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FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

DETECTION OF TRANSMITTANCE DYNAMICS OF A-549 AND MCF-7 CANCER CELL LINES THROUGH VISABLE AND NEAR INFRARED SPECTRUM

By

Ali Dlawar Noori KHDIRBEGI

MSc. THESIS
BIOMEDICAL ENGINEERING PROGRAMME

ISTANBUL, MARCH/2015

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T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

A-549 VE MCF-7 KANSER HÜCRE HATLARININ GÖRÜNÜR VE YAKIN KIZILALTI SPEKTRUMDAKİ GEÇİRGENLİĞİNİN DİNAMİK OLARAK TESPİT EDİLMESİ

Ali Dlawar Noori KHDIR BAGI

YÜKSEK LİSANS
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
Yrd. DoÇ. Dr. HAŞIM ÖZGÜR TABAKOĞLU

ISTANBUL, MART / 2015

T.C.

FATIH UNIVERSITY

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Ali Dlawar Noor KHDIR BAGI, a **MSc.** student of Fatih University **Institute of Biomedical Engineering** student ID 520113014, successfully defended the **thesis** entitled "**DETECTION OF TRANSMITTANCE DYNAMICS OF A-549 AND MCF-7 CANCER CELL LINES THROUGH VISABLE AND NEAR INFRARED SPECTRUM**", 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

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Dedicated to the little kind baby (Katan),

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I would like to thank everyone for their precious support with me including my family and friends; who helped me in all ways during my studying in Istanbul, I ask God to make them delight in all ways as well.

I am so grateful to have **Assist. Prof. Dr. Haşim Özgür TABAKOĞLU** as my supervisor; who helped me during all my master time, in all ways concerning my studying time. Kind thanks to **Assist. Prof. Dr. Nermin TOPALOĞLU AVŞAR** who came from Izmir to be a member of my committee. Special thanks to my teacher **Assist. Prof. Dr. Şükrü OKKESİM**, who was one of the kindest teachers at Fatih University and always encouraged me.

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ABBREVATIONS

Nm : Nanometer

A-549 : Adenocarcinomic human alveolar basal epithelial cells

MCF-7 : Michigan Cancer Foundation-7

mV :Millivolts

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SUMMARY

DETECTION OF TRANSMITTANCE DYNAMICS OF A-549 AND MCF-7 CANCER CELL LINES THROUGH VISABLE AND NEAR INFRARED SPECTRUM

Ali Dlawar Noori KHDIR BAGI

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Biomedical engineering programme

MSc. Thesis

Assist. Prof. Dr. Haşim Özgür TABAKOĞLU

Cancer is one of the most appalling and widespread disease in the world; its curing , prevention and diagnosing steps are developing day by day and even every minute data will be useful for scientists to develop any new drugs and devices for any of the mentioned purposes to be developed.

In this study, transmittance of light within on two types of cancer cell lines A-459 and MCF-7 in the spectrum of 450 nm and 1100nm has been measured and evaluated comparatively. Differences have been tried to be put forward in not only between two cancer cell lines but also in different wavelengths e.g. visible and near infrared of the given spectrum. According to our findings, critical responses are observed regarding each cell types with different concentrations and the comparison between both types is illustrated.

These data will be useful for any other devices that will be designed to diagnose cancer within these two organs; namely breast and lung.

Keywords: Absorption- Reflection spectrophotometer MCF-7 A-549 cancer cell

FATIH UNIVERSITY-INSTITUTE OF BIOMEDICAL ENGINEERING

ÖZET

A-549 VE MCF-7 KANSER HÜCRE HATLARININ GÖRÜNÜR VE YAKIN KIZILALTI SPEKTRUMDAKİ GEÇİRGENLİĞİNİN DİNAMİK OLARAK TESPİT EDİLMESİ

Ali Dlawar Noori KHDIR BAGI

Biyomedikal Mühendisliği Programı

Yüksek Lisans

Yrd. Doç. Dr. Haşim Özgür TABAKOĞLU

Kanser, dünyada en yaygın ve ürkütücü hastalıklardan biri olarak kabul edilmektedir. Korunma, teşhis ve tedavi aşamaları günbegün geliştirilmekte, bu aşamalara katkıda bulunacak en ufak bir bilgi yeni ilaçların ve tıbbi cihazların geliştirilmesinde yararlı olmaktadır.

Bu çalışmada, iki kanser hücre hattının (A-549: akciğer kanseri ve MCF-7: meme kanseri) 450nm den 1100nm ye kadar olan ışık spektrumunda geçirgenlikleri ölçülmüş ve karşılaştırmalı olarak incelenmiştir. İki kanser hücre hattı arasındaki farklılıkların yanı sıra görünür ışıktaki ve yakın kızılaltı bölgedeki farklılıkları da irdelenmiştir. Elde edilen bulgular değişik konsantrasyonlardaki farklı hücre hatları arasında istatistiksel olarak anlamlı farklar olduğunu ortaya koymuştur.

Bu çalışmanın sonuçları, ileride meme ve akciğer kanserin teşhisinde kullanılmak için tasarlanacak cihazlar için faydalı olacaktır.

Anahtar kelimeler: Absorption- Reflection spectrophotometer MCF-7 A-549 cancer cell

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

The aim of this study is to evaluate and differentiate between different kinds of cancer cells (Lung and Breast cancer cells), in different concentrations by applying and using (Absorption/Reflection spectrophotometer). This was achieved by use of absorption and scattering characteristics of the cells, response to different wavelengths. Absorption and scattering of the light may give a key concept for the size of single cell or molecule of the cell's surface leading to absorption and scattering properties.

1.2 Motivation

This study has benefit in any plans regarding the designing of any clinical devices used for detection and diagnosing maybe also treating patients for curing from cancer; as this study gives information on the responses of the cells to given wavelengths that lies between 450nm to 1100 nm.

CHAPTER 2

BACKGROUND INFORMATION

2.1 What is Cancer?

Cancer is a disease that affects individuals all over the world. It arises from defective interaction between previously normal but now abnormal cells of tissues. This malfunctioning of the cells originates from a change or mutation in the building blocks of the genetic materials found within their nuclei.

Cancer is a major worldwide burden and a leading cause of death. About 12.7 million people were affected by cancer in 2008 globally [1]. This number is expected to rise in the future as the average age of world's population is increasing and partly due to inclining trend toward adoption of western lifestyle especially in the developing countries from which the exposure to numerous well documented risk factors will be a major contributing factor. It is estimated that by 2030 the annual rate of cancer will increase to 21.4 new cases [2].

Cancer control strategies should implement a balanced approach which comprises primary prevention aiming at prevention of exposure to risk factors, screening for early detection and effective treatment. Well organized screening programs for specific population groups over regular intervals had proven to have tremendous effect on reduction of some cancer rates [3]. It is proven that some cancers can be detected early and prompt interventions provide promising outcomes like cancers of the breast, cervix, colon and rectum [3]

Cancer screening tests include the use laboratory tests and imaging techniques. The most critical factor in determination of the outcome and prognosis of cancer is the stage of the disease at the time of diagnosis. While the histopathological of the suspicious tissues is the gold standard of diagnosis, obtaining tissue biopsies with

subsequent analysis is an invasive, expensive and time consuming approach. Meanwhile appliance of optical detection technologies provide easy cheap and non-invasive technique not merely for diagnosis but also for post-management monitoring of the patients.

These technologies depend on the interaction of the light with tissue specimens examined. Through observation and comparison of optical properties of normal and cancerous tissues, useful parameters can be formalized to differentiate between them.

Various techniques used for optical detection of cancer include vibrational spectroscopy (Raman spectroscopy) which is used to gain information about the biochemical constituents of the body tissues, fluorescence spectroscopy which analyses the fluorescence from the sample tissues, reflectance spectroscopy which measures the characteristic spectra obtained from sample tissues to determine whether they are normal or not depending on the changes that occur within the cell after or in the period of transition to cancerous cells, optical coherence tomography which a high resolution non-invasive imaging technique used for visualizing biological tissues, and Narrow band imaging (NBI) which used for improving the diagnostic value of endoscopic diagnostic investigational tools [4].

2.2 Development of cancer

Multicellular organism can survive only when their cellular regulatory mechanisms work properly. All of the processes that occur within a cell is well regulated. Cell cycle is the process by which cells divide and duplicate. Although there are many type of cancers, all cancers have the property of that they arise when the normal regulatory mechanism of cell division is disrupted. Not single but many factors over long period of time when accumulated will lead to cancer or inappropriate cell growth i.e. for most of the cancers there is a long interval in terms of time between the initial events preceding and leading to the cancer itself [5].

