method works properly.

After having seen the results that show what we were doing right, we used our software with the actual images that were captured by the EMCCD camera. Figures 22-31 show the actual images taken by the EMCCD camera from chemiluminescence reactions that were taking place in the pits, and the calculations of the averages of irradiation pertaining to each pit for all the 96 pits in each picture.

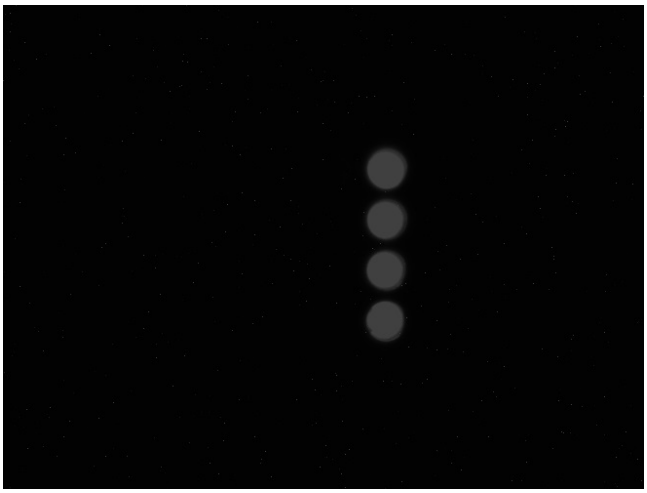


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	1679	1560,81	1706,15	1775,23	1700,17	1803,21	1790,62	1683,66	1713,38	1605,74	1398,43	1254,97
2	1677,1	1616,72	1761,46	1796,68	1777,91	1859,98	1882,17	1783,42	1746,51	1640,2	1452,95	1308,07
3	1694,98	1664,82	1813,62	2169,85	2626,23	3902,17	7513,26	1807,83	1821,31	1699,36	1495,39	1387,82
4	1680,23	1700,89	1836,86	1884,97	2111,4	2499,53	3551,66	1867,82	1821,5	1729,25	1552,94	1429,01
5	1635,79	1662,33	1798,48	1774,82	1814,39	1997,02	2068,01	1810,38	1815,33	1701,98	1544,41	1407,99
6	1641,03	1659,19	1767,07	1706,56	1788,21	1895,07	1865,41	1760,15	1759,37	1647,69	1479,94	1396,68
7	1621,31	1611,5	1746,01	1814,08	1870,29	1992,65	2082,98	1726,97	1725,29	1633,12	1466,11	1388,12
8	1571,23	1583,74	1686,76	1717,95	1692,27	1780,07	1770,56	1634,28	1621,5	1556,02	1398,61	1317,54

(b)

Figure 22: a) An image (Image 1) from the EMCCD camera, and **b)** the calculated average intensity values belonging to the pits

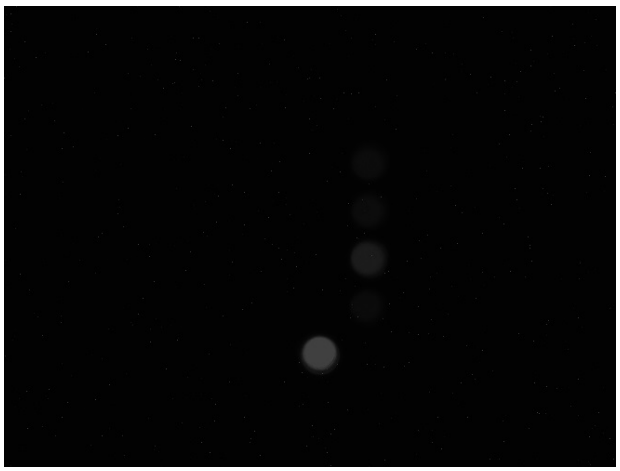


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	518,72	509,83	513,71	539,75	521,44	512,63	525,27	515,84	520,11	517,5	536,41	521,37
2	510,56	498,77	512,74	513,56	518,78	515,63	541,64	559,66	510,24	503,48	533,39	510,55
3	521,41	530,46	520,37	536,29	562,38	570,78	614,09	12130,23	550,6	523,79	528,59	519,72
4	521,38	510,81	535,55	526,23	546,92	568,59	579,25	11728,02	559,07	540,2	535,69	522,68
5	514,64	515,48	526,6	537,58	533,03	571,01	570,52	11454,86	562,26	532,91	548,58	516,05
6	515,04	507,09	529,38	515,4	536,4	543,93	561,53	11431,33	562,95	529,56	509,56	534,24
7	513,42	509,62	510,48	536,32	530,03	526,85	565,5	563,48	546,99	541,89	523,9	520,87
8	528,11	518,6	502,04	514,82	536,19	512,4	531,39	521,73	512,23	525,81	510,71	530,2

