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FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

A NOVEL LED BASED LIGHT SYSTEM FOR PDT APPLICATIONS IN 96 WELL-PLATE CELL CULTURE

MEHMET NECMİ BURGUCU

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

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ASSIST.PROF.DR.HAŞİM ÖZGÜR TABAKOĞLU

İSTANBUL, SEPTEMBER / 2014

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

96'LI HÜCRE KÜLTÜRÜ KAPLARINDA FOTODİNAMİK TEDAVİ DENEYLERİ İÇİN UYGULANABİLECEK LED TABANLI ÖZGÜN BİR IŞIK KAYNAĞI

MEHMET NECMİ BURGUCU

YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
YARD.DOÇ.DR.HAŞİM ÖZGÜR TABAKOĞLU

İSTANBUL, EYLÜL / 2014

T.C.

FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

Mehmet Necmi Burgucu, a **MSc.** student of Fatih University **Institute of Biomedical Engineering** student ID 52011111, successfully defended the **thesis** entitled "**A NOVEL LED BASED LIGHT SYSTEM FOR PDT APPLICATIONS IN 96 WELL-PLATE CELL CULTURE**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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It is approved that this thesis has been written in compliance with the formatting rules laid down by the Institute of Biomedical Engineering.

……

……

……**Prof. Dr. Sadık KARA**

Director

Date of Submission : 29 September 2014

Date of Defense : 22 September 2014

This thesis is dedicated to my parents, who have supported me all the way since the beginning of my studies,

This study was supported by Fatih University Research and Development Management Office with the project number of P50091101_Y (1689) .

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September 2014 Mehmet Necmi BURGUCU

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LIST OF SYMBOLS

C	Correlation index
λi	Lambda factor
Ω	Omega
ψ	Positive number
Δ	Delta function
δ	Distance

ABBREVIATIONS

PDT : Photodynamic Therapy

LED : Light Emitting Diode

LASER : Light Amplification by Stimulated Emission of Radiation

CW : Continuous wave

PW : Pulsed wave

FDA : Food and Drug Administration

IPL : Intense pulsed light

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SUMMARY

A NOVEL LED BASED LIGHT SYSTEM FOR PDT APPLICATIONS IN 96 WELL-PLATE CELL CULTURE

Mehmet Necmi BURGUCU

Biomedical Engineering Programme

MSc Thesis

Advisor: Assist.Prof.Dr. Haşim Özgür TABAKOĞLU

Photodynamic therapy (PDT) is used clinically to treat a wide range of medical conditions, including wet age-related macular degeneration and malignant cancers, and is recognised as a treatment strategy which is both minimally invasive and minimally toxic.

Most modern PDT applications involve three key components; a photosensitizer, a light source and tissue oxygen. The wavelength of the light source needs to be appropriate for exciting the photosensitizer to produce reactive oxygen species. The combination of these three components leads to the chemical destruction of any tissues which have either selectively taken up the photosensitizer or have been locally exposed to light.

This investigation focused on the illumination system used for In-Vıtro PDT (Photodynamic theraphy), usibility of LED (Light Emitting Diodes)'s inside the other coherent and noncoherent light sources, effect of the LED's (Light Emitting Diodes) on some photoactive chemicals, as a result of the investigation design of a light system for in vitro PDT applications.

This light emitting diode light system allows us to do experiment with continuous wave illumimation and pulsed wave illumination with the aid of the microcontroller on it. The light system can be used for long time periods without any temperature change on light emitting diodes.

This investigation allows us to use this product as in-vivo cell culture experiments, animal experiments and at the end human trials of the Photodynamic therapy in future.

Keywords: Photodynamic therapy, Cancer Theraphy Light Sources, Anticancer techniques, In-Vıtro Photodynamic Drug Activation, In-Vıtro Light system.

ÖZET

TEZ BAŞLIĞI

Mehmet Necmi BURGUCU

Biyomedikal Mühendisliği Programı

Yüksek Lisans Tezi

Danışman: Yard.Doç.Dr.Haşim Özgür Tabakoğlu

Fotodinamik tedavi(FDT) içerisinde yaşa bağlı görme kaybı ve kötü huylu kanserlerin tedavisi de olmak üzere birçok medikal durumu tedavi etmek için yaygın olarak kullanılan bir tedavi türüdür.Aynı zamanda en az kesi ile ve kimyasalın organizmaya verdiği zararı minimize etmek için bir tedavi stratejisi olarak da belirlenebilir.

Birçok modern FDT uygulamaları üç temel bileşenden oluşur; fotoduyarlı madde, ışık kaynağı ve dokunun içerisinde bulunan oksijen.Doku içerisindeki oksijen radikallerini oluşturabilmek için kullanılan ışık kaynağının dalgaboyu kimyasalı aktif hale geçirebilecek dalgaboyunda olmalıdır.Bu üç bileşen biraraya gelerek bulunduğu dokuyu yokeder.Bu doku fotoduyarlı maddenin olduğu veya ışığın belirli bir yere uygulanmasıyla belirlenebilir.

Bu araştırma in-vitro çalışmalarda kullanılacak ışık sistemleri üzerine yoğunlaşır.LED ışık kaynaklarının diğer koherent ve koherent olmayan ışık kaynakları gibi kullanılabileceğini gösterir, bazı kimyasallar üzerindeki etkisini inceler. Sonuç olarak 96 hücre kültürü kapları için dizayn edilen ışık sistemini inceler.

Dizayn edilen ışık sistemi üzerindeki mikroişlemcili kart sayesinde sürekli ışıma ve kesikli ışıma şeklinde kullanılabilir.Herhangi bir sıcaklık değişimi olmadan uzun sure kullanılabilir.

Yapılan bu araştırma, sonunda çıkan sonuçlarla, gelecekte bu tür ışık sistemleri ile in-vivo, hayvan ve insan deneylerinin yapılmasına bir ön çalışma olabilecek niteliktedir.

Anahtar kelimeler: fotodinamik tedavi, kanser tedavisinde ışık sistemleri, antikanser teknikleri, in-vitro fotodinamik ilaç aktivasyonu, in-vitro ışık sistemleri

CHAPTER 1

INTRODUCTION

1.1 Literature Survey

The term of 'CANCER' is a general description of disease that effect one or more part of the body. The creation of abnormal cells that grow more than their natural boundaries(cancer), after that with the aid of the vasculature system invades the other organs(metasteses).The major cause of death is metastases.

