Biological Effects of Low Power Microwaves: Experimental Evaluation at Molecular and Cellular levels

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis/project is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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I devoted this thesis to ***my mother and my father*** who have taught me the meaning of life and encouraged me to seize every opportunity in my life.

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SUMMARY

The Radiofrequency (RF) and Microwave (MW) radiation refer to electromagnetic fields with frequencies between 300 kHz -300 MHz and 300 MHz-300 GHz respectively. The most important applications of RF/MW found in various communication systems (mobile telephony), and microwave heating (food technology and medical applications). The RF/MW radiation is non-ionizing because the energy levels associated with it are not high enough to cause ionization of atoms and molecules. But RF/MW can produce resonance interactions with ions and with charged macromolecules, and such interactions can significantly alter biochemical functions. A large body of research has shown that MW/RF causes an increased production of free radicals and reactive oxidant species in living tissues, and that this increased oxidant stress can damage DNA. This damage can and does occur at power levels well below those levels that could produce damage by thermal mechanisms.

The mobile phone system operating at about 900 and 1800 MHz for Global System for Mobile Communication (GSM); 800 and 900 MHz for Wideband Code Division Multiple Access (WCDMA); 1800 MHz for Long Term Evolution (LTE); and 2100 MHz for Universal Mobile Telecommunications System (UMTS) is located in a region of the spectrum that is referred to as both microwave (MW) radiation and RF radiation.

There is a large body of internationally accepted scientific evidence which points to the existence of non-thermal effects of RF/MW radiation. The issue at the present time is not whether such evidence exists, but rather what weight to give it. RF/MW radiation research have shown that RF/MW transmissions of the type used in digital cellular antennas and phones can have critical effects on cell cultures, animals, and people in laboratories and have also found epidemiological evidence of health effects at "non-thermal levels," where the intensity of the RF/MW radiation was too low to cause heating.

This PhD research project is aimed at investigating the effects of low intensity/power MW radiation on selected cells and proteins with the specific focus on the frequencies emitted by mobile phones.

The project includes the following sub-studies:

1. Design and fabrication of the custom-made microwave exposure camera.
2. Modeling/simulation of the generated field inside the custom-made exposure camera.
3. Experimental evaluation of the effects of low level microwaves on selected biological systems.

The experimental evaluation conducted within this project resulted in the following findings:

1. In this study the microwave exposures at the 900 MHz and the powers of 13dBm,

3dBm and -7dBm were used to irradiate S. cerevisiae yeast cells. The yeast samples were exposed and sham-exposed for 6 hours. The results obtained show: (i) MW at 900 MHz and power of -7dBm induced no effect on yeast growth; (ii) MW at 900 MHz and powers of 13dB and 3dB affected significantly (14% and 11% respectively) the concentration of yeast cells.

2. In this study Saccharomyces cerevisiae yeast cells were exposed to MW at two frequencies 500MHz and 900MHz and powers of -17dBm, -13dBm, -10dBm, 0dBm, 10dBm, 13dBm and 17dBm. Similar to *Study 3.1*, TC-5062AUHF TEM cell (100kHz–3GHz) from TESCOM Ltd (Unitechvill, Goyang, Korea) was used to irradiate yeast cells here. The results showed: (i) MW at 900MHz and powers of -13dBm, 0dBm and 10dBm induced the changes in the yeast cell samples. The findings revealed that the yeast cell proliferation rates are increased (21%, 12% and 6% respectively). In contrast, at the same frequency of 900MHz and the powers of -17dBm, -10dBm, 13dBm and 17dBm the opposite effects are observed. The exposures at these particular parameters decreased the growth and proliferation of yeast cells with the different degree of suppression. The maximum decrease in yeast cells growth (38%) is recorded at the frequency of 900 MHz and the power of 17dBm.

3. In this study L-Lactate Dehydrogenase enzyme and Saccharomyces Cerevisiae yeast cells were continuously exposed to microwave radiation at the frequency of 968MHz and power of 10dBm using the designed and fabricated custom-made TEM

cell. The findings reveal that MW at 968MHz and power of 10dBm inhibits the enzymatic activity of L-Lactate dehydrogenase by 26% and increases significantly (15%) the proliferation rate of yeast cells.

4. In this study L-Lactate dehydrogenase and Glutathione peroxidase enzymes irradiated at the frequencies of 1.8GHz, 2.1GHz and 2.3GHz and power of 10dBm using the commercial Transverse Electro-Magnetic (TEM) cell. The results obtained show that MW exposures of L-Lactate dehydrogenase enzyme resulted in the frequency-dependent effects on the observed biological reaction as follows exposures at 1.8 GHz and 10dBm resulted in 20% increase, 2.1 GHz – 51% increase, and 2.3GHz - 44% increase in NADH absorbance. For irradiation of Glutathione peroxidase enzyme, the findings reveal that MW exposures at 1.8 GHz and 2.1 GHz and power of 10 dBm induced the inhibitory effects on the studied biochemical reaction that resulted in 15% reduction of the reaction speed. The experimental findings of this research project clearly demonstrate that applied microwave exposures of selected frequencies and powers can induce modulating effects in the studied model systems. Moreover, the observed effects are frequency- and power-dependent, which provide an opportunity of altering the cellular and protein activities of the selected model systems. This, in turn, can lead to development of novel technologies in medical and food applications by employing the microwave radiation of the specific parameters able to affect/modify selected biological processes of interest.

CHAPTER 1

INTRODUCTION

1.1 Background of Understanding

The electromagnetic spectrum includes radio waves, microwaves, infrared rays, light rays, ultra violet rays, X-rays and gamma rays. All electromagnetic radiation is transmitted through empty space at 3.0×10^8 metres per second (300 thousand kilometres per second). The different forms of electromagnetic radiation are distinguished from each other by: (i) their wavelength, and (ii) the amount of energy they transfer. These properties also determine their ability to travel through objects, their heating effects and their effect on living tissue.

In recent decades, radiofrequency/microwave radiation, a subset of non-ionizing electromagnetic radiation, has been widely used in communication (mobiles phones and towers), medical applications, as well as food technology and other industrial applications. Medical devices used for magnetic resonance imaging, diathermy, hyperthermia, various kinds of radiofrequency (RF) ablation, surgery, and diagnoses may cause high levels of electromagnetic fields (EMF) at the patients position or locally inside the patient's body. In addition, some of these medical applications may produce high fields at certain workspaces. In modern life the use of mobile phones is growing exponentially. Consequently, the exposure to weak radiofrequency/ microwave radiation generated by these devices is markedly increasing. Mobile communication networks cause on average low levels of electromagnetic fields in areas accessible to the general public. However, mobile phones might cause

significantly higher peak levels of exposure during use. Accordingly, public concern about the potential hazards of long-term use of mobile phones and its effect on human health is mounting [1].

Radiofrequency (RF) radiation is electromagnetic radiation in the frequency range of 3kHz - 300GHz. RF exposure is usually specified in terms of *modulation* (continuous wave or pulsed), *incident electric-field* and *magnetic-field strengths*, *incident power density* (when appropriate), *source frequency*, *type* and *zone of exposure* (near or far field), and *duration of exposure*.

The coupling of RF energy into biological systems may be quantified by the induced electric and magnetic fields, power deposition, energy absorption, and the distribution and penetration into biological tissues. These quantities are all functions of its relationship to the physical configuration and dimension of the biological body. Important to note that exposure of a whole body to a given field strength could have outcomes far different for partial body or localized exposure at the same strength (power). The spatially averaged field strength, depending on the region of space over which the fields are averaged, may vary widely for a given body. Current understanding is that induced fields are the primary cause for biological effects of RF exposures, regardless of the mechanism [2].

The mobile phone system operating at about 900 and 1800 MHz for Global System for

Mobile Communication (GSM); 800 and 900 MHz for Wideband Code Division Multiple Access (WCDMA); 1800 MHz for Long Term Evolution (LTE); and 2100 MHz for Universal Mobile Telecommunications System (UMTS) is located in a region of the spectrum that is referred to as both microwave (MW) radiation and RF radiation.