(b)

Figure 23: In **a)** An image (Image 2) from the EMCCD camera, and **b)** the calculated average intensity values belonging to the pits

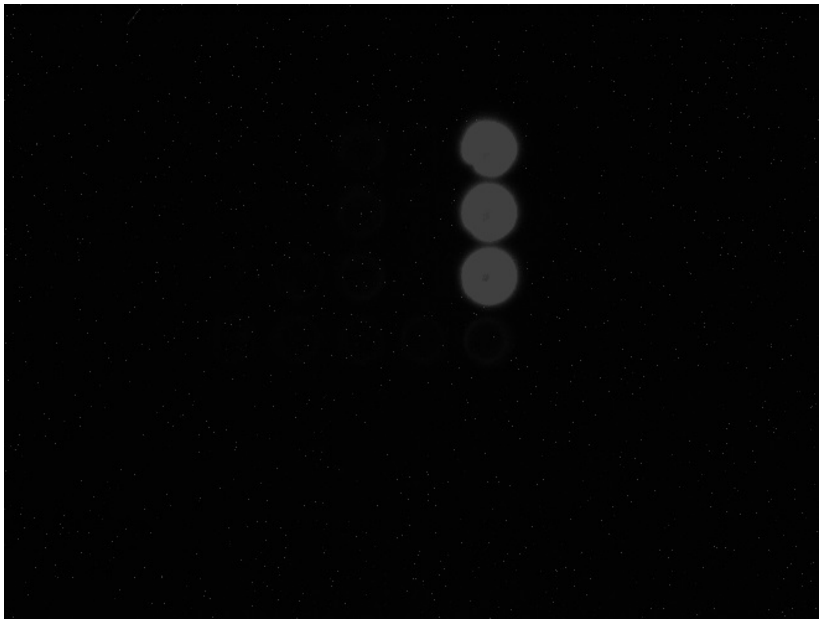


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	517,48	508,53	511,71	537,58	517,55	508,32	518,93	508,47	513,56	512,36	533,34	518,82
2	509,83	496,22	509,8	511,34	515,03	510,44	531,47	509,24	496,75	496,83	530,18	507,97
3	519,4	527,84	515,88	528,58	550,22	550,15	560,13	1841,05	518,27	514,04	524,08	515,69
4	517,71	506,52	531,4	518,5	537,06	550,02	531,59	1863,25	522	528,03	529,75	517,26
5	510,29	510,26	520,51	528,82	523,61	554,59	532,98	4359,31	530,66	521,2	541,71	510,61
6	508,51	500,21	526,42	507,48	525,43	529,07	536,44	1630,74	536,4	517,52	501,6	526,8
7	506,67	502,47	503,05	527,37	522,02	531,25	9979,4	544,36	530,96	531,45	515,2	514,43
8	521,61	509,76	493,05	506,06	527,47	507,75	534,29	514,92	504,34	516,52	502,18	523,06

(b)

Figure 24: a) An image(Image 3) from the EMCCD camera and **b)** the calculated average intensity values belonging to the pits