According to GLOBOCAN 2012 PROJECT (estimates of the incidence of, mortality and prevalence from major types of cancer, at national level, for 184 countries of the world):

8.2 MİLLİON PEOPLE DIED FROM CANCER WORLDWIDE IN 2012.

Table1.1: Major cancer types and amount of deaths worldwide[16]

TYPES OF CANCER(MAJOR)	***AMOUNT OF DEATHS WORLDWIDE***	***PERCENTILE***
LUNG CANCER	1590000	19,3902439
LIVER CANCER	745000	9,085365854
STOMACH CANCER	723000	8,817073171
COLOTERAL CANCER	694000	8,463414634
BREAST CANCER	521000	6,353658537
OESOPHAGEAL CANCER	400000	4,87804878
OTHER CANCER TYPES	3527000	43,01219512

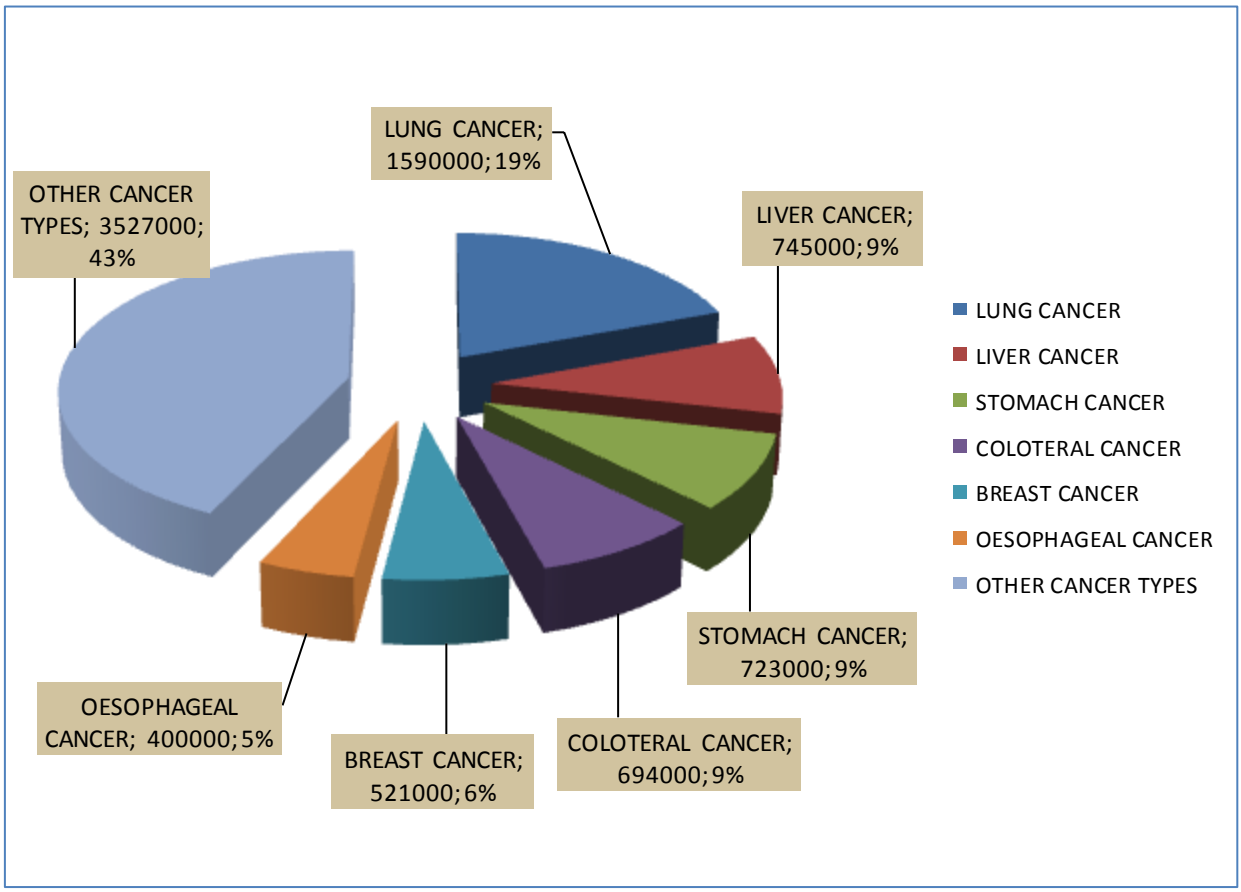

Figure 1.1 Distribution of major cancer types[16]

Therapies of cancer

Surgery

The primary cancer threatment modality is surgery. At the begining a cancer is a local disease. If the cancer occurs in an organ of the patient, removing the tumour is the best way to threatment. Between the adventages of surgery, it is not the one threatment of the cancer.The limitations of this threatment are the stage of the tumour, depth of penetration etc.. It is generally used for early stage cancers. [1, 2]

Chemotherapy

This anticancer technique is using one or more chemicals to threat cancer or reduce the symptoms. In spite of its adventages, it has disadventages as well.

Radiotherapy

This technique uses ionazing energy as a part of a cancer threatment to kill cancer cells. Similar to chemotherapy it has adventages and disadventages.

PDT has several potential advantages over surgery, radiotherapy or chemotherapy.[3]

Photodynamic therapy

Photodynamic Theraphy (PDT) is an technique which is use for (tedavi etmek) several illnesses like destroying wound infecting, antibiotic-resistant bacteria, age-related macular degeneration and cancer threatment(head and neck cancers, skin cancers). [4-6]

The main compounds of this technique are a photoactive drug (photosensitizer) and the light (typically visible (wavelenghts between 400-700 nm) or infrared (wavelenghts between 700-106 nm)) and the oxigen inside the tissue. In this technique when a photosensitizer irradiated with an appropriate wavelenght of light, initiates chemical reactions. As a result of this reactions some cytotoxic species(such as radicals and singlet oxygen) are formed. The reaction of cytotoxic species with the organels and macromolecules like DNA, proteins, mytocondria results to apoptosis or necrosis of the cells that includes PS.[7]

On the other hand PDT has the adventage of dual selectivity. First of them concentration of photosensitizer can be set in target tissue. The other one is the dose and the place of irradiation can be arrange to a specific area. [5]

Selective Destruction:

Between the anticancer theraphies, Photodynamic Theraphy has a very significant place because of the selective destruction. In many cases it is very important for the further life of the patient. The preferencial accumulation of PSs in cancer cells and the localized light delivery helps us selective destruction of cancer cells. In this technique healthy cells are minimally effected by the PS. Even if the photosensitizer binds to the healthy cells, because of the localized light delivery, it does not effect the healthy cells. But clearance time let the healthy cells be alive. [8]