At distances within a wavelength from a RF transmitter is a region known as the near field. Since the RF radiation from a mobile phone has a wavelength of 10-30cm (depending on the type of technology) the users' head will be within this near field region. The head disturbs the field and alters the manner in which RF interacts with tissue. This interaction complicates the absorption of RF energy within the head and makes calculations difficult. Absorptions within the head are therefore determined experimentally or by simulation on a computer. The specific absorption rate (SAR) is defined as the rate at which a mobile phone user absorbs energy from the handset [3]. The Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) Standard specifies exposure limits to RF exposure for mobile phone handsets in terms of the SAR. In the ARPANSA Standard the SAR limit for mobile phone handsets is 2 watts per kilogram (W/kg) of tissue (averaged over 10 grams). A SAR of 4W/kg is associated with a 1 degree temperature rise in humans. In practice, a mobile phone will only cause a temperature rise of a fraction of a degree which is unlikely to be noticed compared with the normal daily variations in body temperature [3].

SAR is set at 1.6 W/kg averaged over 1 g of body tissue in the US and Canada Standards and 2 W/kg averaged over 10 g of body tissue in countries adopting the ICNIRP guidelines. The SAR is used to quantify energy absorption to fields typically between 100 kHz and 10 GHz and encompasses RF radiation from devices such as mobile phones up through diagnostic magnetic resonance imaging (MRI). The biological effects of RF/MW radiation depend on how much of the energy is absorbed in the body of a living organism. Overall, the absorption of RF/MW radiation depends on the following characteristics: frequency of transmission, power density, distance from the radiating source and the organism's size, shape, and water content. Exposure will be lower from towers under most circumstances than from mobile phones because the transmitter is placed directly against the head during cell phone use whereas proximity to a mobile tower will be an ambient exposure at a distance [4]. Exposure guidelines for RF protection had adopted the value of 4 W/kg averaged over the whole body (SARWB) as the threshold for the induction of adverse thermal effects associated with an increase of the body core temperature of about 1°C in animal experiments. This standard is set by International Commission on Nonionizing Radiation Protection (ICNIRP), national Radiological Protection Board (NRPB), and Institute of Electrical and Electronics Engineers (IEEE) [2].

With the rapidly increasing use of mobile phones devices, especially among the general public, there has been a focus on the problems associated with near field RF exposure to the head from the small radiating antenna of mobile phones. In addition,

concerns persist that exposure to pulsed and amplitude modulated RF fields may cause specific health effects.

The established biophysical mechanisms underlying the interaction of RF radiation with cells, tissues and entire bodies include ionization potential, induced charge and dipole relaxation, enhanced attraction between cells for pearl-chains formation and other RF-induced force effects, microwave auditory phenomenon, and thermal effects as manifested in tissue temperature elevations. The heating effect of the RF/MW radiation is already well-known and documented, however a doubt remains on the existence of non-thermal biological effects. The thermal effects are related to the heat generated by the absorption of microwave energy by the water medium or by organic complex systems.

Of particular interest to this PhD project is to study non-thermal effects of RF/MW radiation. Non-thermal biological effects are measurable changes in biological systems that may or may not be associated with adverse health effects. In essence, this research project has two arms:

(i) Computational modelling of the exposure camera and construction of its operational prototype, and

(ii) Experimental evaluation of low power MW radiation on the selected model

systems at the molecular and cellular levels using the commercial and custom-made exposure cameras.

In particular, in this research project the effects of applied MW exposures of different frequencies and powers were studied on the selected proteins (enzymes) and yeast cells. The enzymes and yeast cells were exposed using the commercial Transverse Electro-Magnetic (TEM) cell and the custom-made exposure camera (design, simulation, fabrication and testing are presented in Chapters 3 of the thesis).

The experimental part of the project includes the following sub-studies:

- Study of non-thermal effects of microwave exposures (different frequencies and powers) on the proliferation rate of Saccharomyces Cerevisiae yeast cells.
- Study of non-thermal effects of microwave exposures at 500MHz and 900MHz and different powers on biological activity of L-lactate dehydrogenase (LDH) enzyme.
- Study of non-thermal effects of microwaves exposures at 1.8GHz, 2.1 GHz and 2.3GHz with the power of 10dBm on biological activity of Glutathione Peroxidase and L-Lactate Dehydrogenase (LDH) enzymes.

The findings of this project are presented in the following chapters of this thesis.

1.2 Thesis Composition

To document the details of this research, the thesis has been organized in the following fashion:

- **Chapter 1** presents an introduction to the research project, including research aims and objectives. It also presents the motivating factors behind undertaking this study and briefly outlines the approach taken towards the aim of final outcome of this particular research.

- **Chapter 2** lays focus on the literature review and essential understanding of the effects of non-thermal weak (low power) RF/MW radiation on cells and proteins.

- **Chapter 3** presents Materials and Methods employed in this study. In particular, in Chapter 3 two exposure cameras (TEM cells), used in the *in vitro* experimental studies for irradiation of the selected cells and proteins, are presented in great details. The design, simulation, fabrication and testing of the custom-made exposure camera are also presented.

- **Chapter 4** presents experimental evaluation of the low power microwave radiation on the selected model systems. It includes three sub-studies:

1) This study evaluates the effect of non-thermal weak RF/MW radiation on the proliferation response of the yeast Saccharomyces cerevisiae. S. Cerevisiae strains type II (Sigma-Aldrich). Yeast cells were exposed to the microwaves at 900MHz and the selected powers of 13dBm, 3dBm and -7dBm using the commercial Transverse Electro-Magnetic (TEM) cell. The average specific absorption rate (SAR) for a single cell was 0.12W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells.

2) This study provides experimental data on the effects of low power microwaves at 500MHz and 900MHz on yeast cells' growth. It evaluates the effects of low power microwave radiation on the proliferation rate of yeast Saccharomyces cerevisiae strains type II. Yeast cells were exposed to the microwaves at the frequencies of 500MHz and 900MHz and the selected powers of 0dBm, 10dBm, -10dBm, 13dBm, -13dBm, 17dBm and -17dBm using the Transverse Electro-Magnetic (TEM) cell. The average specific absorption rate (SAR) for a single cell was 0.12W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells. A comparative analysis of changes in the proliferation rate of the irradiated *vs*. non-radiated yeast cells was performed for the selected frequencies and powers, with the results being presented and discussed.

3) This study reports on the effects of low power microwave radiation on biological activity of L-Lactate dehydrogenase enzyme and growth rate of S. Cerevisiae yeast. The selected model systems were continuously exposed to microwave radiation at the frequency of 968MHz and power of 10dBm using the designed and constructed (custom made) Transverse Electro-Magnetic (TEM) cell. The findings reveal that microwave radiation at 968MHz and power of 10dBm inhibits L-Lactate dehydrogenase enzyme activity by 26% and increases significantly (15%) the proliferation rate of yeast cells.

4) This study documents the effects of low power (10dBm) microwaves at 1.8GHz, 2.1GHz and 2.3GHz on L-Lactate dehydrogenase and Glutathione Peroxidase enzymes. In this investigation, the selected enzymes are exposed using the commercial Transverse Electro-Magnetic (TEM) cell.

- **Chapter 5** presents a summary of the research contribution and conclusive remarks critically analyzing the findings of the research project.

- **Chapter 6** provides some ideas and suggestions for a future research. This section is rounded up by a list of peer-reviewed publication yielding form this research project.

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CHAPTER 2

LITERATURE REVIEW

2.1 Electromagnetic Field (EMF) and Electromagnetic Radiation (EMR)

According to a definition, electromagnetic field (EMF) is a physical field produced by charged objects. Electromagnetic field is generated from the interaction of electric field produced by stationery charged particles and the magnetic field produced by moving charged particles. The interaction between electromagnetic field, charges and currents are defined by Lorentz force law shown in Equation 2.1.

$$F = q(E + v \times B) \tag{2.1}$$

where the force, *F*, enforce on a particle of electric charge, *q*, with instantaneous velocity, *v*, E is electric field, B is magnetic field.

Electromagnetic radiation (EMR) is a particular form of the more general electromagnetic field, where a form of energy emitted and absorbed by charged particles as it travel through the space in wave form. EMR is associated with electromagnetic field that moves away from its source. EMR has both electric and magnetic field components oscillating with 90° degree phase difference from each other perpendicular to the direction of wave propagation or energy.