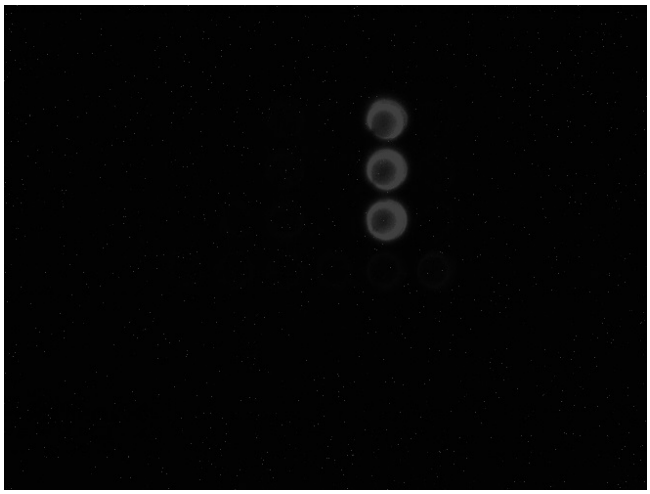


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	549,98	522,01	539,85	556,95	530,27	537,45	547,73	685,6	543,83	535,61	553,39	562,87
2	552,23	525,88	569,76	602,68	587,85	868,96	768,41	14725,24	576,32	534,73	580,46	558,24
3	584,47	575,74	577,64	632,1	627,38	977,91	768,88	15102,7	636,98	580,23	575,87	583,78
4	570,83	535,9	587,63	634,85	749,14	979,64	724,24	15194,96	652,56	599,06	575,49	563,28
5	514,35	531,35	552,34	726,46	747,31	790,18	835,89	1290,48	573,38	578,49	569,66	540,65
6	483,74	467,66	529,74	505,99	551,9	557,78	523,53	577,99	579,83	547,59	500,72	564,36
7	458,98	462,95	472,23	530,1	547,05	541,65	588,86	556,94	545,26	568,85	542,51	557,73
8	517,62	480,14	459,14	506,12	539,19	529,92	556,13	543,8	515,55	521,17	521,47	547,25

(b)

Figure 25: a) An image(Image 4) from the EMCCD camera, and **b)** the calculated average intensity values belonging to the pits

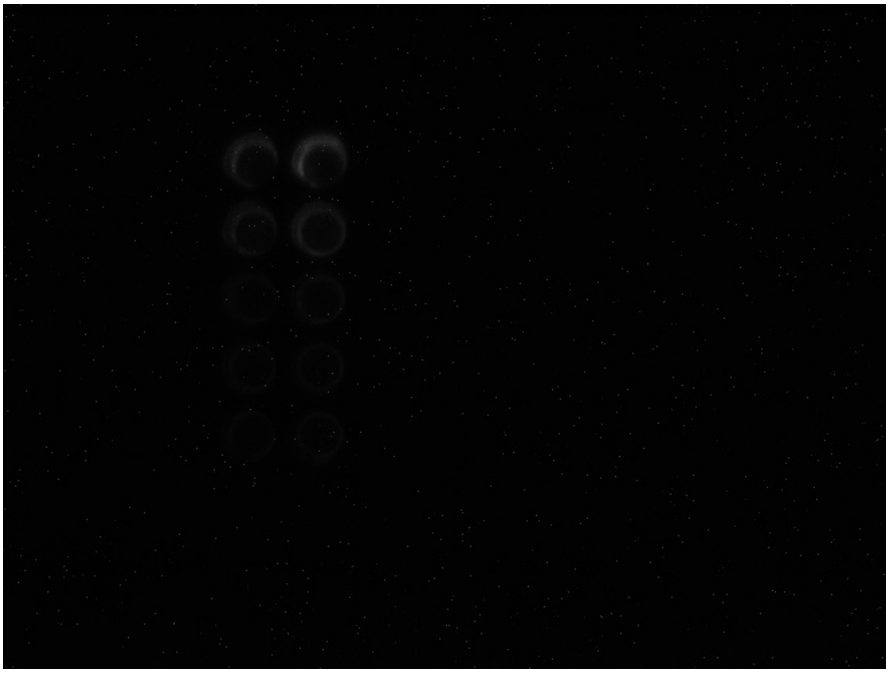


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	546,26	521,82	537,61	553,97	526,01	528,37	530,74	568,78	523,51	525,96	553,98	558,33
2	549,91	523,78	565,92	583,25	572,55	741,74	663,28	10135,87	619,88	524,08	578,87	555,31
3	579,49	567,84	570,04	609,6	601,09	818,96	663,5	10751,47	681,71	561,01	567,5	571,02
4	560,52	521,61	572,8	589,86	674,55	833,9	626,37	11103,45	668,71	574,22	559,57	547,46
5	501,07	516,29	536,22	650,83	668,93	714,78	715,94	1013,77	1129,24	552,8	549,94	526,14
6	474,97	459,05	517,13	489,32	535	537,08	506,24	561,4	567,04	535,67	492,68	552,59
7	445,73	448,56	454,94	509,45	523,7	514,08	566,83	536,6	526,75	559,57	536,73	543,81
8	500,89	462,06	437,2	487,6	516,91	506,22	534,6	520,37	499,21	504,23	512,95	531,53

(b)

Figure 26. a) An image (Picture 5) from the EMCCD camera, and **b)** the estimated average intensity values belonging to the pits