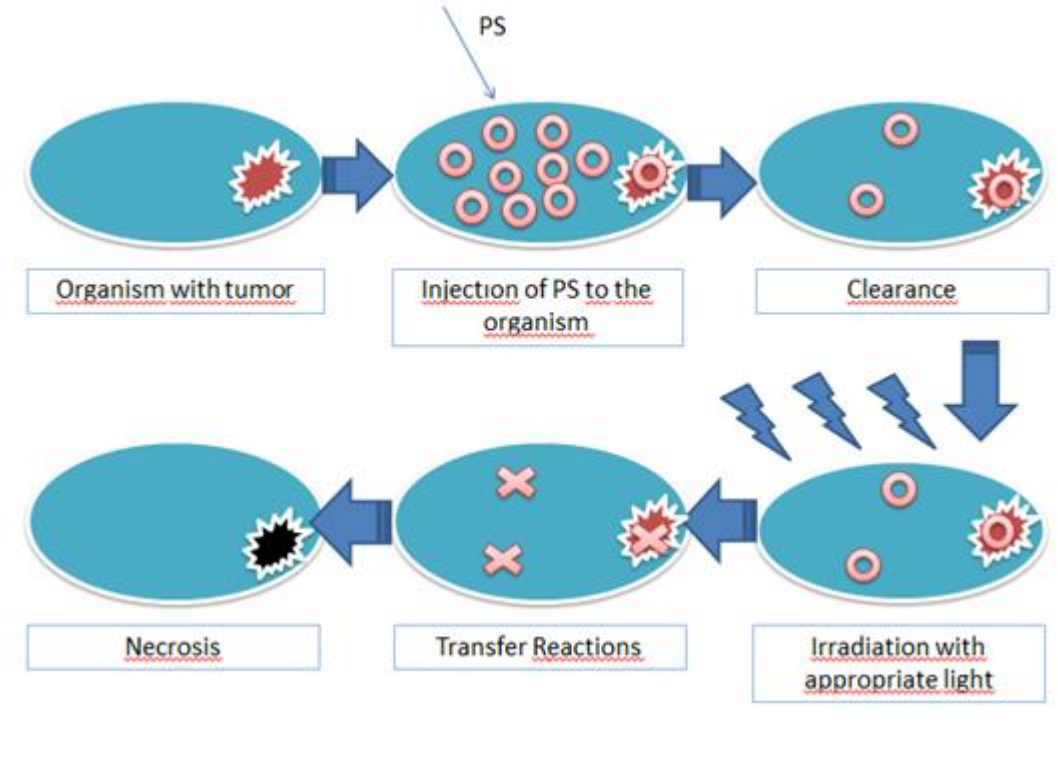

Figure 1.2: Selective destruction of PDT

Tumor Vasculature Targeted PDT(with the aid of neo-angiogenesis targeted PDT)

The tumor vasculature has been mainly targeted in the eradication of vascularized tumors using PDT. For a tumor to survive in a body, blood vessels that develop in and around tumor is vital. This vessels transports the oxygen and nutrition to the tumor. After single PDT application for cancer, the tumor wanted to survive and secondary angiogenic and inflammatory responses occur(results in revascularization of the treated lesions and contributes to tumor recurrence). Angiogenesis targeted PDT interacts with the blood vessels, destroy the reforming vasculature and increase the therepautic efficiacy of PDT. This technique used to suppress tumor recurrence after single PDT. [9]

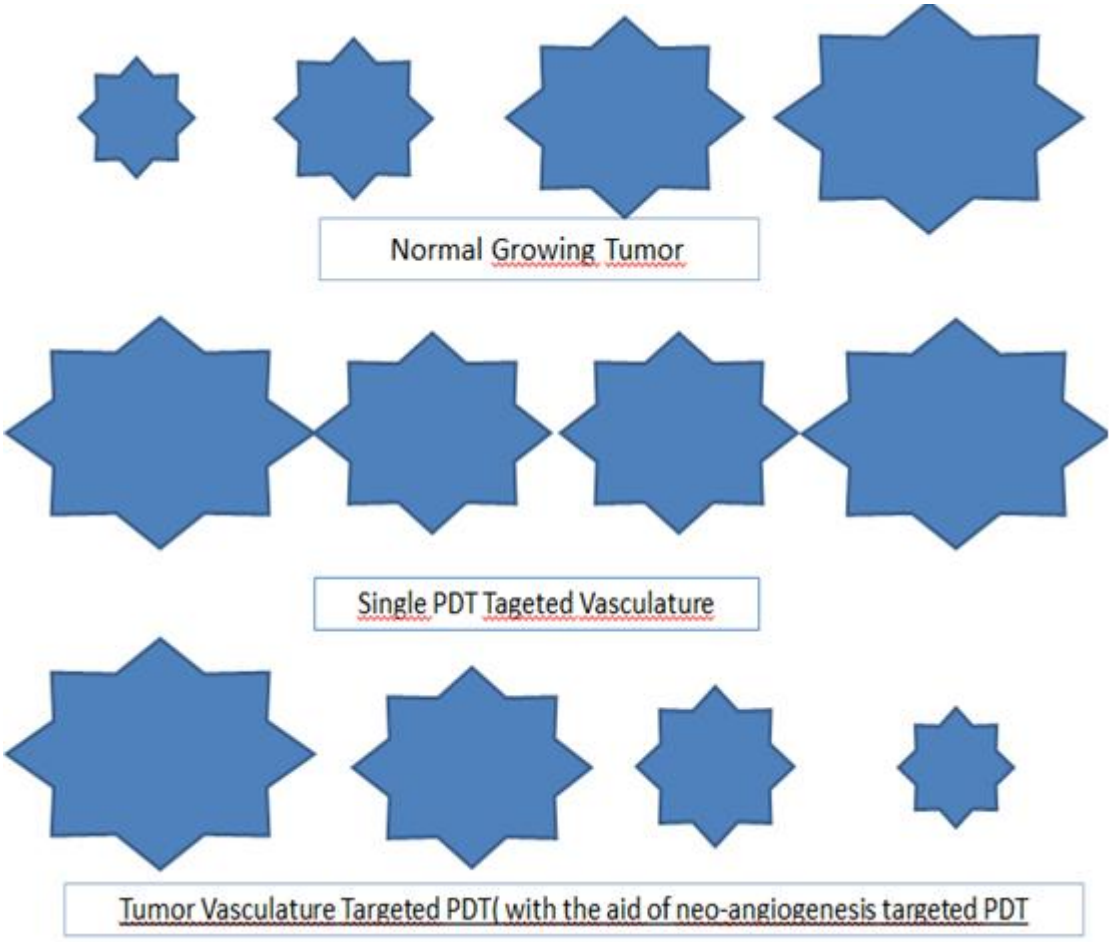

Figure 1.3: Tumor Vasculature Targeted PDT(with the aid of neo-angiogenesis targeted PDT)

1.2 Purpose of the Thesis

Aim of this study is to develop new light source system for *in vitro* photodynamic therapy (PDT) applications. Light system design will be light emitting diode (LED) based. This optical architecture will be designed in accordance with cell culture studies. Power and energy distribution of each well could be controlled by controller based computer. Modulation is achieved by using this system.