A hallmark of cancer is loss of control of the cell cycle. This loss of control is due to inherited and acquired environmental factors that will induce mutation or changes within the genetic material of the cell.

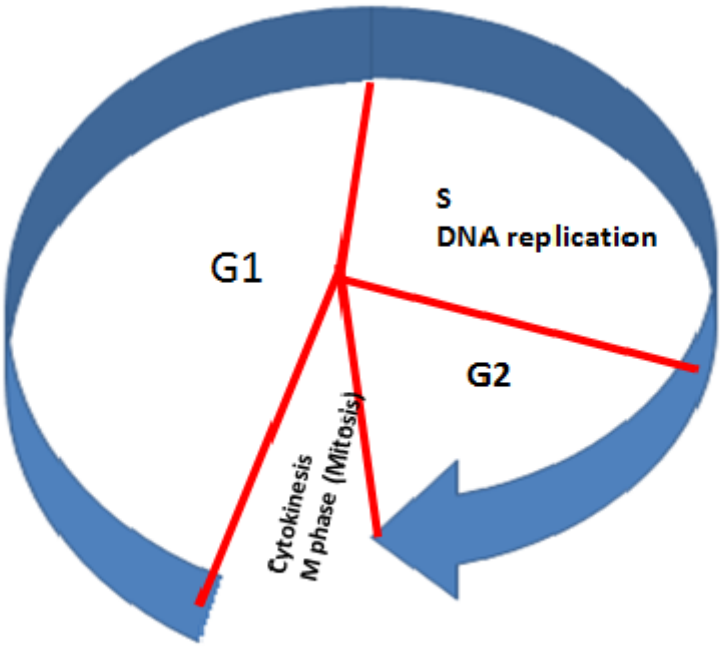

Figure 2.1: schematic of cell cycle; Reproduced from [6]

Although most of the cancers results from combination of both inherited and environmental factors, it's estimated that only 5% of all cancers is strongly hereditary (3). The non-hereditary mutations are also called somatic mutations. Risk factors and causes can further be subdivided to preventable and non-preventable factors. It is estimated that nearly half of all cancers are potentially preventable. Plenty of environmental factors have been contributed to developing cancers. Major risk factors and causes of cancer are depicted in the table 2.1

Table 2.1 Major contributing factors to cancer in developing and developed countries[7]

Causes	**Developed countries**	**Developing countries**
Diet or nutrition	30 %	20%
Infections	8%	26%
Tabacco	16%	10%
Occupational Exposures	5%	--
Environmental Pollutions	2%	--
Other	19%	44%

2.3 Genetic of Cancer

Only a small proportion of the human genome have been associated with cancer, these group of genes are categorized to oncogens, tumor suppressor genes and DNA repair genes.

Oncogens are genes that have the potential to develop into cancer; it arises from its original form which is called proto-oncogen. Proto-oncogens carry the code for the proteins that regulate the normal cell growth and differentiation (8). Proto-oncogens can develop in to oncogens by several mechanism, these include (a) point mutations, deletions or insertions, (b) Gene amplification events, and (c) chromosomal translocations [8]. Some examples of oncogens include HER-2/*neu (erbB-2)* associated with breast cancer, *myc* associated with many cancers like B-cell leukemia and Burkitt's lymphoma, *ras* associated with cancers of lung, bladder, ovary, thyroid and pancreas, *src* associated with certain types of breast, lung and colon cancers [8].

Tumor suppressor genes are involved in suppressing cell division, resolving damaged parts of DNA, and triggering timely programmed cell death otherwise known as apoptosis. While oncogens cause cancer when activated Tumor suppressor cells cause cancer when deactivated. Examples of Tumor suppressor genes include pVHL, APC, CD95, ST5, YPEL3, ST7, and ST14. Most of the mutations in the tumor suppressor genes are acquired [9]. A well-known example is p53 protein which is encoded by Tp53 gene. Mutations in the Tp53 gene have been found in more than half of the cases of cancers [10].

2.4 Tumor Biology

The development of a cancer from a single genetically altered cell is a multistage process, so cells become cancerous through a progressive sequence of changes. The first step in development of a tumor is hyperplasia. Although during hyperplasia the cells have normal appearance, however their number in increased inappropriately. Hyperplasia differs from hypertrophy in that in hypertrophy the size of the cell is increases while in hyperplasia the cells increase in number.

The second step in tumor development is dysplasia, it differs from hyperplasia in that the cells are not looked normal anymore, and there will be changes both at macroscopic and microscopic level. The cell mass become disoriented.

The next step in carcinoma is in situ or the cancer in its original place. Carcinoma in situ is a high grade dysplasia and the cells become more abnormal than before. This is a precancerous stage and for those types with risk of developing in to metastatic cancer surgical removal at this stage is mandatory. Tumors that remain in this stage are termed benign while those that progress to later stage with the ability to spread are termed malignant [5].

The last stage is malignant stage which is the most dangerous and accounts for almost all cases of cancer deaths.

Cancerous cells have different properties from those of normal cells. One of the primary properties of cancer cells is that they don’t show density-dependent inhibition [5]. Normal cells proliferate until they reach a certain density and after that they cease proliferation and become inactive and arrested at G0 stage of cell cycle, while

cancerous cells don’t have this property and proliferate uncontrollably [5]. Also cancerous cells don’t show apoptosis; normal cells have the ability to kill themselves if they are damaged however cancerous cells continue to grow. Cancer cells have reduced requirement for growth factor which is essential for growth and proliferation of normal cells and sometimes cancerous cells produce growth factor of their own and stimulate their own proliferation.

Normal cells have adhesion molecules on the surface of their cell membrane that allows them to attach to other cells, this will allow them to remain in their proper location and also interact with the neighboring cells properly, on other hand cancer cells don’t have this ability due to their inability to code for surface adhesion molecules and this is the reason behind the metastases and spread of these cells away from their original location. Factors that aid the distant spread of cancerous cells include secretion of proteolytic enzymes and also secretion of growth factors important for stimulating angiogenesis to supply them with nutrients. Another cardinal feature of cancerous cells is their inability to differentiate, which is consistent with their continuous proliferation.

2.5 MCF-7

It is one of the types of the breast cancer cell lines that was isolated from a Caucasian old woman at the age of 69 in 1970. This tumor cell line is the acronym of Michigan cancer foundation-7, that refers to the institute that is located in Detroit the place where the cell line was established in 1973 by a researcher call Herbert Soule and his co-workers. Barbara Ann Karmanos Cacer institute, is the current name of the Michigan Cancer Foundation [36]

Here are some of its properties :

- Primary tumor: invasive breast ductal carcinoma
- Origin of cells: pleural effusion
- Presence of estrogen receptors: yes
- Proliferative response to estrogens: yes
- Presence of progesterone receptors: yes [36]

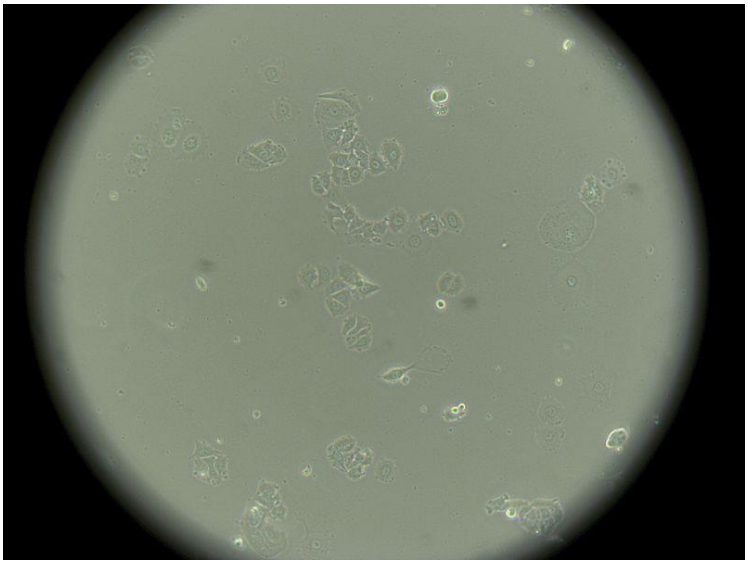

Figure 2.2 MCF-7 cells[36]

2.6 A-549

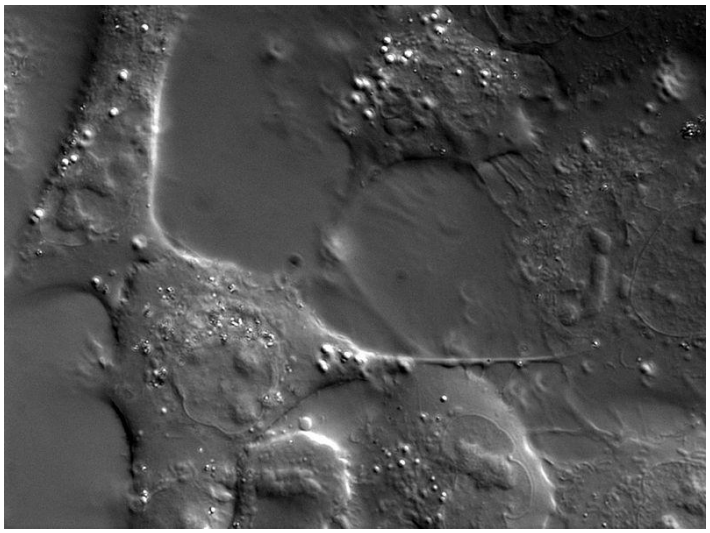

Figure 2.3 A549 cells under DIC microscopy[37]

They are adenocarcinomic human alveolar basal epithelial cells. For the first time A-549 was developed by D.J.Girard et al. in 1972 through the removing and culturing of the lung tissue that was cancerous in the explanted tumor of and old-caucasian man at the age of 58. In their nature, the cells take role in diffusion of some substances like water and electrolytes through the alveoli of the lungs and they are squamous.