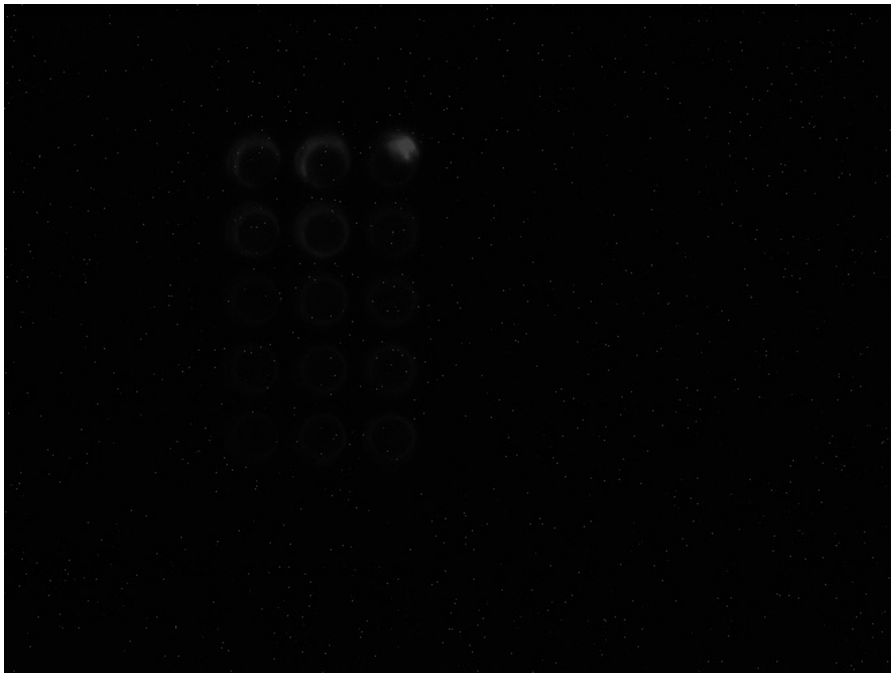


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	542,42	515,18	534,39	556,02	537,91	534,29	515,29	510,13	511,91	520,64	545,28	557,38
2	549,42	525,44	578,14	2548,68	3968,4	548,88	568,21	543,79	507,16	518,11	572,41	554,71
3	582,47	570,51	580,71	2016,22	2684,22	599,66	579,95	560,47	553,57	553,71	569,57	575,96
4	555,35	519,41	573,89	1113,29	1381,72	600,39	549,31	587,21	567,35	567,63	557,02	542,83
5	502,5	519,36	536,31	1088,14	1132,83	592,98	536,38	524,74	535,15	551	553,02	526,75
6	472,59	455,39	522,63	914,23	1097,22	530,4	497,94	551,35	558,22	534,06	489,07	551,59
7	447,16	449,77	457,88	513,57	528,09	514,61	565,53	535,77	529	554,68	531,46	545,39
8	504,83	463,08	443,14	490,86	521,81	504,05	535,61	527,2	505,19	508,95	505,96	535,88

(b)

Figure 27: a) An image (Image 6) from the EMCCD camera and **b)** the estimated average intensity values belonging to the pits