CHAPTER 2

LIGHT SOURCES FOR PHOTODYNAMIC THERAPHY

In PDT studies, coherent and noncoherent many light sources, from lasers to fluorescent lambs, were tried and each have adventages and disadventages compared to each other. [10, 11]

The light sources that are used for photodynamic therapy are one of the most important part of the experiment or the therapy. The wavelength, power measurements and other conditions should be suitable for the chemical to become active. On the other hand it should not affect the organism (or the affects should decrease as soon as possible).For example if the activation wavelenhgt of the chemicals are between the UV region, it makes person cancer in place of cure.

2.1 Laser

As a light source, LASER has been the first choice for Photodynamic Theraphy especially for the last 30 years. Especially metal vapour lasers and argon lasers. Because of their power output, the acceptable wavelenghts that can excites the photoactive drug and coupling with optical fibers to achieve some inner parts of the body with endoscopes.[11]

Table 2.1: Laser types with respect to wavelenghts

FAR ULTRAVIOLET	NEAR ULTRAVIOLET	VISIBLE	NEAR INFRARED	FAR INFRARED
238-284 nm	308-364 nm	416-700 nm	700-1350 nm	1540 nm
Krypton SHG	Argon	Ruby	Cr:Forsterite	Er:Glass
Argon SHG	XeF	Krypton	HeNe	
KrF	N2	Cu	Argon	
	XeCl	InGaAlP	Nd:YAP	
		HeNe	Nd:YAG	
		HeCd	Nd:Glass	
		N2+	Nd:YLF	
		DPSS	Ti:Sapphire	
		Argon	InGaAs	
			Krypton	
			Cr:LiSAF	
			GaAs/GaAlAs	

2.2 Lamp

The other light source to use in photodynamic theraphy is lamps. In some PDT applications filtered light sources are used. Altough Lasers are the first choice for he Photodynamic Therapy lamps are easy to use and cheaper than Lasers. For large areas Lamps are good choices.

There are some types called Tungsten Filament Quartz Halogen Lamps(From UV to IR), Xenon Arc Lamps(from 300 to 1200 nm), Metal Halide Lamps(),Phosphor-coated Sodium Lamp and Fluorescent Lamps.[10]

Some investigations shows that microlamps can use for photodynamic applications and dosimetry.[12]

2.3 Light Emitting Diodes(LED)

Beside the adventages of Laser, there are some disadventages. In order to eliminate these disadventages there is a third light source for PDT: Light Emitting Diodes(LED).Due to its emission band, size, weight, flexibility, ease of operate and the most important part ‘It’s COST’.[13]

A Light Emitting Diode(LED) is an electronic circuit element which emits light when current passing on it.

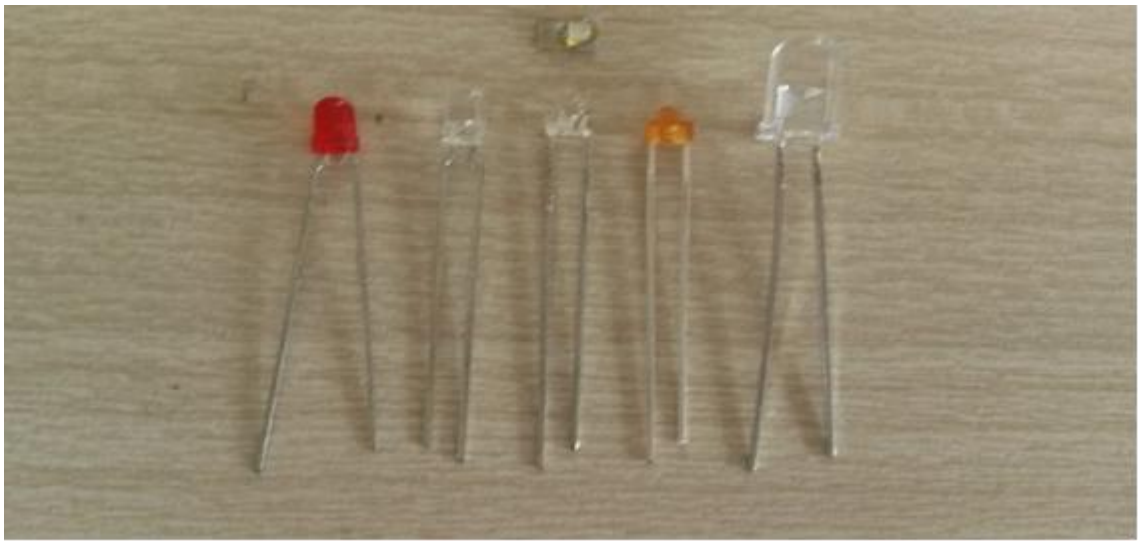

Figure 2.1: Light Emitting Diodes

General design of light emitting diode:

• The semiconductor layer spread on a substrate, when it exposed current, emits light all arount the layered area.

• The structure positioned in a small reflectice aparatus

• This aparatus allows user to reflect the light toward the desired exit direction and the angle.[10

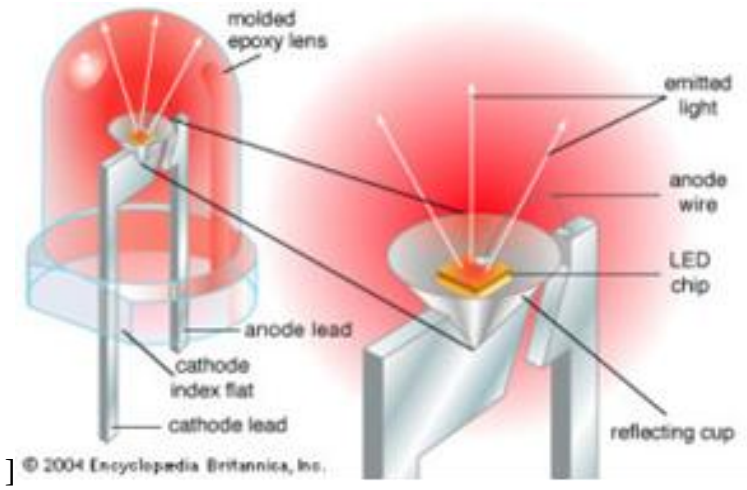

Figure 2.2: Light Emitting Diode working principle.[17]

Light Emitting Diodes become the new favorite in the field of medical treatment and phototheraphy since the begining of 1980. Treatment of rhinitis, arthiritis, jaundice, joint-tissue inflammation, skin abnormality, and for the relief of stress, seasonal affective disorder, as well as biological clock disorders. LEDs are the first light source to provide the capability of true spectral composition control.In order to optimize the effectiveness Light emitting diodes allows wavelengths to match the requirement of the medical treatment.[6]

The adventages of using Light Emitting Diodes in photoactivation are

- Compactibility(very easy to reach distal area)
- Easily integrated to control systems (phototheraphy or threatment stage)
- Safer to operate than other light sources
- Need low voltage power supply…

CHAPTER 3

A NOVEL LED BASED LIGHT SYSTEM FOR PDT APPLICATIONS IN 96 WELL-PLATE CELL CULTURE

In vitro photodynamic therapy (PDT) applications in different cancer cell lines are preliminary studies for in vivo clinical trials. Parameters, such as optical power, duration and amount of photosensitizer, determined in these studies are practiced on animal studies, and then it becomes possible to start human trials. In this study, effect of a novel LED-based light source developed for 96-well-plates cell culture applications, was tried on AGS stomach cancer cell line, in combination with Poly(amido amine) (PAMAM) modified – porhyrin molecule.