Synthesizing lecithin, containing high level of unsaturated fatty acid that take role in maitaining the membrane phospholipids, are of the remarkable characters of this cell

line. This type of cell line, they are used in a wide range for type II pulmonary epithelial cell model for drug metabolism [37].

2.7 Various optical detection technologies

2.7.1 Raman spectroscopy

Raman spectroscopy is one of the most common vibrational spectroscopies used for determination of molecular motions. This method depends on the inelastic scattering or Raman scattering, in which interaction between a monochromatic light directed at a sample with the sample yields a specific spectra which can be visualized.

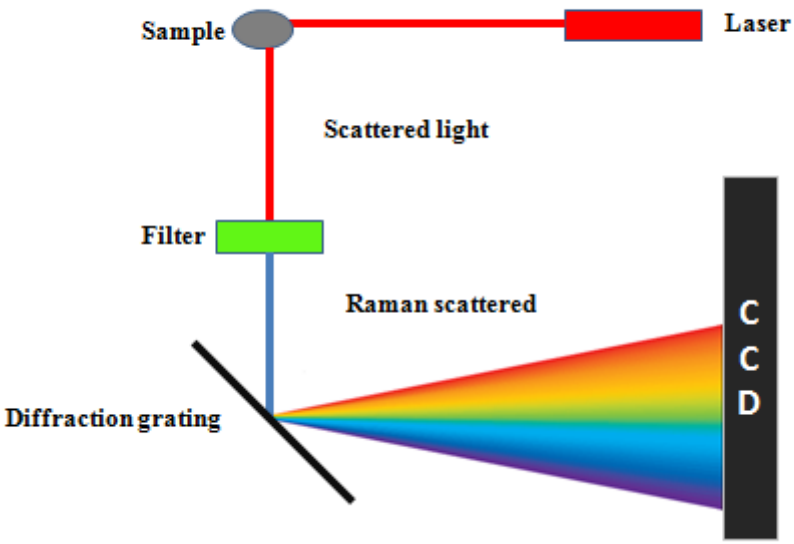

Figure 2.4 Raman spectroscopy; Reproduced from [11]

The photons emitted from the monochromatic source of the light will interact with the excited state of the sample resulting in the change of the energy of the photons respective to the change of the wavelengths; this will provide information on the vibrational modes of the sample. These changes then can be used for analysis and interpretation or identification of changed elements within a system. Some of the applications of Raman spectroscopy include investigation of biopsy samples, deep tissue studying, measuring drug efficacy, treatment monitoring, cytology

investigations and etc. In another meaning it can be used to differentiate between normal and cancerous cells.

Role of Raman spectroscopy in cancer

Raman spectroscopy can be used in detection of many cancers. Raman spectroscopy can play an important role in distinguishing between tumor and non-tumor brain tissue [12]. According to the Brain Research Trust, brain tumours are diagnosed in ~13000 people in Britain each year (of which 60 % are metastatic). Using Immersion Raman spectroscopy researchers were able to differentiate between normal, primary and secondary metastatic brain tissue tumors [13].

Raman spectroscopy can be very useful in cases of breast cancer. Patients diagnosed with breast cancer and requiring surgery for removal of the confirmed cancerous breast tissue require further assessment of the regional lymph nodes before deciding whether to remove these nodes or not. This often requires taking samples during the first surgery and sending them to an expert pathologist for assessment before deciding whether a second operation of nodal removal is necessary or not. An effective, rapid and timely approach would be an intraoperative assessment. This can be provided by Raman spectroscopy as a group of researchers from UK found out that it has specificity and sensitivity near to that of histopathological assessment [14].

2.7.2 Fluorescence Spectroscopy

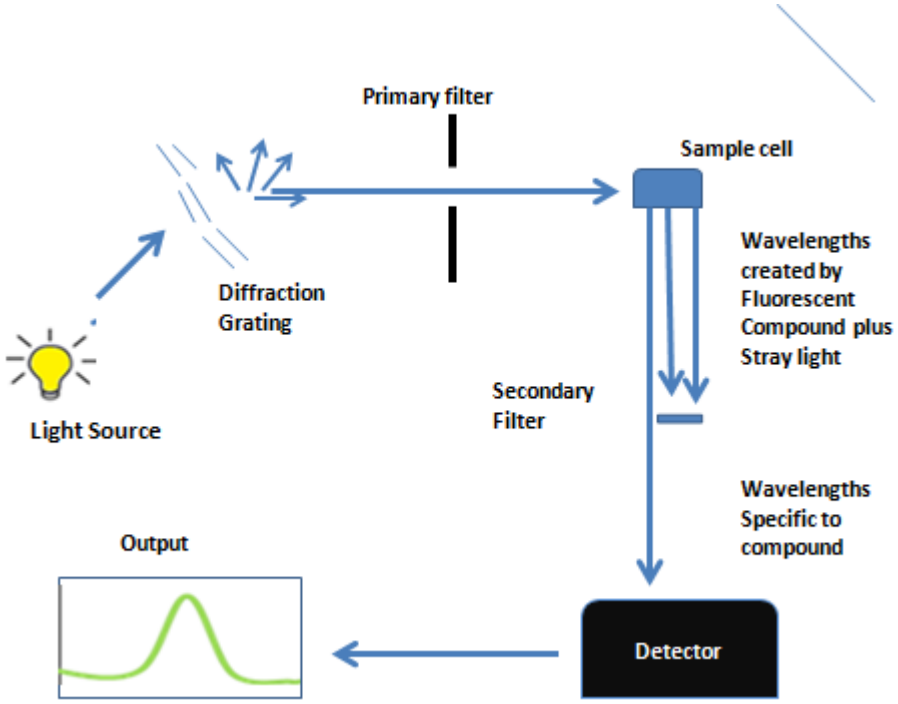

Figure 2.5 Fluorescence spectroscopy; Reproduced from [15]

Fluorescence spectrometry is a type of electromagnetic spectroscopy. As it is known light emissions from bodies are called luminescence and florescence spectroscopy is used to analyze a type of luminescence that is called fluorescence. When these emissions occur these bodies lose energy and for this process to be continuous there must be an external source of energy supply. When this source is from an infrared, visible or ultraviolet light, it is called photoluminescence and this is the principle of any fluorometric analysis. These processes also occur naturally in nature in from various minerals and various biological states.

Fluorescence spectroscopy in cancer

1-In vivo laser induced fluorescence spectroscopy can be a valuable diagnostic tool for basal cell carcinoma and squamous cell carcinoma of the skin. Furthermore actinic keratosis especially in light colored skin individuals can be diagnosed with this technique [16].

2-light induced fluorescence spectroscopy can be used to detect adenocarcinoma of stomach and esophagus, also for squamous cell carcinoma of esophagus [17].

3- Difference in fluorescence of nicotinamide adenine dinucleotide dehydrogenase and collagen can be used as cost-effective, reliable method for differentiating the cervical dysplastic tissues from normal tissues in cases with a high specificity and sensitivity [18].

4-Studies on animal models have validated that fluorescence spectroscopy can be a useful tool for diagnosis of cancers within the oral cavity [19].