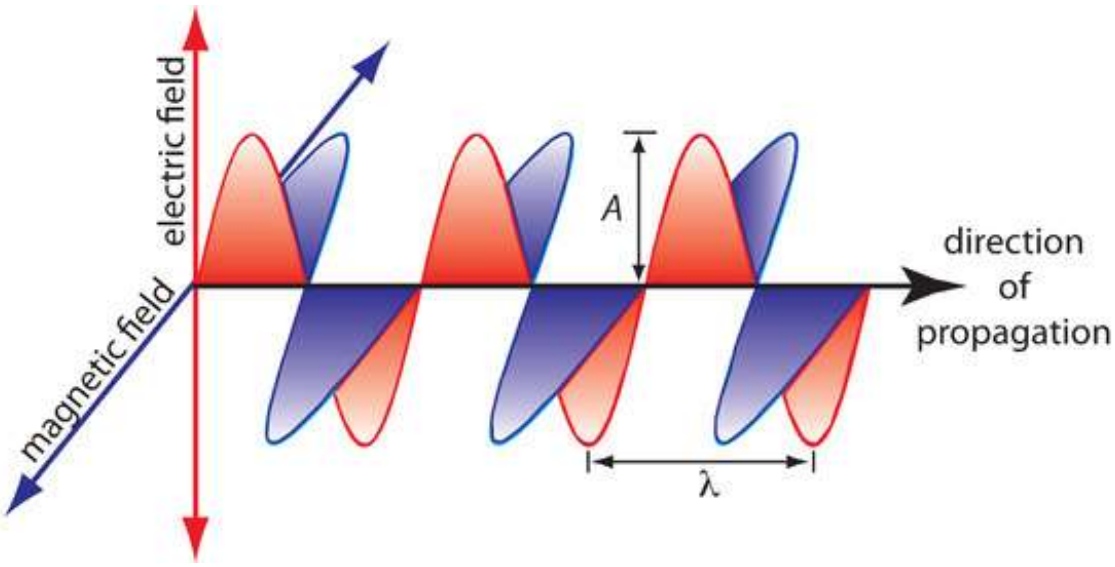

Figure 1. Electromagnetic radiation – the propagation of energy in the form of electromagnetic waves through a medium: plane-polarized electromagnetic radiation is showing the oscillating electric field in red and the oscillating magnetic field in blue [1]

In Figure 1 the radiation's amplitude, A, and its wavelength, λ, are shown. Normally, electromagnetic radiation is unpolarised, with oscillating electric and magnetic fields present in all possible planes perpendicular to the direction of propagation [1].

The electromagnetic environment consists of natural radiation and man-made electromagnetic fields that are produced either intentionally or as by-products of the use of electrical devices and systems. The natural electromagnetic environment originates from terrestrial and extra-terrestrial sources such as electrical discharges in the earth's atmosphere and radiation from sun and space.

Characteristic of natural fields is a very broadband spectrum, where random high peak

transients or bursts arise over the noise-like continuum background. This natural background is in order of magnitude below local field levels produced by man-made RF-sources considered here [2].

EMR is a type of energy transmitted in the form of waves. These waves correspond to spatial and time variations of the electric and magnetic field. Electromagnetic fields are divided into different categories (called spectra) depending on their frequency (measured in cycles per second (Hertz)), wavelength (measured in meters), and certain characteristics and applications that each division has. The electromagnetic spectrum is generally divided into 7 broad categories of Radio, Microwave, Infrared, Visible, Ultraviolet, X-ray, Gamma ray. Some of these classifications are further divided into subcategories.

EMR is transmitted through empty space at 3.0×10^8 metres per second (300 thousand kilometers per second). The different forms of electromagnetic radiation are distinguished from each other by:

- Their wavelength, and
- Amount of energy they transfer.

These properties also determine their ability to travel through objects, their heating effects and their effect on living tissue. EMR is described as a stream of mass-less

particles called photons. Each photon has a certain energy level and is travelling in a wave-like pattern at the speed of light. The energy level of each type of photon is defined through oscillation rate in Hertz. Rate of oscillation is inversely proportional to the distance each photon travels in meters. Higher photon energy means higher frequency of oscillation and shorter wavelength. Thus, radio waves contain photons with the lowest energy level, while Gamma rays have the highest energy level in the spectrum [2].

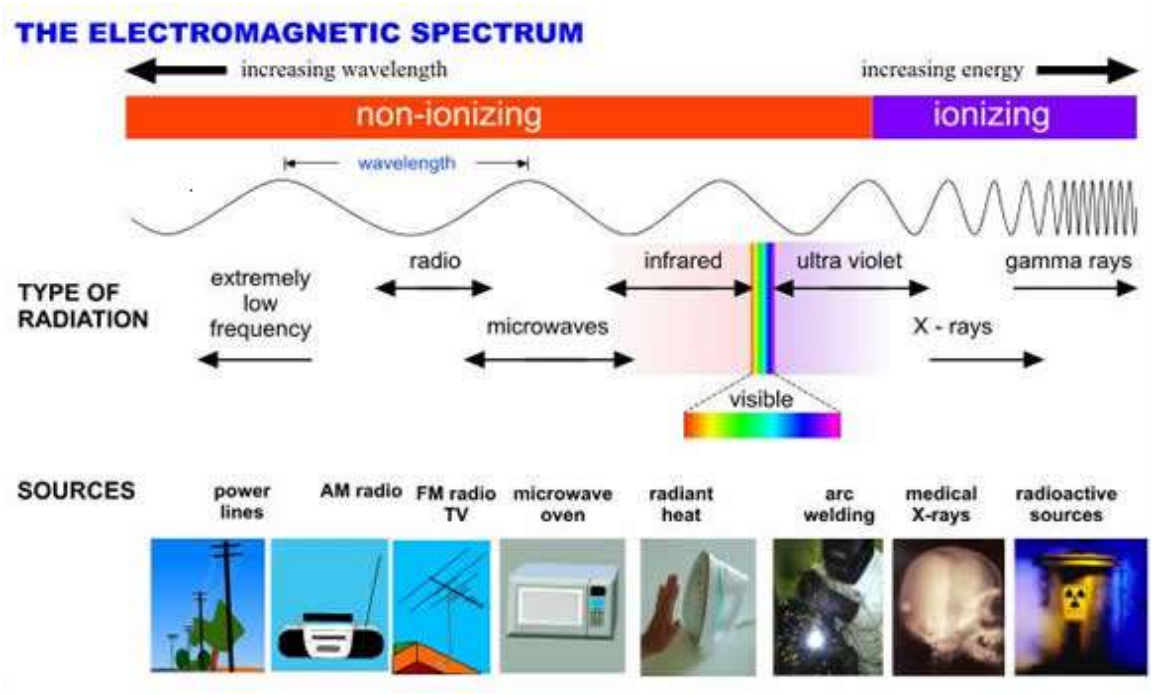

Figure 2. Electromagnetic spectrum (ARPANSA) [3]

In the electromagnetic spectrum, radiation from EMFs with a high order of frequencies is classified as ionizing radiation. Low frequency EMFs, whose quanta are

insufficient to break molecular bonds, are called non-ionizing radiation. Gamma rays, X- rays are examples of ionizing radiation whereas radiations from microwaves, radiofrequency fields and ELF are found at the relatively long wavelength and are classified as non-ionizing radiation. Non-ionizing radiation ranges from 0 to approximately 3×10^{11}Hz. Similarly, radiation above 3×10^{11}Hz is considered as ionizing radiation [3].

2.2 Radiofrequency Electromagnetic Radiation and Mobile Phones

RF EMR is part of everyday life, emitted by natural sources like the Sun, the Earth and the ionosphere. RF EMR is also emitted by artificial sources such as mobile phone base stations; broadcast towers; radar facilities; remote controls, and electrical and electronic equipment. As was mentioned above, RF EMR is non-ionizing radiation. This means that it is not able to directly impart enough energy to a molecule or atom to break chemical bonds or remove electrons. Mobile phones are now an integral part of modern telecommunications. In many countries, over half the population use mobile phones and the market is growing rapidly. At the end of 2009, there were an estimated 4.6 billion subscriptions globally. In some parts of the world, mobile phones are the most reliable or the only telephony [4].