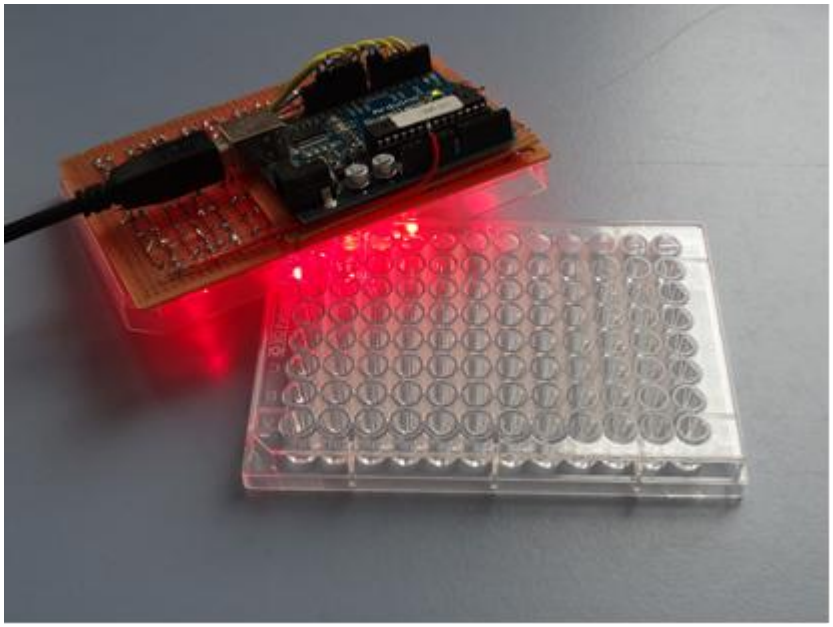

Figure 3.1: In-Vitro experiment cell seed on 96 well plate

LEDs have low power consumption and high light –power output, as well as do not warm in long term usage. Although LED irradiation is not monochromatic, they have quite narrow irradiation spectrum. In this study, a novel LED-based system by taking the advantages mentioned above was designed especially for 96-well-plates. System was designed for in-vitro PDT studies. Every LED, assembled on perforated plate such that one LED for one well. System was controlled by ARDUINO® control card and irradiation time and modulation frequescies can be entered from computer user interface.

3.1 Desing

Light source is composed of a 12x8 LED array and every well is paired with a LED which is fixed on a perforated plate. LED system is connected to ARDUINO® control card that can be controlled by a computer.

3.2 Microcontroller and Sortware

Microcontroller:

The system uses a microcontroller called Arduino Duemilanove. This is a open-source microcontroller. It has 6 analog and 14 digital input-output. This board connect to compiter with Universal Serial Bus(USB) cable.

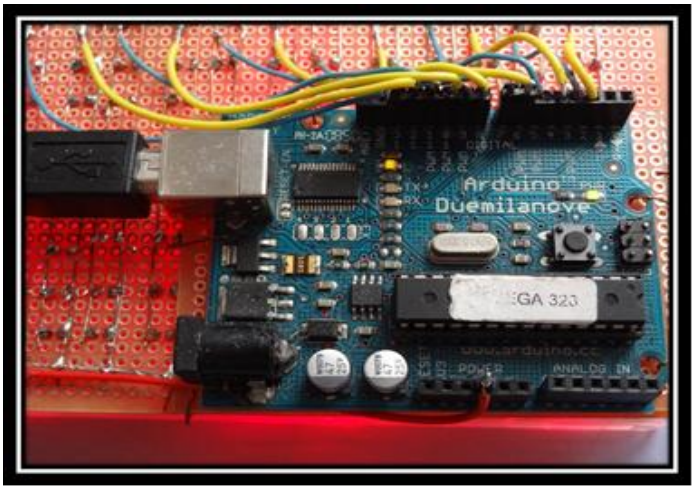

Figure 3.2: System Control Card ARDUINO

Table 3.1 : Properties of Arduino Control Card

Summary

Microcontroller	ATmega328
Operating Voltage	5 V
Input Voltage (recommended)	7 to 12 V
Input Voltage (limits)	6 to 20 V
Digital I/O Pins	14 (of which 6 provide PWM output)
Analog Input Pins	6
DC Current per I/O Pin	40 mA
DC Current for 3.3V Pin	50 mA
Flash Memory	32 KB of which 2 KB used by bootloader
SRAM	2 KB
EEPROM	1 KB
Clock Speed	16 MHz

Software:

Appendix A includes program code.

3.3 Measurement of Power

The point that seperates this light system from the other light systems is the power measurement of the cells. The power measurements of whole cells should be the same to have an effective results.

Light emitting diodes allows us to illuminate small areas effectively. The angle of the light emitting diodes that is used for the illumination system should be very small. Because the cells of the 96 well plate are very close to each other. If the angle is greater than we need the light emitting diodes on the cell effects the other cells.

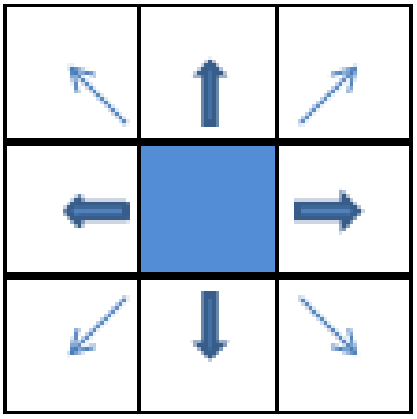

Figure 3.3: Effects of neighbour cell

If the angle of the light emitting diode used for light system are not small we have 3 different power measurements.

***Cells with 3 neighbour cells

***Cells with 5 neighbour cells

***Cells with 8 neighbour cells

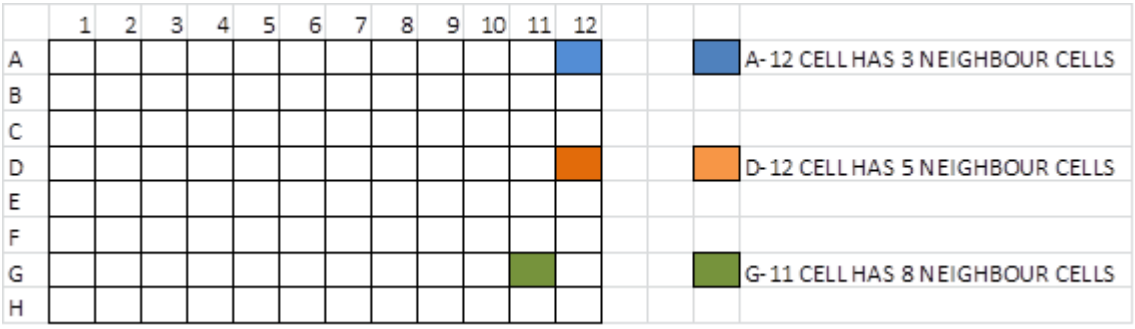

Figure 3.4: Amount of neighbour cells at 96 well plate

Whole the power measurements have been done by the Thorlabs Power Meter.