2.7.3 Optical Coherence tomography

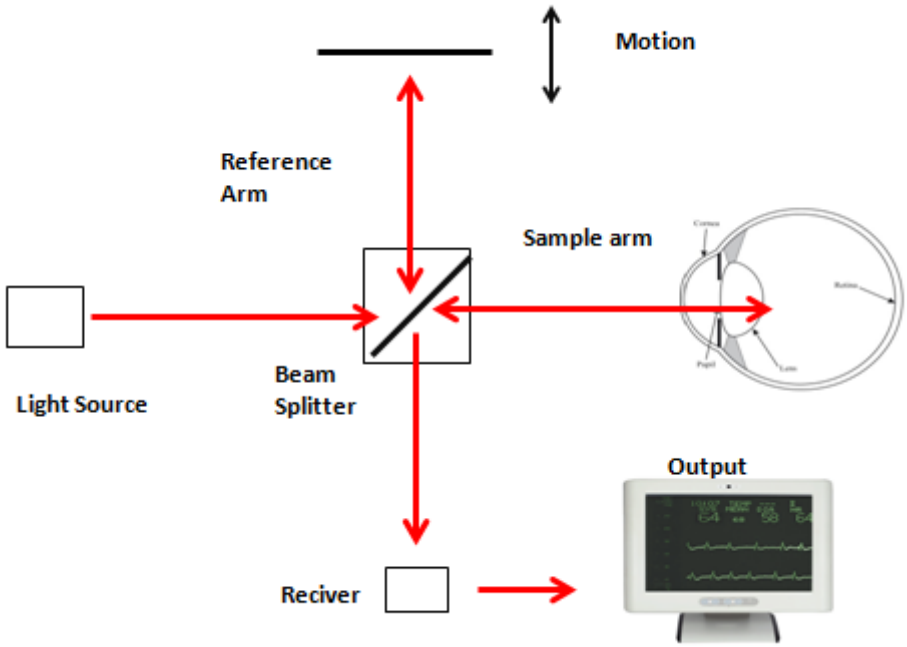

Figure 2.6 Optical coherence tomography; Reproduced from [20]

OCT is a non-invasive diagnostic technique that typically uses near infrared light to obtain two dimensional and three dimensional high resolution images from light scattering media such as biologic Medias.

OCT depends on the concept of interferometry. In interferometry waves which are usually electromagnetic are manipulated to obtain information about the waves and it is a technique employed in many fields from which optics is one of them.

OCT is able to provide real time images at a resolution ten times better than endoscopic ultrasound, thus it will allow us to identify microscopic characteristics of many biological samples as villi, glands, crypts, lymphatics, and blood vessels.

Although OCT provide high resolution images but it is limited to 1-3 mm depth of penetration.

Optical coherence tomography in cancer

1-Ovarian cancer represents a leading cause of cancer related deaths in females, particularly in US. This is mainly due to the fact that this type of cancer can not be diagnoses and managed at an early phase. Optical coherence tomography can characterize the microscopic features of human ovaries. Laparoscopic OCT device has the potential of being used as an effective technique to diagnose this type of cancer due to its ability to differentiate between normal and surface epithelial neoplasms of ovaries [22].

2-OCT is also useful for imaging of the cases of breast cancer. OCT represent a potential to determine the margins of the tumors in vivo. Through combination with the histologic examination which is considered the gold standard for this purpose, researchers were able to obtain high resolution images of breast tumor boundaries and also obtaining the microstructural details of them [22].

3-Because OCT can work with a fibro-optic probe, it can be used to access any anatomic structure. This is particularly important for cases cancerous or pre-cancerous oral lesions. The challenge in these particular types is that the lesions are usually multifocal and also require multiple biopsies before the definitive diagnosis. A study was done and has shown that OCT is capable of detection of these types of cancers and also provides detailed complex images [23].

2.7.4 Narrow band imaging

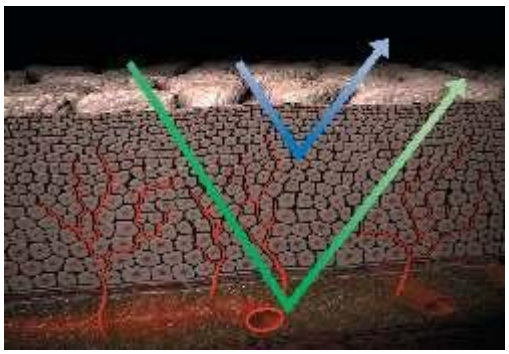

Figure 2.7 Narrow band imaging; Reproduced from [24]

Narrow band imaging is a method that is used to increase the diagnostic value of the endoscopic diagnostic tools [25]. NBI can be a valuable addition to widely used

conventional white light endoscopy. Both NBI and standard endoscopy can be used simultaneously and yield great results together.

NBI uses special filter to direct light with a specific wavelength at tissues. It will produce the greatest contrast between the vessels and surrounding mucosa, this will enhance the characterization of abnormal tissues and differentiate them from normal ones. In addition to that NBI can determine which area is the best for taking biopsies for further investigation [26].

Narrow band imaging in cancer

Application of narrow band imaging in cases of bladder cancer has significantly improved and established proper care for these patients [26]. With NBI better quality care can be provided by improving the visualization of lesions and also early diagnosis with early intervention. Moreover NBI will be at a lesser cost and also provide requirements at the demand of the patients. NBI provide new treatment modalities for urologists, it has been approved by FDA to be used in cases of Non muscular invasive bladder cancer for both treatment and diagnosis and assisted by cystoscopy its better than conventional light imaging for detection of bladder tumors [26].

NBI also is an effective technique in cancers of head and neck. Recently, NBI was used for diagnosis of oral, oropharyngeal, hypopharyngeal, nasopharyngeal and laryngeal pathologies [27].

CHAPTER 3

MATERIALS AND METHOD

3.1 Absorption / transmission/ Reflection Spectrophotometer

The system of ZLX- AS uses for measuring high quality of light absorption performance, and have a wide range of solid and liquid sample's reflection and transmission.

Ability of the system used from UV to IR with high affinity can be achieved with appropriate light source of monochromator grating and detector section which are difficult to obtain with other conventional designs of spectrophotometers.

Integration optical sphere can be used to for measurements of diffuse reflections and other requirements