Given the large number of mobile phone users, it is important to investigate, understand and monitor any potential impact on public health. Mobile phones communicate by transmitting radio waves through a network of fixed antennas called base stations. Mobile phones are low-powered radiofrequency transmitters, operating at frequencies between *450MHz and 2700MHz* with peak powers in the range of *0.1 to 2watts*.

Tissue heating is the principal mechanism of interaction between radiofrequency

energy and a human body. At the frequencies used by mobile phones, most of the energy is absorbed by the skin and other superficial tissues, resulting in negligible temperature rise in the brain or any other organs of the body. A number of studies have investigated the effects of radiofrequency fields on brain electrical activity, cognitive function, sleep, heart rate and blood pressure in volunteers [5-14]. To date, research does not suggest any **consistent evidence** of adverse health effects from exposure to radiofrequency fields at levels below those that cause tissue heating. Further, research has not been able to provide support for a causal relationship between exposure to electromagnetic fields and self-reported symptoms, or "electromagnetic hypersensitivity".

Epidemiological research examining potential long-term risks from radiofrequency exposure has mostly looked for an association between brain tumors and mobile phone use [15-18]. However, because many cancers are not detectable until many years after the interactions that led to the tumor, and since mobile phones were not widely used until the early 1990s, epidemiological studies at present can only assess those cancers that become evident within shorter time periods. However, results of animal studies consistently show no increased cancer risk for long-term exposure to radiofrequency fields [19].

Several large multinational epidemiological studies have been completed or are ongoing, including case-control studies and prospective cohort studies examining a

number of health endpoints in adults. The largest retrospective case-control study to date on adults, *Interphone*, coordinated by the International Agency for Research on Cancer (IARC), was designed to determine whether there are links between use of mobile phones and head and neck cancers in adults. The international pooled analysis of data gathered from 13 participating countries found no increased risk of glioma or meningioma with mobile phone use of more than 10 years. However, there are some indications of an increased risk of glioma for those who reported the highest 10% of cumulative hours of cell phone use, although there was no consistent trend of increasing risk with greater duration of use. The researchers concluded that biases and errors limit the strength of these conclusions and prevent a causal interpretation. While an increased risk of brain tumors is not established, the increasing use of mobile phones and the lack of data for mobile phone use over time periods longer than 15 years warrant further research of mobile phone use and brain cancer risk. In particular, with the recent popularity of mobile phone use among younger people, and therefore a potentially longer lifetime of exposure, WHO has promoted further research on this particular group of mobile phone users. Several studies investigating potential health effects in children and adolescents are underway [2, 20].

Interestingly, limitations of the methodology used by the Interphone study and collated analyses and findings, generated by other studies, pointed out that the possible effects of long-term heavy use of mobile phones require further investigation [4]. In 2011 IARC reviewed all the available evidence in relation to RF fields and cancer.

Based on the limited association between wireless phones (mobile and cordless phones) and glioma and acoustic neuroma and inadequate evidence for other types of cancers, IARC classified RF fields as a "possible human carcinogen" [21, 22].

3G mobile phones operate at lower power levels than both GSM and CDMA handsets. The maximum power from a 3G phone (2100MHz) is 0.125 watts produced over a 5 MHz bandwidth, whereas GSM phones (900 and 1800MHz) emit an average power of 0.25 and 0.125watts over a 0.2 MHz bandwidth and CDMA handsets (800MHz) have a maximum power of 1watt. With adaptive power control technology, handsets operate at the lowest power necessary for good radio communications. Typically, handsets are held against the head, while a call is made. The distance from the antenna to the head is only about 2cm or less. Therefore, the user is in the near-field of the source and simple field calculations are not appropriate to assess exposure. Radiofrequency exposure limits for mobile phone users are given in terms of Specific Absorption Rate (SAR) – the rate of radiofrequency energy absorption per unit mass of the body [23, 24].

During talks, GSM users are exposed to microwaves at different frequencies. There are 124 different channels/frequencies, which are used in GSM900 (Global System for Mobile Communication). They differ by 0.2MHz in the frequency range between 890 MHz and 915MHz. Frequency is supplied by base station to a mobile phone user depending on amount of connected users. The frequency can be changed by base

station during the same talk. Contrary to GSM phones, mobile phones of the 3rd generation irradiate UMTS (Universal Global Telecommunications System) wide-band signal. UMTS MWs may result in higher biological effects because of eventual "effective" frequency windows [25].

Currently, two international bodies have developed exposure guidelines for workers and for the general public (except patients undergoing medical diagnosis or treatment). These guidelines are based on a detailed assessment of the available scientific evidence:

(i) **International Commission on Non-Ionizing Radiation Protection (ICNIRP).** *Statement on the "Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields (up to 300 GHz)", 2009*, and

(ii) **Institute of Electrical and Electronics Engineers (IEEE).**

IEEE standard for safety levels with respect to human exposure to radio frequency electromagnetic fields, 3kHz to 300GHz, IEEE Std C95.1, 2005

2.3 Radiofrequency Electromagnetic Radiation Exposure Limits and Health Protection

The most stringent western exposure limits for RF radiation at 900MHz (*carrier frequency*), set by International Radiation Protection Association (IRPA) and International Commission on Non-Ionizing Radiation Protection (ICNIRP), were established basically to protect biological tissue from temperature increases (thermal effects). These refer to occupational exposures to RF EMR at a power density of 2.25mW/cm^2 or whole-body mean specific absorption rate (SAR) of 0.4W/kg. In terms of electric field intensity (used in the near field of an antenna), the occupational exposure limit is 90V/m. All the above exposure limit values are to be averaged over any 6-min period during the working day. For the frequency of 217Hz (*modulation frequency*), the IRPA–ICNIRP limits for occupational exposure are 2.3kV/m electric field intensity and 1.15G (0.115mT) magnetic field intensity for a few hours exposure during the day and for the general population 1.15kV/m and 0.23G for up to 24-hr exposure during the day [23, 24].

In Australia, the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) specified exposure limits to RF EMR at the frequencies used by mobile phones. The absorption of RF radiation energy, measured by SAR in units of Watts per kilogram (W/kg), is defined as the rate at which RF energy is absorbed per unit mass of a biological body. In the ARPANSA Standards, the SAR limit for mobile

phone handsets is 2watts per kilogram of tissue. With the growing use of mobile phones, most RF safety concerns have focused on RF absorption by the head, particularly from mobile handsets. The dose of RF exposure is linked to exposure time: maximum SAR is normally averaged over a 6 minutes period during the 24 hours day.

To address public and governmental concern, World Health Organization (WHO) established the International Electromagnetic Fields (EMF) Project in 1996 to assess the scientific evidence of possible adverse health effects from electromagnetic fields. The WHO 2010 RF Research Agenda recommends a number of very different research items, ranging from dosimetry and low level cellular studies to research aimed at better communicating the current state of RF bioeffect knowledge. WHO also conducted a formal risk assessment of all studied health outcomes from radiofrequency fields exposure in 2012. In addition, and as noted above, the International Agency for Research on Cancer (IARC), a WHO specialized agency, has reviewed the carcinogenic potential of radiofrequency fields, as from mobile phones in May 2011. WHO developed public information materials and promotes dialogue among scientists, governments, industry and the public to raise the level of understanding about potential adverse health risks of mobile phones.

Commencing in 1996, the Australian Government provides $1 million dollars per year for the Electromagnetic Energy (EME) Program. This program supports research into

and provides information to the public about health issues associated with mobile phones, mobile phone base stations and other communications devices and equipment. The program recognizes public concern, and the need to ensure standards and public health policies continue to be based on the best available scientific information. The program receives one million dollars funding annually acquired through a levy on radio communication licensees that is collected by the Australian Communications and Media Authority (ACMA) [3].

The EME program consists of three components:

- Australian research program (managed by the NHMRC) which aims to conduct research into EME issues of relevance to Australia and to complement overseas research activities;
- Australian participation in the WHO International Electromagnetic Fields (EMF) project through the ARPANSA, with a role as a WHO Collaborating Centre for Radiation Protection, and;
- Public information program (managed by the ARPANSA) which aims to provide information to the public and the media.