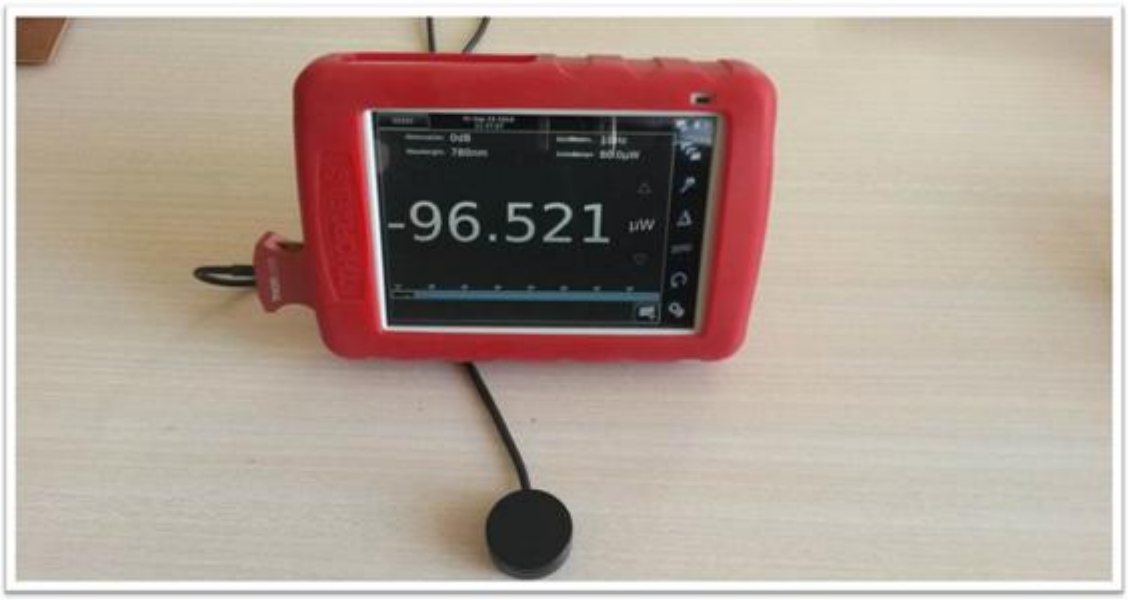

Figure 3.5: Thorlabs Power Meter

CHAPTER 4

RESULTS

Experiments with LED based light sources(Ended):

MEHMET NECMI BURGUCU-650 nm peak wavelenght-effective dose experiment:

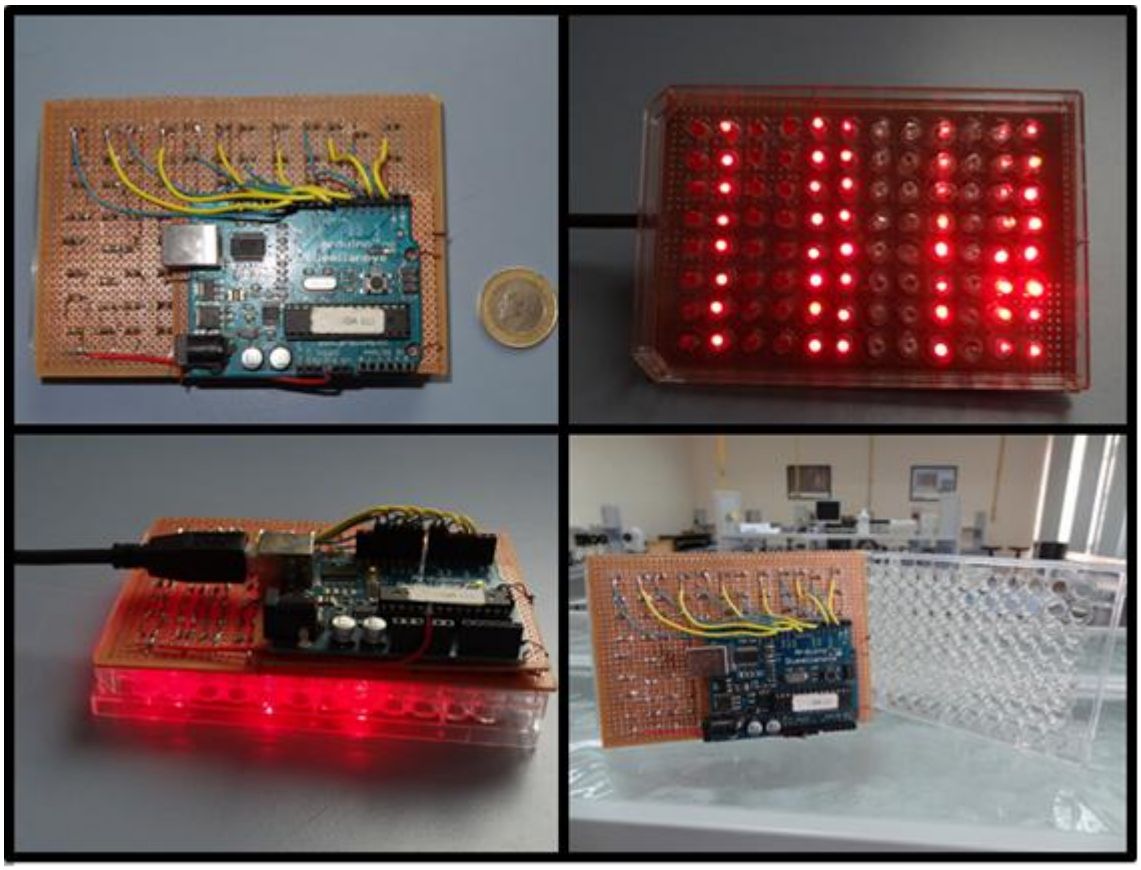

Figure 4.1: 650 nm illumination system

Cell culture

AGS cells were cultured in DMEM (Dulbecco's Modified Eagle Medium from GIBCO) medium containing 10% (v/v) fetal bovine serum (FBS), 1% penicillin-streptomycin, in a humidified atmosphere of 95% air and 5% CO2 at 370C. Cells were subcultured twice a week for continuous culture.