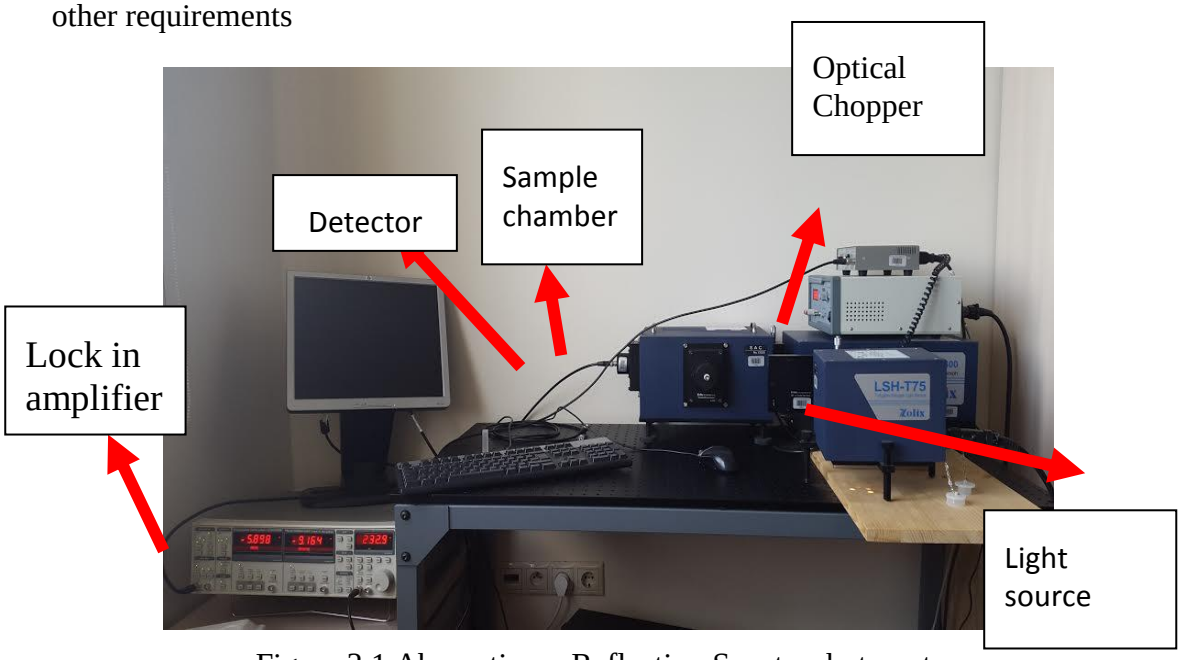

Figure 3.1 Absorption – Reflection Spectrophotometer

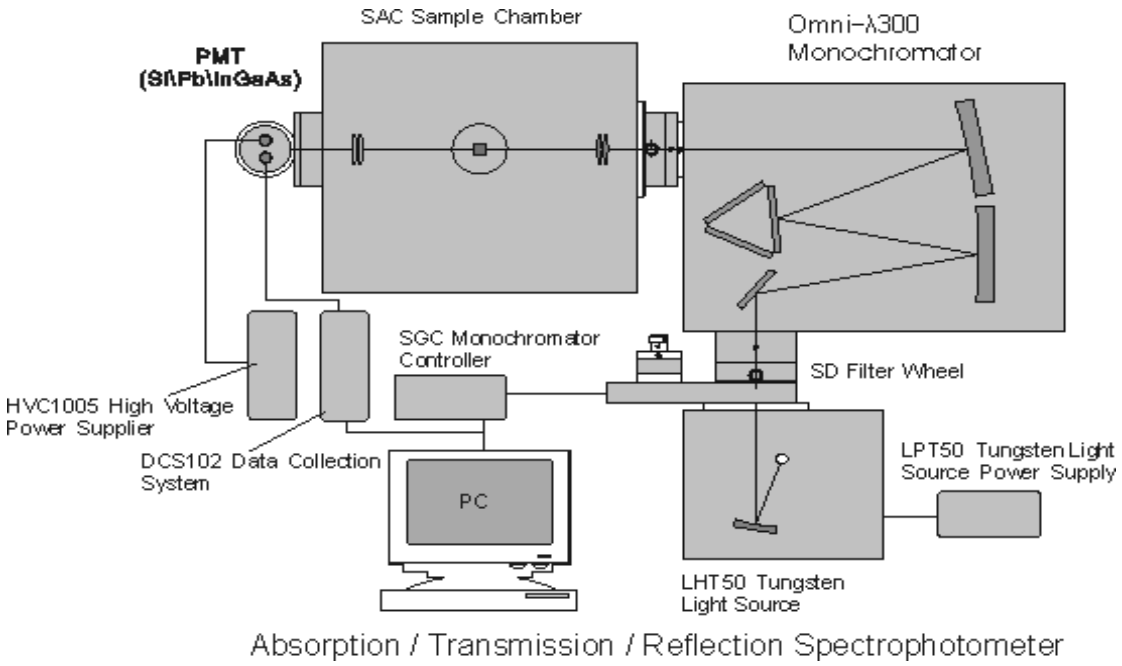

Figure 3.2 Sketch of Absorption Reflection Spectrophotometer[28]

3.1.1 Monochromator

Very sensitive and fully automatic system of 300 nm is used for multi-purposes. Device has manually adjustable slits for precision control. The multi-purpose monochromator are applied to systems with extended resolution and flexible grating options from UV to IR, are excellent optic performance.

Changing the wavelength controls stepping and motor scanning system. Optional internal form experiment requires attenuation of signal set up to USB2.0 computer installation interference.

There are different imaging spectroscopy, related spectroscopy and Zolix spectroscopy

3.1.2 Sample chamber

Evaluation of sample chamber by SAC port is configured; and it is used for the application for measuring, transmission, reflecting and absorption.

The SAC contain different quartz lens which is designed to make beam parallel and converges while passing through samples. Application of the parallel beam was for measuring transmission, reflection and absorption of interference filter. While application of converge beam is for measuring reflection, absorption and density of

gas and liquid sample. Also quartz lens focuses beam and turns 360 degree properly of sample.

Figure 3.3 Sample Chamber [29]

3.1.3 DSi Silicon Detector

There are different kinds of detectors in Zolix instrument each are specific for different wavelengths. Silicon detectors are sensitive photodetectors, with the range of spectral of UV between 200-1100 nm.

Silicon detector composed of DSi200 built-in UV sensitive Si detector, DSi300 for the low drack current detector and 1-vamplifier for the current to voltage conversions.

Specifications of detector is given below Table 3.1.

Table 3.1: Specifications of DSi200 detector.

SPECIFICATION OF DETECTOR	DSi200 (UV sensitive)
	Import UV Enhanced
Effective receiving Area (mm^2)	100(Φ11.28)
Spectral range (nm)	200-1100
Peak wavelength (nm)	-------
Peak Responsivity (A/W)	0.52
Response @254nm (A/W)	0.14(>0.09)
Response time (μs)	5.9
Operating Temperature (℃)	-10～+60
Storage Temperature(℃)	-20～+70
Shunt resistor R_{SH}(MΩ)	10(>5)
Noise equivalent power NEP (W/√Hz)	4.5×10^{-13}
Dark current (25℃；-1V)	-------
Junction capacitance (pf)	4500
Signal output	Current
Output signal polarity	P

Figure 3.4 DSi Silicon Detectors [20]

3.1.4 Tungsten-Halogen light source

Long life of the Zolix light is about 4000 hours and the wavelength is spectral ranged from 300-2500nm.

Figure 3.5 Tungstem-Halogen Light Source [32]

3.1.5 Lock in amplifier

Lock in amplifiers is used for very small signals up to little nano-volts detection.

Phase-sensitive detection is known by a technique that is used for detection of signal out of specific reference and phase components.

Noise signals do not accept the measurement at frequencies other than reference frequency.

The external reference of lock-in amplifier generates internal signals mostly by their phase-locked-loop.

Figure 3.6 Lock in Amplifier [33]

3.1.6 Optical chopper SR540

The intermodulation, double beam and singe measurements are held on the optical chopper SR540. It has four frequency digits which are analogue voltage frequency control, front-panel frequency control and two reference outputs with selectable operation mode. The blades are composed of two aluminum blades, that supplies for two different slots with different frequencies: up to 400 Hz frequencies are by 5/6 slot blade whereas up to 3.7 frequency by 25/ 30 slot blade.

Figure 3.7 Optical Chopper SR540 [34]

3.2 Sample Preparation

Subculture of MCF-7 and A-459 cells (75cm^2 flask)

1. Aspirate the medium.

2. Wash the flask with PBS 5 ml

3. Add trypsin to remove adherent cell layer 4 ml

4. Incubate at 37˚C for 2 min.

5. Add 5 ml medium (DMEM+10% FBS)

6. Collect the cells into a 15 ml falcon tube.

7. Centrifuge at 1500 rpm for 10 min.

8. Discard the supernatant, leave the pellet alone.

9. Add 2 ml DMEM.

10. Re-suspend the pellet.

11. Count the cells using hemacytometer.

Cell;Seed 7.5 x 105 cells into each 75cm^2 flask.

DMEM + 10% FBS + CELL SUSPENSION: 20 mL

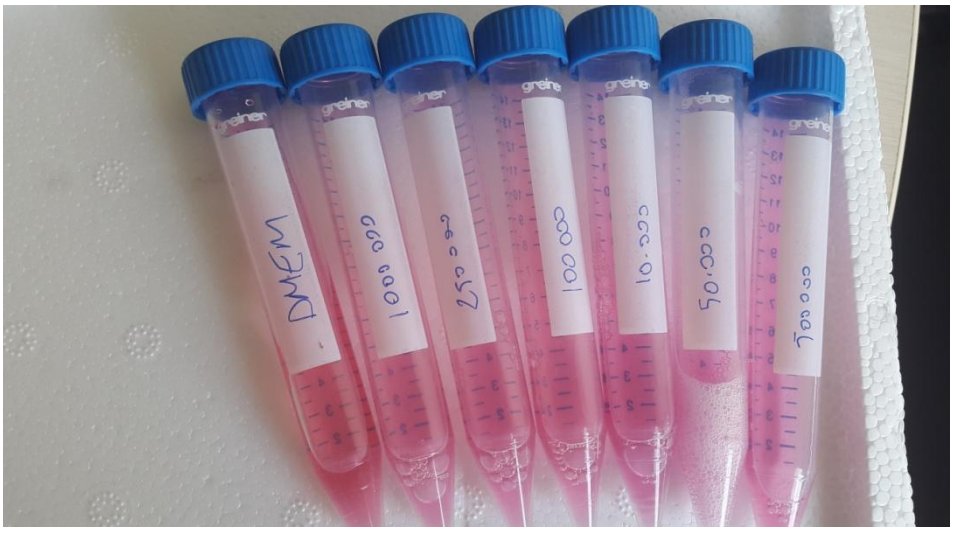

Figure 3.8 samples of MCF-7 cells at different concentrations and the medium

3.3 Instrumental Protocol

Preparing Zolix device by filtering the light to adjust the frequency of light which is 223 Hz in the Optical chopper part of the device and separation of light in the monochromator which have three different grade, for measurement we have to choose a suitable grade which is grade two, after ongoing light through grading system that light will adjusts from (450-1100 nm), and will travel to chamber part, which some of light beam will transmit through and some beam will scattered while exposing to the testing samples on the cuvette. Both transmission and scattered light are detects by special detectors.