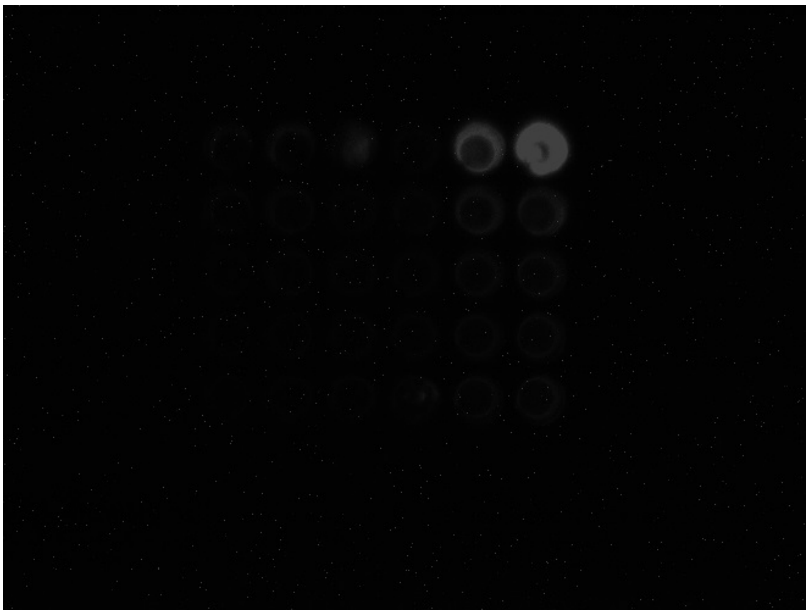


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	543,79	526,89	536,14	555,73	533,69	545,16	520,97	511,65	513,06	518,9	548,84	557,94
2	546,82	518,9	569,55	1784,72	2473,12	3466,31	572,56	540,99	504,82	514,76	568,64	549,97
3	574,08	567,09	573,83	1471,34	1813,11	1140,58	577,57	557,51	551,26	551,1	562,79	571,37
4	556,35	517,62	571,65	937,21	1148,75	1118,21	551,67	589,22	568,54	567,09	556,68	544,78
5	499,72	515,14	536,97	917,27	980,53	1116,41	536,59	523,28	532,93	551,34	546,83	525,54
6	471,99	459	522,9	792,45	958,25	1080,44	497,01	556,94	565,1	539,53	488,9	553,36
7	448,88	450,69	459,52	510,38	526,89	516,48	565,49	536,79	529,45	558,28	529,16	547,47
8	505,01	469,46	444,48	492,53	527,42	511,19	537,13	527,58	504,15	511,06	510,89	536,78

(b)

Figure 28: a) An image (Image 7) from the EMCCD camera, and **b)** the estimated average intensity values belonging to the pits

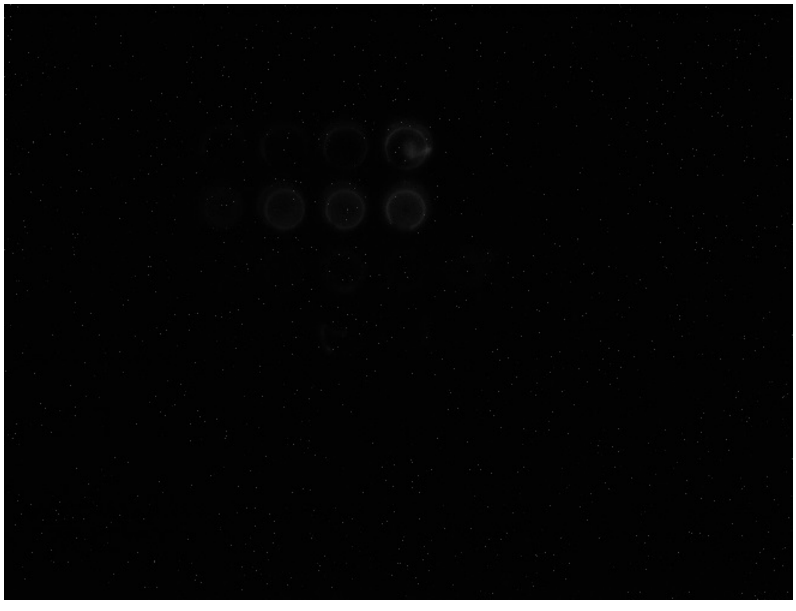


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	546,91	519,42	538,67	555,24	527,77	529,75	530,81	551,44	582,7	529,25	552,47	561,48
2	551,76	526,14	572,64	988,35	1195,31	2055,77	919,39	6782,19	12590,66	572,27	578,92	558,53
3	582,84	572,91	577,69	888,52	995,28	847,39	930,53	1988,97	2415,91	570,26	574,1	584,32
4	557,9	519,32	570,77	705,21	822,23	841,58	838,53	1304,86	1511,85	574,37	556,69	545,3
5	484,78	503,19	520,98	675,58	712,93	807,85	819,79	1106,14	1328,44	540,48	538,66	509,02
6	472,97	453,52	515,29	605,71	704,82	773,7	1087,68	1214,64	1419,56	531,74	486,75	548,34
7	448,24	451,62	458,96	510,57	528,9	516,34	565,91	540,35	530,18	558,17	530,71	547,36
8	501,78	463,31	439,94	491,1	521,78	506,88	534,97	525,41	502,13	504,04	506,58	532,18

(b)

Figure 29: a) An image (Image 8) from the EMCCD camera, and **b)** the estimated average intensity values belonging to the pits