2.4 Biological and Health Effects of Radiofrequency Exposures

2.4.1 *In vitro* studies

A biological effect occurs when a change can be measured in a biological system after the introduction of some type of stimuli. However, a biological effect does not necessarily suggest the existence of a biological or health hazard. A biological effect only becomes a biological hazard, when it causes impairment to the health of the individual or his or her offspring. It has been known for many years that exposure to sufficiently high levels of RF EMR can heat biological tissue and potentially cause tissue damage. This is because the human body is unable to cope with the excessive heat generated during exposure to very high levels [5].

Three major physical parameters of RF EMR are frequency, intensity, and exposure duration. The well-understood and studied effect of microwave radiation is dielectric heating, in which any dielectric material (such as living tissue) is heated by rotations of polar molecules induced by the electromagnetic field. In the case of a person using a cell phone, most of the heating effect will occur at the surface of the head, causing its temperature to increase by a fraction of a degree. In this case, the level of temperature increase is an order of magnitude less than that obtained during the exposure of the head to direct sunlight. The brain's blood circulation is capable of disposing of excess heat by increasing local blood flow. However, the cornea of the

eye does not have this temperature regulation mechanism and exposure of 2–3hours duration has been reported to produce cataracts in rabbits' eyes at SAR values from 100-140W/kg, which produced lenticular temperatures of 41°C. There were no cataracts detected in the eyes of monkeys exposed under similar conditions [26]. Cataract in the eyes of anesthetized rabbits remains a well-established thermal effect of RF exposure. However, primates appear less susceptible to cataract induction than rabbits, and opacities have not been observed in primates following either acute or prolonged exposures [27].

A study performed by Acar *et al*. showed that RF radiation emitted from a mobile phone can cause temporary facial nerve dysfunction that can be due to temporary temperature increase in the soft tissue around the facial nerve [28]. Some researchers have argued that so-called "non-thermal effects" could be reinterpreted as a normal cellular response to an increase in temperature. The German biophysicist Roland Glaser, for example, [29] has argued that there are several thermo-receptor molecules in cells, and that they activate a cascade of second and third messenger systems, gene expression mechanisms and production of heat shock protiens in order to defend the cell against metabolic cell stress caused by heat. The increases in temperature that cause these changes are too small to be detected by studies such as REFLEX, which base of their whole argument on the apparent stability of thermal equilibrium in their cell cultures [30]. The REFLEX project (QLK4-CT-1999-01574 / REFLEX / Final Report) has made a substantial contribution to the database on biological effects of

both ELF-EMF and RF-EMF on *in vitro* cellular systems. The study was designed to investigate whether or not EMF exposure below the energy density reflected by the present safety levels generates *in vitro* critical cellular events. Gene mutations, deregulated cell proliferation and suppressed or exaggerated programmed cell death (apoptosis) that are caused by or result in an altered gene and protein expression profile are such critical events, the convergence of which is required for the development of chronic diseases.

Genotoxic effects and a modified expression of numerous genes and proteins after EMF exposure could be demonstrated with great certainty, while effects on cell proliferation, cell differentiation and apoptosis were much less conclusive. Since all these observations were made in *in vitro* studies, the results obtained neither preclude nor confirm a health risk due to EMF exposure, but they speak in favor of such a possibility. Because of their fundamental character the findings will be presented to WHO, IARC and ICNIRP. It will be up to these organisations to make use of them for risk evaluation, in combination with findings from animal and epidemiological studies [30].

Other researchers believe that effects observed in heat-shock (stress) proteins are not related to thermal effects, since they were detected for exposures at both extremely low frequencies (ELF) and radiofrequencies (RF), which have very different energy levels [32, 32]. Another study conducted using fluorodeoxyglucose injections and

positron emission tomography concluded that exposures by radiofrequency radiation within parts of the brain, closest to the mobile phone antenna, resulted in the increased levels of glucose metabolism, but the clinical significance of this finding is unknown [33].

There are several reports indicating that EMR from mobile phones at non-thermal levels may elicit a biological effect in target cells or tissues. Whether or not these biological effects lead to adverse health effects, (including cancer) is unclear. To date there is a limited scientific evidence of health issues, and no mechanism by which mobile phone radiation could influence cancer development [34-40]. In [32], French *et al*. developed a theoretical mechanism by which radiofrequency radiation from mobile phones could induce cancer, via the chronic activation of the heat shock response. Up-regulation of heat shock proteins (HSPS) is a normal defense response to a cellular stress. However, chronic expression of HSPS is known to induce or promote oncogenesis, metastasis and/or resistance to anti-cancer drugs. The authors suggested that repeated exposure to mobile phone radiation acts as a repetitive stress leading to a continuous expression of HSPS in exposed cells and tissues, which in turn affects their normal regulation, and thus cancer can result. This hypothesis provides the possibility of a direct association between mobile phone use and cancer, and thus provides an important focus for future experimentation [32, 41].

Studies at cellular level have shown that non-thermal effects of MWs at lower levels

than the ICNIRP (International Commission for Non-Ionizing Radiation Protection) safety standards depend on several physical and biological parameters. Frequency-dependent effects of non-thermal microwaves from GSM mobile phone on 53BP1/γ-H2AX foci and chromatin conformation in human lymphocytes were observed. UMTS MWs induce significant adverse effects in human lymphocytes similar to effects of heat shock and GSM MWs at effective frequencies. The obtained results are in line with hypothesis that UMTS MWs may affect cells more efficiently than GSM MWs because of the nature of signal [42]. The effects of MWs from mobile phones on 53BP1/γ-H2AX foci persisted up to 72 h following exposure of lymphocytes. This long-lasting adverse effect on these important cells of the immune system can have strong relationship with health risk from mobile telephony [43].

Based on current literature, it can be summarized that RF exposures can change gene and/or protein expression in certain types of cells, even at intensities lower than the standard recommended exposure levels [44]. *However, the biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored to make an evidence-based conclusion on their health effects*.

To date, a limited number of studies of non-thermal effects of RF radiation on cells, tissue, bacteria and molecules report often conflicting results [44-47]. There have been numerous experiments to study the growth pattern of yeast cells exposed to microwaves. In a research by Grundler [48, 49], a diploid wild strain of

Saccharomyces cerevisiae was suspended in a stirred aqueous medium. The cells were then exposed to microwave frequencies of 40-60GHz with the power level of up to 50mW, which were transmitted by a waveguide. The outcomes of this study showed that the exposures at high frequency waves induced alterations in the growth rate of the yeast cells. In a later study involving 83 yeast cells, Grundler *et al*. reported that the yeast cell growth rate was affected by low intensity microwave irradiation. It was also concluded that these effects depend on frequency, showing a strong resonance-like behavior, and are not correlated to the microwave power used. The frequency range of 41.64-41.835GHz was used in the study [48, 49].

In another experimental study [50], the budding yeast, Saccharomyces cerevisiae, was preserved on malt extract agar slopes at room temperature. The microwave irradiation was applied using a quasi-optical set-up for more accurate results. The frequency of the signal generator was set to 192, 203, 213, 222, 231, 248, 263, 268, 294, 326, and 341GHz with the powers of 0.4, 0.4, 0.4, 0.4, 0.4, 2.8, 4.5, 4.5, 11, 9, 30, and 23mW respectively. The test was performed in 3 different groups according to the micro-colonies' size (small, medium, and large), and for each group, a variation of exposure time was applied, ranging from 30 to 150minutes with multiplication factor of 30 minutes. The results demonstrated that a statistically significant difference was observed between the control and the exposed groups. That was apparent for all of the exposure times except for that of 150min. Furthermore, it was clear that the greatest difference occurs within the first 30min of exposure. These results suggest that

exposure to radiation has the greatest effect, when the yeast cells are at an early growth stage [50].

In a study conducted by Jelinek and his colleagues [51], Transverse Electro-Magnetic (TEM) cell was used to expose b-tubulin mutant yeast cells Saccharomyces cerevisiae. The authors investigated the possibility of using a device to detect electromagnetic emission of yeast cell at 42GHz. The device was composed of the wave resonator, wave low noise amplifier, temperature controller, as well as the spectrum analyser. The yeast cells were kept inside the waveguide resonator, and then exposed to 42GHz microwaves. The main finding of the experiment was that the system could provide threshold conditions for electromagnetic waves emitted by the yeast cell [51].