First empty cuvette should put into the device to insure the proper adjustment of the light.

Lights will inter Lock in amplifier which measures the signals by millivolts and also transfer into digit data in the computer by specific Zolix software.

3.4 Experimental Procedure

Step-1: Preparation of 6 different concentrations for each breast and lung cancer cell lines (10.000, 50.000, 100.000, 250.000, 500.000 and 1.000.000) Cells in 5 ml of the medium.

Step-2: Suffusion grid uses to set the wavelength of the light which is grid 2 to adjust the wavelength to 450-1100 nm. The wavelengths that start from 450 nm is increasing 5 nm in the next wavelength till it reached 1100 nm, that is 450 nm,455nm, 460 nm,…1100 nm.

Step-3: Setting optical chopper to 233 Hz., and the power of light source on 6.45 Ampere (A).

Step-4: Measuring both types cancer cells separately (study groups), only medium+cuvette (without cancer cells) and as a control.

Step-5: Recording and comparing the result of both types of the cancer cells with different concentrations.

3.5 Statistical Methods: T-Test

As it is clear there many times of statistical data analysis procedure for testing of hypothesis; İn this study T-Test was used. And also there some kinds of T-Test , but the most used one is the ‘’Two- sample T-Test’; which is also known as the ‘’ Student’s T-Test’’ or the ‘’ Independent sample T-Test’’.

It is used to observe the change between two groups of having different scores. But this scores may not represent a real difference between the twoo populations, so it may be only a difference in our particular sample that might not be the real representative of the populations.

Thus the T-Test is done to answer this question when having different results to find the real answer. Thus by using T-Test we get the P-Value, this p-value will indicate whether there is a difference between the groups of differentiatings or not. İf the p-value was less than 5% so we can say that there is a significant difference between the groups; but if p-value was not less than 5% so there is no difference and we can call the result at this time as’’Null Hypothesis’’[35]

CHAPTER 4

RESULTS

TRANSMITTANCE EXPERIMENTS RESULTS

A-549 CELL LINE

Optical response of A-549 cell line to the light spectrum from 450nm to 1100nm was given below figure (Figure 4.1).

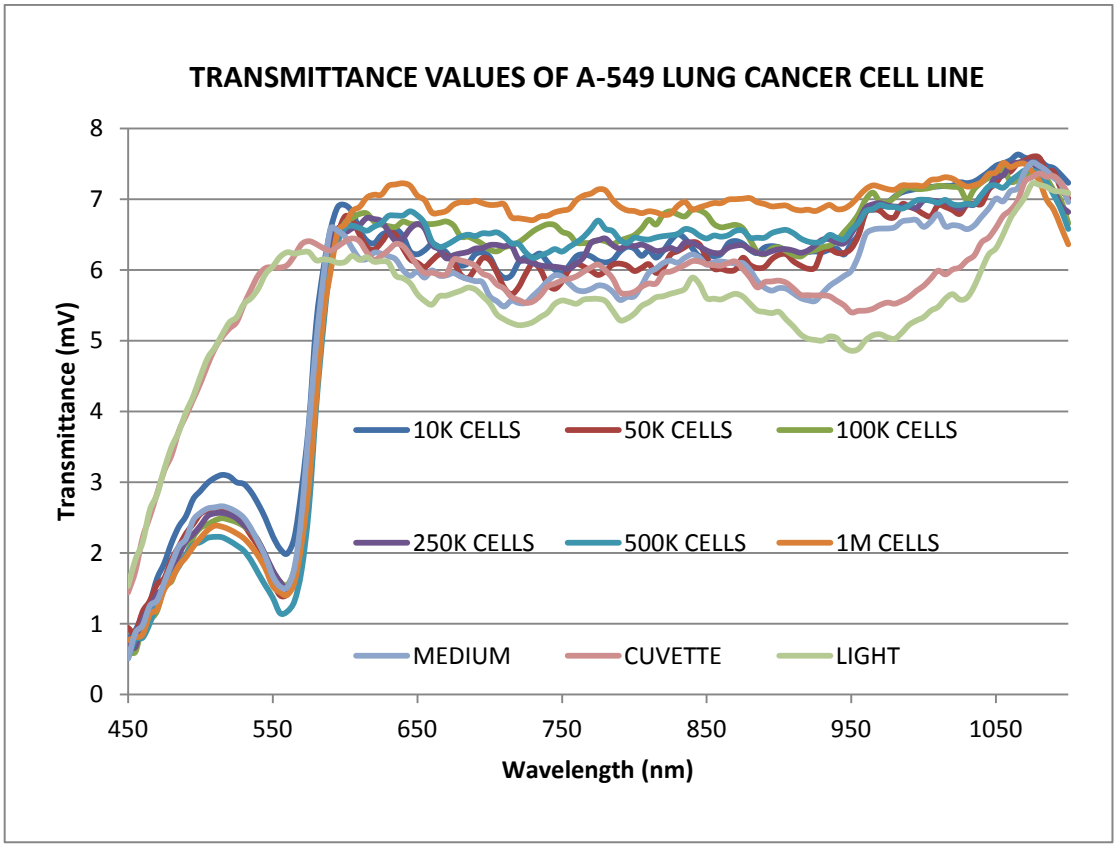

Figure 4.1: Raw data of the transmittance values of A-549 lung cancer cell line of different concentrations and of medium+cuvette, of only cuvette, of only light. Measurements of only cuvette and only light intersect at around 595nm with experimental groups.

In the figure above it was observed that in the VIS region the light and the cuvette transmittance are much higher than others, but in the NIR regions the situation was observed vice versa. Although there is no statistical difference of detected intensities between experimental groups and medium+cuvette, fluorescence from cells may contribute such an increase of detected intensity of the light from experimental groups.

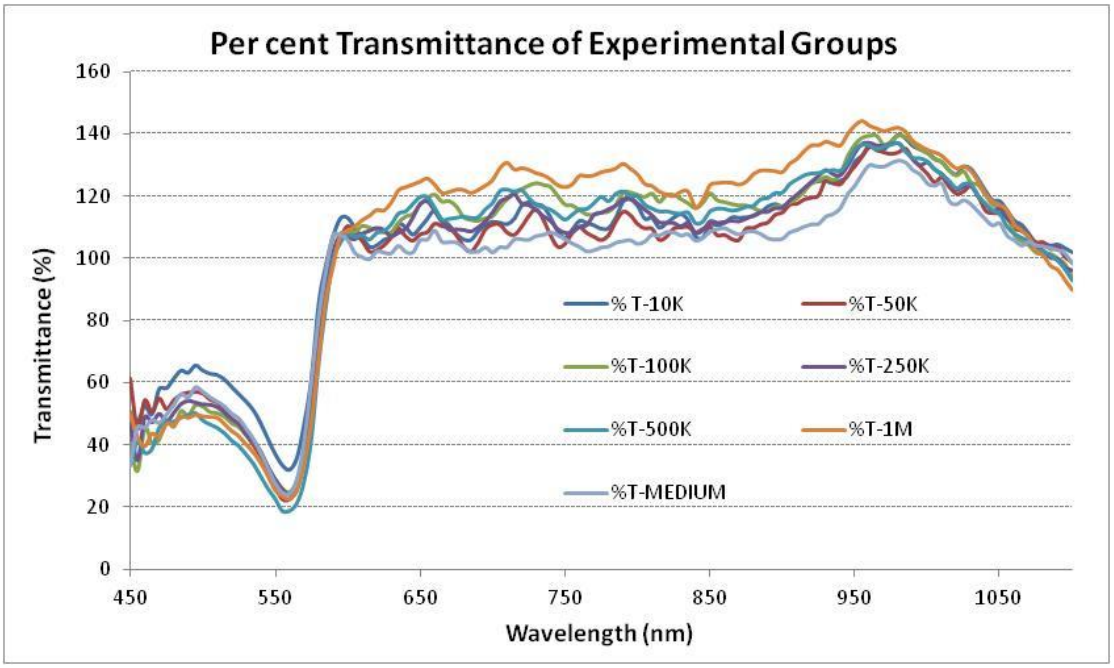

Figure 4.2: Transmittance as percent of different concentrations of A-549 lung cancer cell lines and medium.

Detected light intensity was regarded as reference and transmitted intensities of experimental groups were compared with the light and expressed as percent transmittance, i.e (100*experimental group light intensity) / light intensity at corresponding wavelength.

In order to observe the cells` response to the spectrum scanned, transmittance of the medium was neglected (Figure 4.3).