Cell Viability Assay

AGS cells were seeded in 96 well plates (1×104 cells / well) and preincubated for 24 hrs before the experiment. The chemicals PPIX 0, 1, 2, 3 were added into the culture at a ratio of 1:1, 1:10, 1:25, 1:50 and 1:100 dilutions and incubated for 24 hrs.

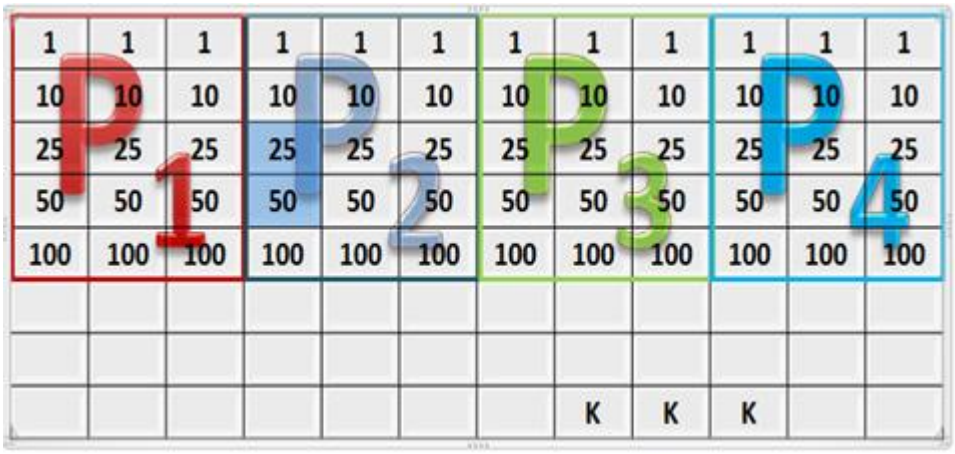

Figure 4.2: Effective dose experiment cell doses

After incubation with chemicals, one plate was left in dark for 30 min and another plate was irradiated for 30 min. LEDs irradiate at 650 nm peak wavelength and have 4 mW optical powers (changing according to the LED used). Following exposure to light, proliferation and cell viability assay was performed according to the manufacturer's instructions (WST-1 reagent, ROCHE) in order to observe change in cell number. Afterwards, absorbance was measured at 450 nm using an ELISA reader. Results showed that ***50 μM and 100 μM*** concentrations were effective doses.

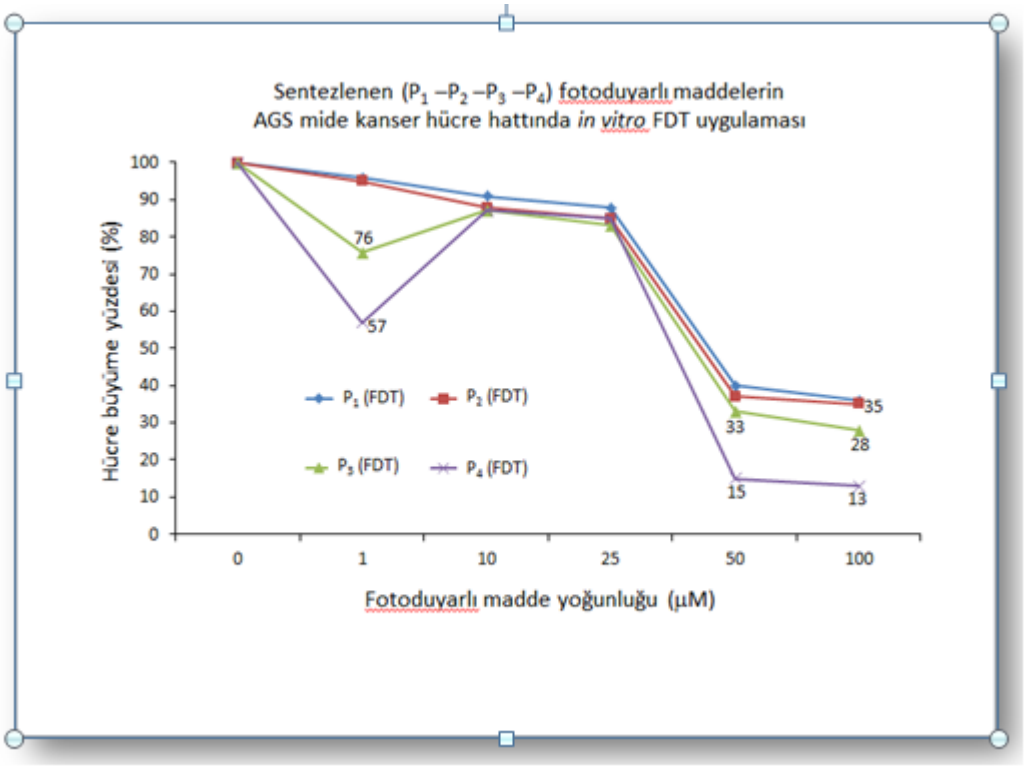

Figure 4.3: Result of efficient dose experiment with 650 nm illumination system

AYŞENUR KIRIS-780 nm peak wavelength[14]

‘COMBINED EFFECTS OF CHEMOTHERAPY AND INDOCYANINE GREEN MEDIATED PHOTODYNAMIC THERAPYON EX-VIVO HUMAN PRIMER BREAST CANCER CELLS’

In order to perform this experiment, due to the amount of photosensitizer and cells Aysenur KIRIS needs to use a 5*4 well plate. So the design of the illumination system changed according to the plate.

Light emitting diodes that is used has 18 mw output power and 780 nm wavelength.(Thorlabs Led code: LED780E).

Approximately 45 minutes of illumination was performed without any temperature increase on LED’s.

The results are published as master thesis.