In another *in vitro* study, reported by Vrhovac and colleagues, the potential effects of 905MHz radiofrequency microwave radiation, which is similar to that emitted by mobile phones, were evaluated on yeast cells' growth [52]. The researchers used Saccharomyces cerevisiae strains including the wild-type (FF18733), the irradiated strain (FF1481) and D7 strain, which are commonly used to detect reciprocal and nonreciprocal mitotic recombination. The yeast cells were exposed to radiation for 15min, 30min and 60min, and the obtained results showed that the wild-type yeast cells did not exhibit statistically significant changes in colony growth compared to the control samples. On the other hand, the irradiated strains FF1481 and D7 demonstrated statistically significant reduction of colony growth compared to non-

irradiated strains at all exposure times, with the observed effects are being time-dependent. Interestingly, their findings also indicated that strain FF1481 was more sensitive to RF/MW radiation than strain D7 [52].

Noteworthy, the effects of microwave radiation on mammalian cells are controversial and no consensus has been reached [36, 38, 44]. For example, the results of some studies, investigating the effects of microwave exposures on mammalian cells, could not demonstrate microwave-induced DNA damage and cell proliferation [40, 47]. In contrast, other studies have reported that modulated microwave radiation is capable of causing DNA lesions and inhibition of cell proliferation [36, 37, 53]. Various studies have shown that even at low levels of this radiation, there is evidence of damage to cell tissue and DNA, and it has been linked to brain tumors, cancer, suppressed immune function, and neuroendocrine disruption, chronic fatigue syndrome, and depression [54-56].

Oncogenesis studies at molecular and cellular levels due to RF EMR are considered particularly important [57]. Orientation, navigation, and homing are critical traits expressed by organisms ranging from bacteria through higher vertebrates. Across many species and groups of organisms, compelling evidence exists that the physical basis of this response is tiny crystals of single-domain magnetite (Fe_3O_4) [58]. All magnetic field sensitivity in living organisms, including elasmobranch fishes, is the result of a highly evolved, finely-tuned sensory system based on single-domain,

ferromagnetic crystals. Animals that depend on the natural electrical, magnetic, and electromagnetic fields for their orientation and navigation through earth's atmosphere are confused by the much stronger and constantly changing artificial fields created by technology and fail to navigate back to their home environments [58].

The effects of GSM 900 MHz mobile phone radiation on the reproductive capacity of Drosophila melanogaster were investigated by Panagopoulos and his colleagues [59]. The results revealed that GSM radiation affects the reproductive capacity of both female and male insects. The reason why female insects seem to be more affected than males is possibly due to the fact that the exposures started a few hours after the eclosion, thus a few hours after oogenesis starts in female flies, while at the same time the first mature spermatozoa are about to be completely developed in male flies of the same age (spermatogenesis starts earlier than oogenesis). It was shown that the reproductive capacity is much more decreased with modulated emission (50%–60%), than with non-modulated emission of EMR (15%–20%). In addition, the power density of modulated emission is increased by about one order of magnitude in relation to non-modulated emission. Thus, the effects are not linearly correlated with the emitted power density, but it is better correlated with the electric field intensity [59]. In this study, the authors have chosen to refer to the radiation in terms of the power density, which can be measured objectively, rather than in terms of SAR, which cannot be accurately estimated, especially for small insects. They did not detect any temperature increases, caused by the GSM field, within the glass vials (in the food).

They used an Hg thermometer with a 0.05°C accuracy. The researchers reported that even if there were some temperature increase, certainly smaller than 0.1°C, this would not have any observable effect on the insect's ovipositing. Therefore, the recorded effect cannot be attributed to any possible temperature increases caused by the radiation; in other words, it is considered as a non-thermal effect [59]. Actual GSM signals are never constant. There are always changes in the intensity and frequency of these signals. Electromagnetic fields with changing parameters usually are more bioactive than fields with constant parameters, possibly because it is more difficult for living organisms to get adapted to them [60]. As stated in [60], experiments with constant GSM signals can be performed, but they do not simulate actual conditions. To simulate actual conditions, the researchers in [61] used a common GSM mobile phone in their experiments.

A number of studies reported that electromagnetic fields alter the proliferation rate of cells, as well as the rate of DNA, RNA, and protein synthesis [6, 16, 18, 30-32, 34]. The biochemical processes are strongly affected by changes in cytosolic ion concentrations (especially calcium), and such changes can be induced by RF-microwave radiation [62, 63]. It has been shown that RF fields modulated by extremely low frequencies (ELF) decrease cytosolic calcium ion concentration [64-66]. In some experiments, this effect was at the maximum power densities between 0.6 and 1mW/cm^2 [64]. In [67] the GSM signals tested were the RF carrier signals, pulsed at ELF and the power densities 0.436-0.060mW/cm2. It is known that cell

proliferation, DNA, RNA, and protein synthesis are connected with increased cytosolic ion concentrations (especially calcium) and with depolarization of the plasma membrane. The effects of external electromagnetic fields on the cytosolic ion concentrations seems to be connected with interaction between the external field and the cation channels of the plasma membrane, which result in irregular gating of these channels [67].

A biophysical mechanism for this interaction has been proposed in [67]. According to this mechanism, ELF fields of the order of a few V/m are able to irregularly gate electro-sensitive channels on a cell's plasma membrane and therefore disrupt cell function. In addition, pulsed fields are shown to be more bioactive than continuous ones. Therefore, according to [68], the ELF component of a GSM signal, due to the pulse repetition frequency of 217Hz, with a mean electric field intensity of the order of 6V/m, can possibly disrupt cell function and consequently affect the reproductive capacity of a living organism. Two important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, [69] reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50Hz amplitude-modulated 900MHz RFR, whereas a continuous-wave 9000MHz field produced no effect. In [70], the authors showed increased DNA strand breaks in trophoblasts after exposure to a 217Hz modulated 1.8GHz RFR, but a continuous field at the same carrier frequency produced no effect.

2.4.2 *In vivo* studies

Despite using RF exposures, with SAR values far above those that people are normally exposed to, and in some cases exposures for the duration of the animal's lifetime, about 93% of *in vivo* studies published since 1990 have shown no significant short- or long-term effects of applied irradiation. Furthermore, the average survival of irradiated groups of animals was not affected in some 96% of studies [2]. No convincing evidence has been presented for acute or chronic effects of RF on other physiological and biochemical parameters in animals. Thus, the general conclusion, after more than 20 years of *in vivo* studies, is that no consistent or important effects of RF could be demonstrated in intact animals below the international safety standards. There seems to be no important patho-physiological effect of RF fields, apart from thermal effects caused by exposures to fields many times larger than those encountered in our living and working environments [2].

Human provocation studies have investigated mostly possible effect on the nervous system, including many cognitive and behavioral responses, in response to low-level RF fields emitted by mobile telephones near children as well as in adults. It is now generally accepted that there are no significant effects of cell phone usage or reasonable proximity to radiating antennas of base stations on them [2].

Other research studies investigated the effects of RF EMR on pain, vision, hearing and

vestibular function, as well as on the endocrine and cardiovascular systems, were mostly negative. Taste and olfaction have not been studied, so far. Even in studies that reported a mild effect, they were not considered as detrimental to health [2]. However, their significance from long-term exposures could not be verified. Studies using functional imaging of the brain and deep infrared thermographs have shown that there is no significant heating caused directly by RF exposure in the bone or brain [2].

In the so-called "RF hypersensitivity symptoms", 4 to 5% of the population report being sensitive to RF fields, while some of these intolerant individuals report ill health and a number of distressing subjective symptoms during and after using a cell phone and from exposure to other radiofrequency-emitting devices, or being near an RF antenna site [71]. These symptoms are quite non-specific and are present in many diseases, such as cold and flu (headache, nausea, fatigue, muscle aches, malaise, etc.). However, several studies, systematic reviews and meta-analyses in the last 15 years have concluded that hypersensitivity and the observed symptoms have no correlation to RF exposures of individuals. There is presently no scientific basis for characterizing RF hypersensitivity as a medical syndrome [2, 71-74].