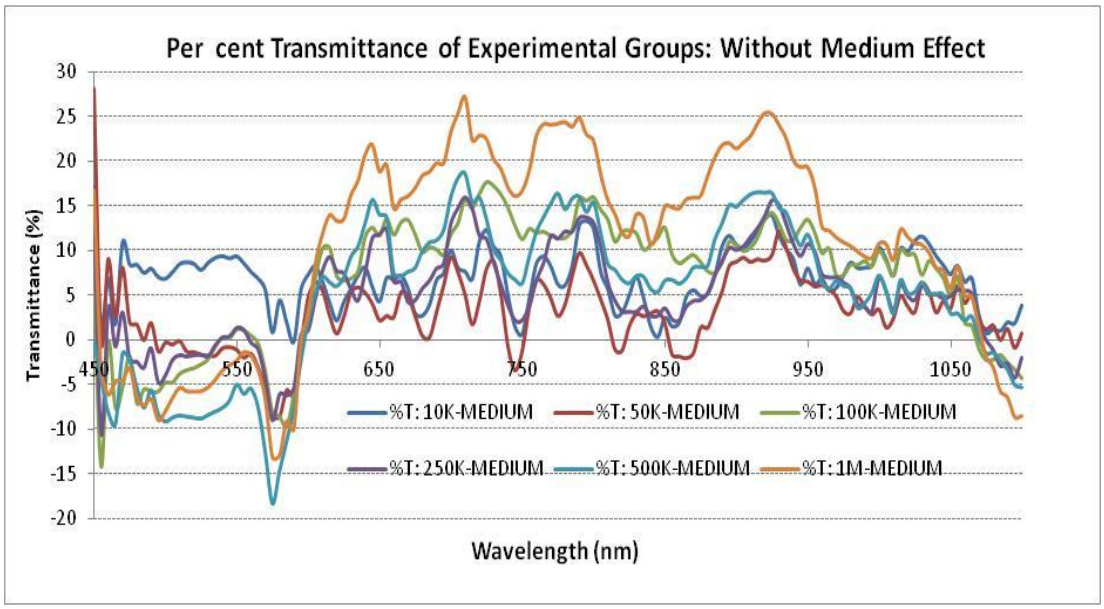

Figure 4.3: Percent transmittance of all concentrations of A-549 lung cell lines without the medium effect; i.e transmittance of medium group was subtracted from transmittance of experimental groups.

In order to observe significant changes t-test have been performed on graphical data, and given tables below:

Table 4.1 T-TEST for each concentrations of A-549 Lung cell lines at different wavelength starting by 450 nm and ending with 1100 nm: Comparison of VIS and NIR regions.

TTEST 10K: VIS-NIR	TTEST 50K: VIS-NIR	TTEST 100K: VIS-NIR	TTEST 250K: VIS-NIR	TTEST 500K: VIS-NIR	TTEST 1M: VIS-NIR
3.32951E-10	2.73949E-10	2.834E-10	4.45589E-10	3.47185E-10	4.28795E-10

It is observed that there is significant difference within each concentration ($p<0.05$) at variable wavelengths. VIS region was taken from 450nm to 700 nm and NIR region was taken from 705 nm to 1100 nm. For every concentration VIS and NIR region transmittance values were found significantly different.

Table 4.2 TTEST for observing the difference between different concentrations of A-549 lung cell lines for the spectrum: 450nm-1100nm

TTEST concentrations	50K	100K	250K	500K	1M
10K	0.333728577	0.981445	0.647805	0.815134	0.227525
50K	NA	0.349119	0.627133	0.611806	0.086293
100K	0.981444825	NA	0.649173	0.688528	0.428904
250K	0.627132778	0.649173	NA	0.968778	0.213692
500K	0.611806107	0.688528	0.968778	NA	0.243376
1M	0.086293324	0.428904	0.213692	0.243376	NA

It is observed that there is no significant difference between different concentrations of the cells ($p>0.05$). This shows that concentration difference from 50K to 1M is not a distinctive parameter.

Table 4.3 t-test between medium and different concentrations of A-549 lung cell lines.

TTEST MEDIUM-10K	0.088841425
TTEST MEDIUM-50K	0.483218504
TTEST MEDIUM-100K	0.103649781
TTEST MEDIUM-250K	0.235957648
TTEST MEDIUM-500K	0.238002204
TTEST MEDIUM-1M	0.016880348

It was observed that only between 1 million cells and medium a significant difference exist ($p<0.05$), while among others difference was absent. Maximum fluorescence intensity arises from 1M cell group

MCF-7 CELL LINE:

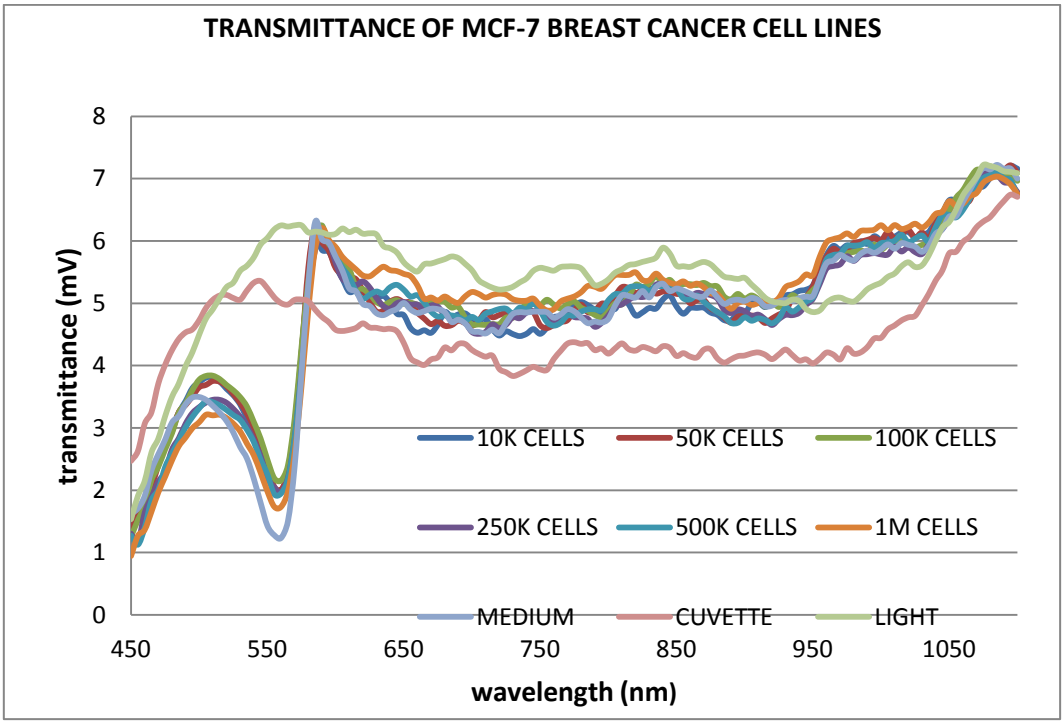

Figure 4.4 raw data of the transmittance of different concentrations of MCF-7 breast caner cell lines with medium ,cuvette and light.

In this experiments, similar results were obtained as in the A-549 cell line.

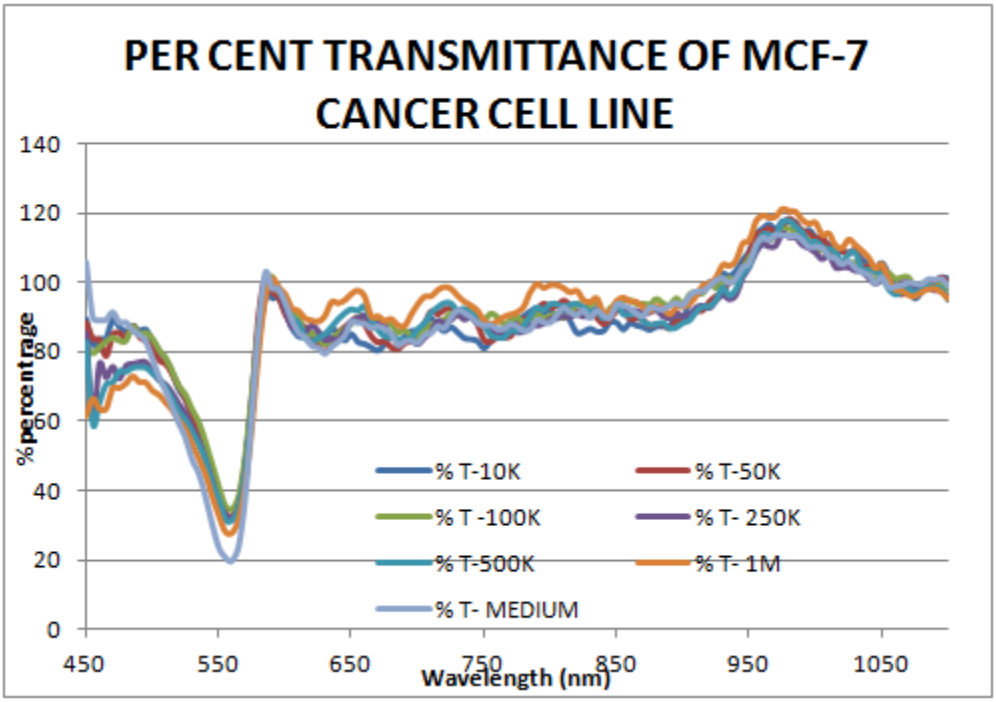

Figure 4.5: Transmittance as percent of different concentrations of MCF-7 breast cancer cell lines and medium. Detected light intensity was regarded as reference and transmitted intensities of experimental groups were compared with the light and expressed as percent transmittance, i.e (100*experimental group light intensity) / light intensity at corresponding wavelength.