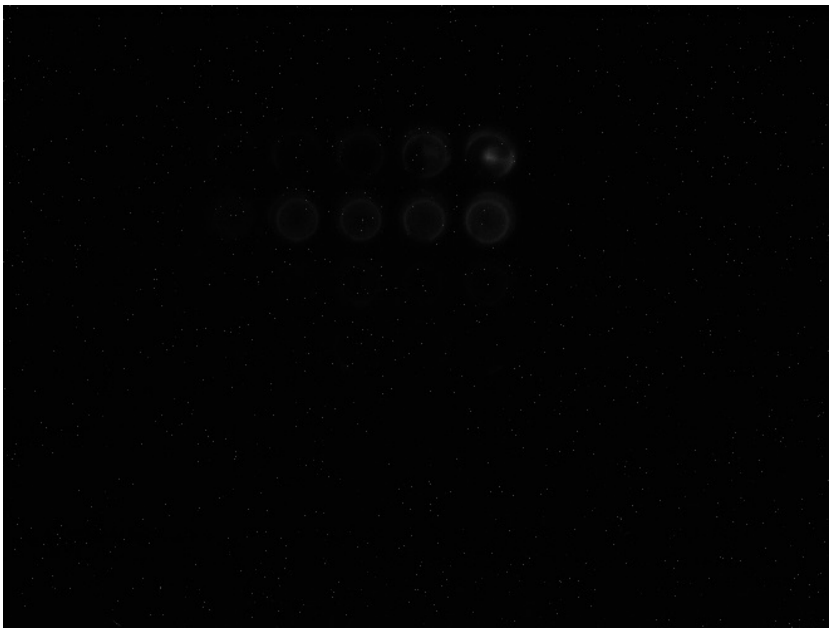


(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	534,82	515,07	541,51	553,72	532,25	542,07	524,46	525,23	505,09	521,06	559,23	555,75
2	562,27	523,85	557,54	693,69	780,35	1111,94	2739,62	534,49	503,15	520,24	568,11	551,63
3	576,4	561,6	573,66	1043,56	1953,34	2175,93	2776,61	556,75	554,93	558,05	563,16	556,48
4	550,02	522,06	568,27	605,18	681,91	909,98	652,18	794,79	565,61	564,69	563,09	549,66
5	492,67	501,5	541,35	558,8	545,73	733,02	591,57	522,19	536,09	557,35	551,1	520,16
6	473,21	453,93	514,87	484,31	534,2	530,84	505,92	537,94	553,28	533,81	485,55	544,47
7	455,19	460,32	457,63	512,4	537,88	513,93	556,19	536,83	547,49	560,85	537,35	546,14
8	501,42	460,99	441,33	481,85	510,92	510,94	531,16	516,36	506,32	507,73	487,14	537,56

(b)

Figure 30: a) An image (Image 9) from the EMCCD camera, and **b)** the estimated average intensity values belonging to the pits



(a)

	A	B	C	D	E	F	G	H	I	J	K	L
1	537,96	518,44	543,25	554,39	533,39	550,07	520,08	530,82	509,28	521,32	559,57	553,34
2	563,56	530,41	555,13	650,47	709,44	886,05	1784,28	2769,56	506,04	521,53	567,1	557,1
3	581,83	562,33	573,38	916,19	1630,2	1659,71	1999,36	2733,72	559,58	563,21	566,03	562,42
4	551,19	517,05	564,94	585,81	641,79	821,58	636,87	806,98	566,21	567,24	567,55	552,01
5	497,58	502,71	542,07	557,19	543,82	640,24	577,7	564,13	538,1	559,47	553,93	523,29
6	472,48	456,7	514,76	486,43	534,03	528,74	503,02	538,13	551,41	528,89	484,84	542,85
7	459,82	462,52	459,55	514,79	540,53	517,12	558,83	538,49	548,12	559,14	541,31	551,15
8	512,39	472,59	446,42	485,06	510,98	511,58	533,67	517,75	504,64	535,04	491,63	540

(b)

Figure 31: a) An image (Image 10) from the EMCCD camera, and **b)** the estimated average intensity values belonging to the pits

By investigating the Figures from 22 to 31 we confirmed that our software locates and segments the pits correctly within images of 96 pits, and performs the irradiation

average calculations of 1900 pixels corresponding to each pit correctly and our declarations of the detections for the pits where harmful microorganism are present were correct.

The average irradiation values pertaining to each pit containing nearly 1900 pixels are calculated by using the algorithm given in Figure 32.