Biological effects of microwave radiation (300MHz - 300GHz) depend on the exposure duration, the distance from the source, and the power level of the emitting device. The power of microwave oven radiation in fact is less energetic than that of ordinary visible light. The Whole Body Averaged - Specific Absorption Rate (WBA -

SAR) depends on the frequency of the incident wave for a given incident power density. This formula is related to the core temperature elevation and WBA - SAR.

$$\mathbf{M + PRF - P_{conv} = S}$$

Where **M** is the rate at which thermal energy is produced through metabolic processes; **PRF** is the RF power absorbed in the body; $\mathbf{P_{conv}}$ is the rate of heat exchange with the convection; and **S** is the rate of heat storage in the body.

It can be concluded from the currently published human experimental studies that there is no consensus on the topic of adverse health effects in humans below thermal thresholds. Some studies have reported on no hazardous influences on the well-being and health status of users and non-users of mobile phones and people living near base stations. However, on the other hand there are also a number of studies reporting on adverse cognitive, behavioral and neuro-physiological and other physiological effects induced by RF exposures [2].

2.5 Effects of Electromagnetic Interference on Medical Devices – possible health concerns

The study [75] investigated for any possible adverse effects of electromagnetic intereference (EMI) on human subjects. The interest in low level EMI is well justified because this might be one of only a few documented study, albeit indirect detrimental effects of low level RF fields on the health of exposed people. This is especially the case for patients using implanted cardiac pacemakers or defibrillators, or hooked up to life support devices, such as mechanical ventilators, which are vital for their continued survival. Since their invention, these medical devices were known to be susceptible to external EMF, such as those used for metal detection in airports and for shop security against theft; and a number of warnings and protection measures were implemented since then [75].

Initial studies reported that these adverse effects of EMI were indeed possible, at least for the mobile handsets in close proximity to medical devices and that there was almost no in-built protection from RF interference in current (i.e., 1980's generation) medical devices [76, 77]. The ongoing exponential growth in the use of mobile phones, both inside and outside healthcare facilities, was also a major motivation for such studies, because it could increase the incidence of heretofore-rare events of EMI. Due to the extremely low level of signals from base stations, however, most of experimental studies focused on EMI for handsets. Medical devices that might, in

theory, be susceptible to RF emitted by communication equipment in its proximity [77], are given below:

1. ***Implantable***: cardiac pacemakers, defibrillators, chronic neural and gastric stimulator packs, artificial cochleas, etc.
2. ***Wearable***: hearing aids, Holter and MAPA monitoring devices, TENS (transdermal electronic neural stimulator), etc.
3. ***External***: bedside signal monitoring equipment, anesthesia machines, renal dialysis and heart-lung pump machines, infusion pumps, external cardioverters and pacemakers, mechanical ventilators, signal recording equipment (ECG, EEG, etc.), imaging terminals, computers with telecommunication capabilities, telemetry equipment and several others.

Two types of research studies have been conducted: *in vivo*, with implanted or wearable devices used by patients, and *in vitro*, with detached or external devices. In both situations, mild to extremely deleterious interference events were observed during tests under laboratory and clinical conditions. The following effects were observed: the sudden malfunction of pacemakers, arbitrary and unexpected change of parameters and resetting of devices, triggering of false alarms, sensor artifacts, alteration of readings and tracings; many of which could cause serious harm or even death in case a real patient would be plugged to any of these devices [78].

In 2004, recommendations from Medicines and Healthcare products Regulatory

Agency (MHRA) classified risks of interference according to more recent knowledge, into three levels: *high*, *medium and low*. Analogue emergency service radios and private business radios (two-way communication radios, used by porters, maintenance and security staff), were classified as being at a high risk of interference with many medical devices, and MHRA recommended its use in hospitals only outside clinical areas, only in an emergency and never for routine communication. An experimental analysis of walkie-talkie radios by [79] determined that these devices usually emit at a higher power output, typically 4W or more, and that they interfere much more with medical equipment than mobile phones, to the point that hardware component failure may occur. Thus, MHRA recommended that these walkie-talkie radios should be changed to lower risk technologies, with a power below 2W, such as mobile phones. These, together with TETRA (Terrestrial Trunked Radio Systems), laptop computers, palmtops and gaming devices, equipped with higher power wireless communication devices, such as GPRS, 3G and HYPERLAN, were classified as having a medium risk of interference. MHRA recommended to use them only in designated areas and to switch them off near critical care or life support equipment. Finally, cordless phones (DECT) and low power wireless networks, such as RLAN and Bluetooth have been classified as low risk of interference with medical devices, and require no action in relation to their use in health care facilities. These recommendations were supported by several studies, such as one carried out with several kinds of ventilators used in intensive care [80].

Many countries and regional governments now have adhered by revising their guidelines to a more restricted use of mobile phones in healthcare facilities and their specialized units. For example, the Health Department of NSW, Australia cites a guideline circular for mobile phones and wireless communication devices in health care facilities [81] across NSW, and recommend a general 2m distance to be observed at all times between the RF emitting mobile equipment and sensitive medical equipment in certain areas, such as Intensive Care Units (ICUs) in hospitals, emergency rooms (ERs), orthotics (OTs), etc., and a 0.5m distance in wards and general areas, and that two-way emergency and security radios should not be turned off, but used only in the stricly required situations.

Another interesting recommendation by the International Standards Organization (ISO) technical report is to "*issue particular mobile wireless equipment to doctors and staff for healthcare specific communication and health information access. This would allow the full benefit of wireless technology operating compatibly throughout the healthcare facility, even in sensitive areas in proximity of life-critical medical devices*". This recommendation has probably been superseded by the new digital cell phones, which emit very low power at frequencies to which most medical devices are considered now immune, and which have largely substituted alphanumeric pagers used by medical personnel. Use of Voice Over Internet Protocol (VOIP) handset devices based on very low power NANOCELL WIFI data communication networks are now being deployed. Indeed, the evolution of wireless communication technology

on one hand, and of radiation protection of medical devices on the other, has greatly changed the situation. For example, in contrast to the HRMA and Irnich studies in the 1990s, [82] could demonstrate almost a decade later that the EMI risks were significantly reduced. The authors carried out a systematic review of seven published research studies between 1996 and 2004 on EMI of cell phones evaluating twenty eight different types of external medical devices. The authors found that clinically relevant EMI potentially endangering patients occurred in 45 of 479 (9.3%) devices tested at 900MHz and 14 of 457 (3%) devices tested at 1800MHz, mostly occurring when mobile phones were used within 1 m of medical equipment [82]. Overall, the prevalence was low, but the authors observed that all studies still recommend some type of restriction of mobile phone use in hospitals, with use greater than 1m from equipment and restrictions in clinical areas being the most common [83].

The trend continued, as demonstrated by an experimental study carried out by [84], on a total of sixty one medical devices in seventeen categories and twenty seven different manufacturers. The authors studied novel digital transmission technologies, such as GPRS-1 and UMTS signals used by third generation (3G) mobile phones. The distance to achieve an interference effect was reduced to 3cm (i.e. with the handset practically in close contact with the medical device), with only one hazardous incident occurring beyond 100cm.

More recent experiments with modern implantable stimulators, for instance, have

demonstrated no effects of GSM mobile phone transmissions nearby [85-87]. Mechanical invasive and non-invasive ventilators have not suffered any malfunction from GSM mobile phones, and a few effects with two-way communication radios at less than 1m distance [88]. In Sweden, [89] tested the interference of General Packet Radio Service (GPRS), Universal Mobile Telecommunications System (UMTS), Wideband Code Division Multiple Access (WCDMA) and IEEE 802.11b (WLAN) signals on seventy six medical devices, including during eleven surgical operations with a total duration of 100 hours. The researchers concluded that UMTS and WLAN signals caused little interference and that "*devices using these technologies can be used safely in critical care areas and during operations, but direct contact between medical devices and wireless communication devices ought to be avoided. GPRS can be used safely at a distance of 1m. Terminals/cellular phones using these technologies should be allowed without restriction in public areas because the risk of interference is minimal*".