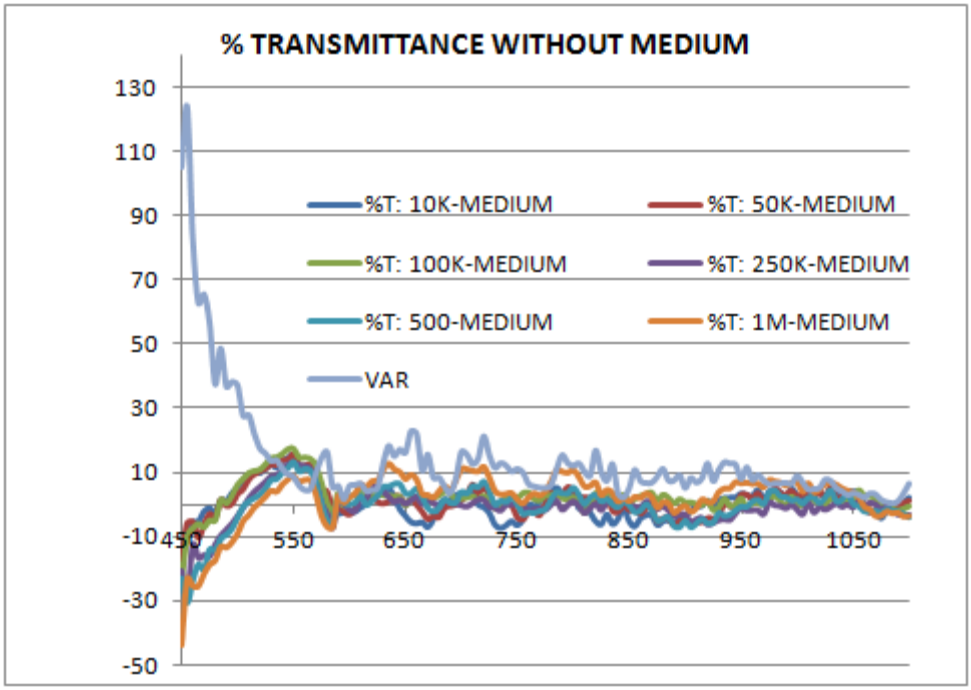

Figure 4.6: Percent transmittance of all concentrations of MCF-7 breast cell lines without the medium effect; i.e transmittance of medium group was subtracted from transmittance

Table 4.4 T-test for each concentrations of MCF-7 breast cancer cell lines at different wavelength: from 450 nm to 1100 nm: Comparison of VIS and NIR regions.

TTEST 10K: VIS - NIR	TTEST 50K :VIS- NIR	TTEST 100K : VIS -NIR	TTEST 250K :VIS-NIR	TTEST 500K: VIS- NIR	TTEST 1M :VIS-NIR
1.5664E-10	1.82347E-10	6.756E-10	1.00973E-09	9.25642E-10	5.53013E-10

It is observed that there is significant difference within each concentration ($p<0.05$) at variable wavelengths. VIS region was taken from 450nm to 700 nm and NIR region was taken from 705 nm to 1100 nm. For every concentration VIS and NIR region transmittance values were found significantly different.

Table 4.5: *t-test* for observing the difference between different concentrations of MCF-7 breast cancer cell lines for the spectrum: 450nm-1100nm

TTEST CONCENTRATIONS	50K	100K	250K	500K	1M
10K	0.859483596	0.530180347	0.674932422	0.880032166	0.479739206
50K	NA	0.654047274	0.55365349	0.747360339	0.58885235
100K	0.654047274	NA	0.299181104	0.44740757	0.899253228
250K	0.674932422	0.299181104	NA	0.796184658	0.277144918
500K	0.880032166	0.44740757	0.796184658	NA	0.407348157
1M	0.58885235	0.899253228	0.277144918	0.407348157	NA

It was observed that there is no significant difference between different concentrations of the cells ($p>0.05$). This shows that concentration difference from 50K to 1M is not a distinctive parameter

Table 4.6 T-TEST between the medium and different concentrations of MCF-7 breast cancer cell lines.

TTEST MEDIUM -10K	0.74670399
TTEST MEDIUM -50K	0.622942383
TTEST MEDIUM -100K	0.354923603
TTEST MEDIUM - 250K	0.932991915
TTEST MEDIUM -500K	0.865853547
TTEST MEDIUM -1M	0.325965655

It was observed that there is no significant difference between groups as the $p>0.05$.

COMPARISION BETWEEN A-549 and MCF-7:

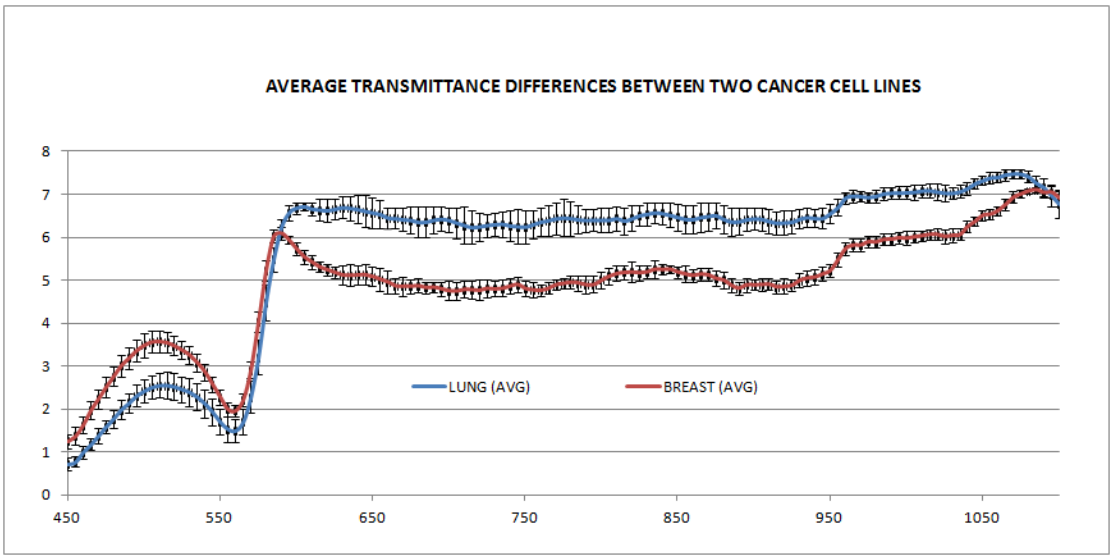

Figure 4.7 Average transmittance differences between A-549 and MCF-7 cancer cell lines for each wavelength.

It was observed that MCF-7 cell line has higher transmission up to 595 nm than A-549 cell line ($p<0.05$); after 595 nm, A-549 cell line has higher absorption. There was no significant difference from 1085 nm to 1100 nm ($p<0.05$).

CONCLUSIONS

In this study, valuable results have been taken in order to contribute for further researches. Three main conclusions could be come up with:

1- Visible and Infrared Regions of the light spectrum could be used as distinctive marker for transmittance.

2- Transmittance detection was not a suitable method for differentiating unlike concentrations of cells; at least in between 50K and 1M.

3- Absorption-Reflection system can differentiate MCF-7 cancer cell line and A-549 lung cancer cell line from each other except for very few wavelengths.

The reason of taking the measurements only time is due to the accuracy of the Absorption Reflection device that is very correct like Raman spectroscopy and others which gives the accurate result and there is no need for repeating.

In clinic these information can be used to the exact response of these kinds of cells to the transmission while they are excited with these ranges of wavelengths; so during the designing of devices which rely on these ranges of wavelengths these data will be useful.

The advantages of this type of devices on other types of devices is that this device is not as much expensive as others; its working principle is easy; and the data can be observed in a short time.

For further studies; primary human cancer cell lines could be tested comparatively with non-cancer human cell lines.

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