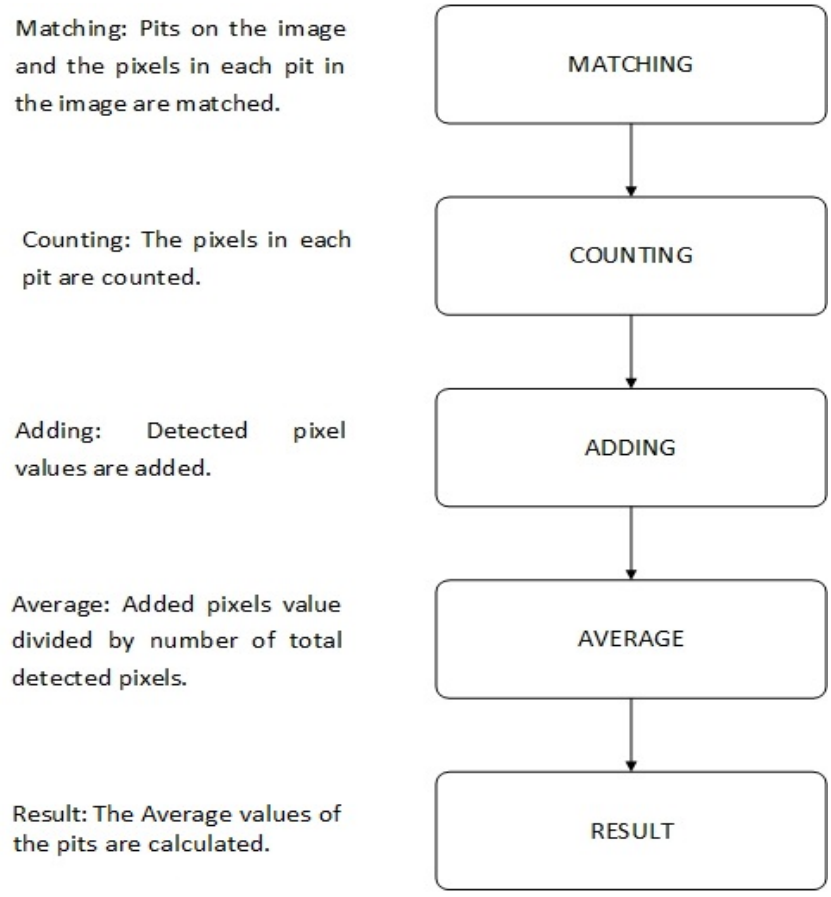


Figure 32: Average irradiation calculation algorithm for each pit.

To implement the algorithm defined in Figure 32 and detect if microorganisms are present in any pit, a code is developed in the MATLAB, and given in Figure 33.

AnalysisMain.m

```
clc;
clear all;

[fileName,pathName,filterIndex] = uigetfile({'*.tiff';'*.bmp';'*.*'},'Select analysis
image','C:\');
```

```
if(filterIndex == 0)
   msgbox('Analysis image not selected!', 'Warning','warn');
   return;
end

mask = imread('plateMask.bmp');%Reading the mask
selectedImage = imread([pathName , '\' , fileName]);%Reading the captured image to
be processed

if( size(mask) ~= size(selectedImage) )
   msgbox('Size of the image selected does not fit the analysis.', 'Warning','warn');
   return;
end

pitValues = calculateValues(selectedImage,mask);

xfilename = [[pathName,'\',fileName(1:end-4)],'xls'];
xlswrite(xfilename,pitValues);

msgbox('Analysis of the image has been completed. Excel file is ready.', 'Process has
been completed','Help');
```

calculateValues.m

```
function [resultMatrix] =  calculateValues(selectedImage,mask)
   rotatedMask = imrotate(mask,5,'crop');
   labeledMask = bwlabel(rotatedMask);
   mask = imrotate(labeledMask,-5,'crop');

   resultMatrix = zeros(8,12);

   pitNumber = 1;
   for column = 1 : 12
      for row = 1 : 8
         pixelValues = selectedImage(mask == pitNumber);
         pitPixelCount = size(pixelValues);
         pitPixelCount = pitPixelCount(1);
         totalPitValue = sum(pixelValues);
resultMatrix(row,column)=str2double(sprintf('%.2f',totalPitValue/pitPixelCount));
         pitNumber = pitNumber + 1;
      end
   end
minValue = min(min(resultMatrix));
         maxValue = max(max(resultMatrix));
         ave_minmax = (minValue+maxValue)/2;
         for column = 1 : 12
            for row = 1 : 8
               if(resultMatrix(row,column)>=ave_minmax)
                disp('Critical value has been detected in')
                  fprintf('row ')
                  fprintf('%.2f',row)
```

```
                fprintf('\n')
                fprintf('column ')
                fprintf('%.2f',column)
                fprintf('\n')
            end
        end
    end

end
```

Figure 33: MATLAB code to implement the algorithm in Figure 32

The threshold value for T is taken as the irradiation value of the intensity, which is half of the sum of the maximum and the minimum irradiations of the 96 pits in the frame. This is our threshold irradiation value (T) for the particular image. In any of the pit, if the average irradiation of the Pixels is greater than this T value we declare that we detected the harmful microorganism in this particular pit.