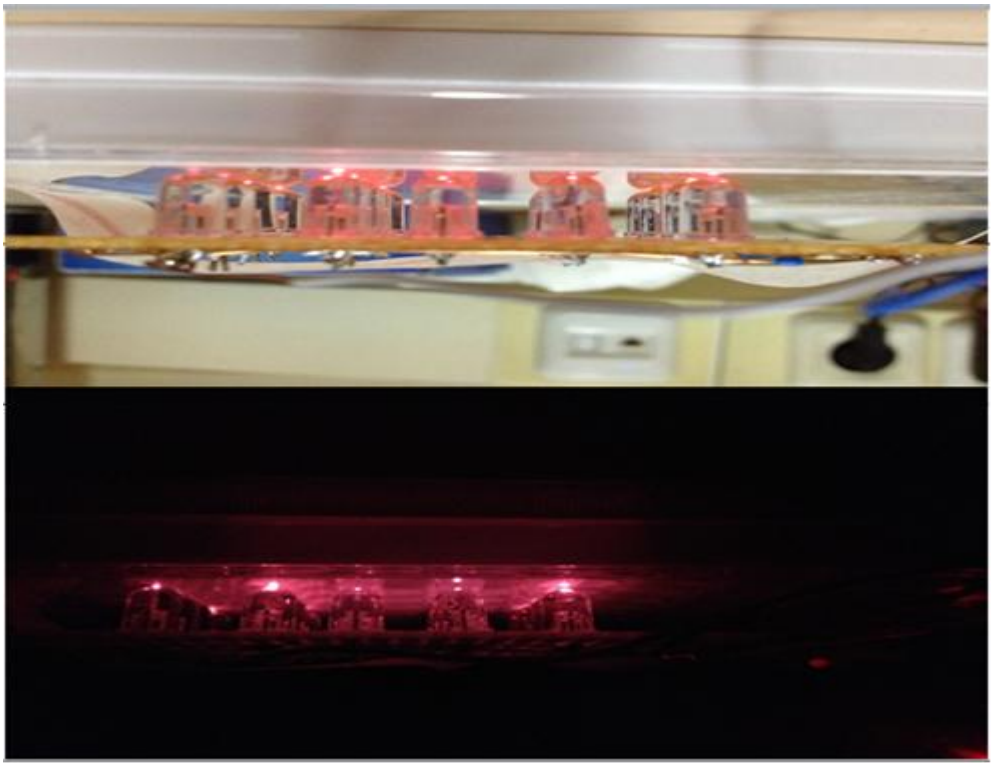

Figure 4.4: 780nm illumination system

TUGBA KIRIS-465 nm peak wavelength[15]

PAMAM MODIFIED PORPHRIN MEDIATED PHOTODYNAMIC THERAPY EFFECTS ON AGS STOMACH CANCER CELL LINES: IN VITRO STUDY

Light emitting diodes that is used has 447.5 nm wavelength. Approximately 30 minutes of illumination was performed without any temperature increase on LED's.The results are published as master thesis.

Figure 4.5: 447.5nm illumination system

DISCUSSION

The results of the experiment shows us‘A NOVEL LED BASED LIGHT SYSTEM FOR PDT APPLICATIONS IN 96 WELL-PLATE CELL CULTURE’ is useful for In-Vitro Photodynamic Theraphy applications. Continouos wave and pulsed wave experiments can be done by this system. The adventages like temperature, ease of operation and cost let us do the experiments efficiently like other light sources with low cost.

CONCLUSIONS AND RECOMMENDATIONS

Not only ın-vıtro and in-vivo studies this light system can be used for animal and human trials with appropriate design.

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Master thesis

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APPENDICES

APPENDIX A

```
int led1 = 13;              // LED connected to digital pin 13
int led2 = 12;
int led3 = 11;
int led4 = 10;
int led5 = 9;
int led6 = 8;
int led7 = 7;
int led8 = 6;
int led9 = 5;
int led10 =4;
int led11= 3;
int led12= 2;
int value1 = LOW;           // previous value of the LED
int value2 = LOW;
int value3 = LOW;
int value4 = LOW;
int value5 = LOW;
int value6 = LOW;
int value7 = LOW;
int value8 = LOW;
int value9 = LOW;
int value10 = LOW;
int value11 = LOW;
int value12 = LOW;
long time1 = millis();
long time2 = millis();
long time3 = millis();
long time4 = millis();
long time5 = millis();
long time6 = millis();
long time7 = millis();
long time8 = millis();
long time9 = millis();
long time10 = millis();
long time11= millis();
long time12= millis();
long interval1 = 1000;      // interval at which to blink (milliseconds)
long interval2 = 1000;
long interval3 = 1000;
long interval4 = 1000;
```

```
long interval5 = 1000;
long interval6 = 1000;
long interval7 = 1000;
long interval8 = 1000;
long interval9 = 1000;
long interval10 =1000;
long interval11= 1000;
long interval12= 1000;
void setup()
{
  pinMode(led1, OUTPUT);      // sets the digital pin as output
  pinMode(led2, OUTPUT);
  pinMode(led3, OUTPUT);
  pinMode(led4, OUTPUT);
  pinMode(led5, OUTPUT);
  pinMode(led6, OUTPUT);
  pinMode(led7, OUTPUT);
  pinMode(led8, OUTPUT);
  pinMode(led9, OUTPUT);
  pinMode(led10, OUTPUT);
  pinMode(led11, OUTPUT);
  pinMode(led12, OUTPUT);

}

void loop()
{
  unsigned long m = millis();

  if (m - time1 > interval1){
    time1 = m;

    if (value1 == LOW)
      value1 = HIGH;
    else
      value1 = LOW;

    digitalWrite(led1, value1);
  }

  if (m - time2 > interval2){
    time2 = m;

    if (value2 == LOW)
      value2 = HIGH;
    else
      value2 = LOW;
```

```
  digitalWrite(led2, value2);
}

if (m - time3 > interval3){
  time3 = m;

  if (value3 == LOW)
    value3 = HIGH;
  else
    value3 = LOW;

  digitalWrite(led2, value2);
}

if (m - time4 > interval4){
  time4 = m;

  if (value4 == LOW)
    value2 = HIGH;
  else
    value4 = LOW;

  digitalWrite(led4, value4);
}

if (m - time5 > interval5){
  time5 = m;

  if (value5 == LOW)
    value5 = HIGH;
  else
    value5 = LOW;

  digitalWrite(led5, value5);
}

if (m - time6 > interval6){
  time6 = m;

  if (value6 == LOW)
    value6 = HIGH;
  else
    value6 = LOW;

  digitalWrite(led6, value6);
}

if (m - time7 > interval7){
```

```
  time7 = m;

  if (value7 == LOW)
    value7 = HIGH;
  else
    value7 = LOW;

  digitalWrite(led7, value7);
}

if (m - time8 > interval8){
  time8 = m;

  if (value8 == LOW)
    value8 = HIGH;
  else
    value8 = LOW;

  digitalWrite(led8, value8);
}

if (m - time9 > interval9){
  time9 = m;

  if (value9 == LOW)
    value9 = HIGH;
  else
    value9 = LOW;

  digitalWrite(led9, value9);
}

if (m - time10 > interval10){
  time10 = m;

  if (value10 == LOW)
    value10 = HIGH;
  else
    value10 = LOW;

  digitalWrite(led10, value10);
}

if (m - time11 > interval11){
  time11 = m;

  if (value11 == LOW)
    value11 = HIGH;
```

```
    else
      value11 = LOW;

    digitalWrite(led11, value11);
   }

  if (m - time12 > interval12){
    time12 = m;

    if (value12 == LOW)
      value12 = HIGH;
    else
      value12 = LOW;

    digitalWrite(led12, value12);
   }
}
```

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

*__Mehmet Necmi Burgucu__ , Haşim Özgür Tabakoğlu : 'LED-Based Light Source Design for 96-well-plate Used in In-Vitro Photodynamic Therapy Studies'. Tiptekno 2012 National Congress. November 1-3, Antalya Turkey.

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