CHAPTER 6

CONCLUSION AND FUTURE WORK

In this thesis we developed an image processing method to detect the harmful microorganism in a given well (pit) from a picture captured by a sensitive camera. This software runs on the single board computer (SBC) that resides in a device called the Biosensor System, which was developed within the framework of a SANTEZ Project.

In order to enable the accurate and rapid identification of the microbial contaminants in the food sector, the validated method is to associate such bacteria to the common food poisoning in meat products. Another important criterion in identification of food poisoning is that the device that makes this identification should be portable and effective and reliable enough to make the detection reliably and quite rapidly. This device can also be moved to the location easily. In the SANTEZ Project, for which we developed the image processing software, such a device is developed and a prototype is built by the SANTEZ project team.

In the biosensor system antibody-based detection technology is used, where the luminescence Sandwich ELISA (sELISA) method is selected. The use of antibodies, which are the smallest and lightest sensors that exist in the nature as biosensor, would be appropriate. The photons generated by the enhanced chemiluminescence (ECL) reaction takes place between the antibody and microorganisms are measured by a radiation cooled Electron Multiplying Charge Coupled Device (EMCCD) camera. In the biosensor system, by enabling the simultaneous measurement of many reaction wells (pits) by the EMCCD camera, we were able to reduce the test duration as much as possible. The device possesses the capability of measuring of more than one pathogens, more sensitive, more time-saving, less power consuming, and lighter compared to the other systems available in the market.

Within the developed biosensor system, there exists an automation system which carries out some of the activities during the sELISA process. This automation system automatically carries out the sample transfer, well washing, enzyme and buffer transfer steps of the sELISA process and the luminescence radiation obtained at the end of the process is recorded by an EMCCD camera and the result is obtained after its analysis by an embedded software developed within the scope of this thesis.

In developing the biosensor device the SANTEZ project team aimed to make the innovations listed below:

• To achieve the detection and identification of one or all agents by using a MicroDisc and chemiluminescence method in a single test procedure.

• By using the cooled EMCCD camera, to attain a better signal to noise ratios (SNRs) than the other devices available,

• Using multi-well Colorimetric Assay Method (sandwich Elisa)(sELİSA) to increase the sensitivity.

• To provide data analysis and processing capability, and to better make use of commonality of the software available on the market, using a capable on board computer.

In this thesis study, we developed an image processing software module that can be used in the biosensor system to detect the harmful microorganisms in the pits within the image frames captured by the EMCCD camera. The software first associates the pits in a mask generated and the image. Each image contains 96 pits, and each pit contains about 1900 pixels. After associating the pits in the mask and the image the locations of the pits are determined correctly. When the pits are determined then the pixels within each pit are determined and counted. Then the irradiation values in the pixels related to the photons generated by the chemiluminescence mechanism in each pit are calculated. We then calculate the average irradiation for each pit within the image. The next step is to determine a threshold irradiation value (T) among the pits in the frame. We calculate the T value by taking the half of the sum of the minimum and maximum irradiation values among the pits in the frame. This value is taken as the threshold irradiation value (T) for the particular image(frame). Then we declare the detection of the harmful microorganism in a pit if its average irradiation value is greater than this T value.

We tested and verified our image processing software by using more than 10 pictures (images) captured by the EMCCD camera, where some of the pits were containing the harmful microorganisms. We showed that our software correctly detected the pits with harmful bacteria in all the images.

We recommend for a future study :

- to search for more powerful association (matching) methods to locate the pits within the image frame and also to locate the pixels in the pit,
- and to develop an alternative method to determine a threshold irradiation value to declare a detection of the harmful microorganisms in a pit.

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APPENDICES A

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EDUCATION

Degree	Institution	Year of Graduation
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WORK EXPERIENCE

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2013- Present	Turkish Armed Forces	Engineer
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FOREIN LANGUAGES

Advanced English.

PROJECTS

1. SANTEZ Project, Development of a Portable System for the Detection of the Microorganisms in Meat Product , Project Number: 00973.STZ.2011-2, Ministry of Science, Industry and Technology, 2014.

HONOURS AND AWARDS

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Avionics, Travel, Books, Swimming, Fitness.