The particularly large *in vivo* study by [90], which tested 679 implanted pacemakers, arrived at an overall 5.5% figure of incidence of EMI per patient. Although [91] demonstrated that current mobile phone technologies in use, such as GSM, CDMA, TDMA and IDEN are still able to cause malfunctions on external medical equipment over short distances, clinically relevant EMI occurred only 1.2% of the tests made. Only four years before that, the same authors [92] had detected a 7.4% incidence of clinically relevant EMI events in cardiopulmonary monitors. Finally, in 2007, the

same authors again, working at the Mayo Clinic [91] determined, in more than 300 tests involving a total of 192 medical devices, that the incidence of clinically important interference was 0% (95% confidence interval, 0%-4.8%). Thus, they concluded that "*although cellular telephone use in general has been prohibited in hospitals because of concerns that these telephones would interfere with medical devices, this study revealed that when cellular telephones are used in a normal way no noticeable interference or interactions occurred with the medical devices*" [93].

2.6 Use of Transverse Electromagnetic (TEM) Cells for Studying Biological Effects of Radiofrequency/Microwave Exposures on Biological Media

Usually in biological experiments with RF fields, exposure chambers/cameras of special constructions are used to ensure constant exposure conditions of different samples simultaneously (e.g., Transverse Electromagnetic (TEM) field cells, radial transmission lines, etc.).

The first use of TEM cell dated back to 1974, when Myron L. Crawford from National Bureau of Standards (NBS) designed a shielded chamber for an electromagnetic susceptibility and emissions testing [94]. The idea was to produce a uniform electric field inside the cell and to put the tested object inside of it to measure how much energy was absorbed by the object. Crawford constructed the cell in several different sets of dimensions, with the largest being 20.3cm x 48.3cm x 63.5cm that can accommodate the smallest frequency of 150MHz. The smaller operating frequency requires to construct a camera of bigger dimensions, due to interference from higher order modes [94].

In another study [95], the performance of the commercial TEM cell (model IFI CC 104 SEXX) was tested. The parameters considered were the test volume, microwave frequency limit, and electric field distribution. A 3D model based on the finite element method (FEM) using COMSOL Multi-physics software was created and simulated.

The results were then validated by a simulation using Finite-Difference Time-Domain (FDTD), as well as experimental testing of the scattering parameters and voltage standing wave ratio. A dosimetric estimate was also done using COMSOL software by placing a Petri dish filled with 55 corn seeds on the Septum. The seeds were exposed to 900MHz electromagnetic wave. The parameters investigated included the specific absorption rate (SAR) and Eigen frequency [95].

As reported in the literature, when designing a TEM cell, several important considerations have to be taking into account in order for the exposure device to give a better performance for the intended purpose. For example, when a higher frequency of operation is required, the dimensions of the TEM cell have to be smaller for minimising interference from the higher order modes. On the other hand, reducing the size of the test area limits the bandwidth of the TEM cell.

Deng came with a solution to suppress higher order modes, while still using larger dimensions TEM cell [96]. TEM cells are normally designed to operate in the frequency range of up to 1GHz. When higher frequencies are applied, higher order modes start to propagate due to the reflections and it will cause disruptions in obtaining information from the TEM cell measurements. Deng and his colleagues had proposed a number of methods to suppress the higher order modes without changing the cell size or the test board size, such as a modified TEM cell with slotted walls, resistors between the traces, lossy absorbing materials wrapped around the cell, and an

outer shielding box. The proposed designs were simulated using full-wave simulations and verified by measurements of the modified cells, and they passed the immunity testing up to 2.5GHz [96].

Another experiment was conducted by Malaric *et al*. where he investigated a way to improve the test area of a TEM cell without sacrificing the other parameters such as characteristic impedance and resonance of higher order modes [97]. The simulations were run using finite element method (FEM) and finite integration technique (FIT). The modifications were mainly calculated numerically on the dimensions of the TEM cell, with the initial step involving a selection of the ratio of length and width of the cell, in relation to the characteristic impedance.

Another possible solution for these problems is the construction of Gigahertz Transversal Electromagnetic Mode (GTEM) cell. In his thesis, Icheln designed and constructed a GTEM cell [98]. The objective of his study was to create an electromagnetic susceptibility testing chamber for electronic equipment with the size of at least a mobile phone in the frequency range of 30MHz to 1GHz. The GTEM cell was also required to be portable, for demonstration purpose. This requirement had led to construction of a TEM cell of 2.2m length, yielding in a testing volume with the dimensions 23cm x 17cm x 10cm. To verify the possibility to use the cell for radiation susceptibility tests and measurements, two experimental testings were conducted [98]. In the first test, a patch antenna was measured at a frequency of 1.885GHz; and in the

second - a noise radiator with a frequency range of 10-1000MHz was measured. The results showed that the constructed GTEM cell could be used for both purposes after some calibrations to the testing object placement as shown in the Figure 3.

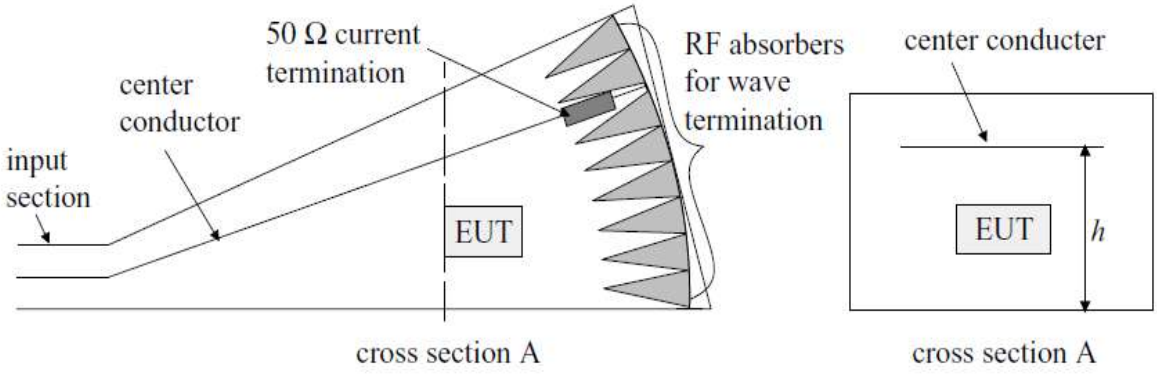

Figure 3. The cross section of GTEM cell [98] Equipment Under Test (EUT)

In 2008, Satav and Agarwal constructed a low-cost and lightweight TEM cell using printed circuit board (PCB) as the conductors [99]. The advantages of using PCB include its lightweight characteristic, better voltage standing wave ratio (VSWR) relative to the thick metallic version, less field-fringing and easy fabrication. The trade-off, however, is that the device can handle lower maximum power; therefore, this is only suitable for low radiated emission testing. The dimensions were adjusted to suit 50Ω characteristic impedance and all the calculations were done manually (without any software). After the fabrication and testings, the authors concluded that the device can be used for in-house pre-compliance testing for products during the development cycle, but is not suitable for a formal compliance testing, due to the

presence of a small window that could possibly produce electromagnetic leakage [99]. Another example of the developed TEM cell was from a master thesis by Boriraksantikul [100]. In that thesis, a TEM cell was used to experiment the mobile phone electromagnetic field strength in a confined area. The TEM cell was designed and simulated using computer simulation technology (CST) Microwave Studio using discrete port as the input signal. The design is shown in Figure 3 After some simulations, the TEM cell was fabricated by breaking the model into pieces. The material of the conductor was not disclosed in the thesis [94].

There are commercial TEM cells available on the market, but they are expensive and most of the time they don't exactly suit the specific experiment the researchers intend to do, due to their general form and dimensions. Therefore, in this study a custom-made TEM cell, which can suit the required operating frequency as well as the dimensions of the testing objects, was designed and constructed. Two TEM cells were employed in this project to study non-thermal effects of low power microwaves of different frequencies at cellular and molecular levels. The details of these exposure cameras are presented in details in Chapter 3.

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CHAPTER 3

MATERIALS AND METHODS

This project aims at *in vitro* experimental evaluating of the biological effects of low power electromagnetic radiation on cells and proteins at the selected radio-/microwave frequencies, which are used in Australian 2G, 3G, and 4G mobile telephone networks.

Table 1. Frequencies commonly used in Australia by mobile network providers

Type	Frequency	Comments
2G	900MHz 1800MHz	Main frequency (900MHz) and supplementary frequency (1800MHz) for Australian GSM (2G) mobile carriers
3G	850MHz 900MHz 2100MHz	Telstra and Vodaphone USB networks Mainly in Rural area Main frequency for 3G networks
4G	1800MHz 2300MHz 2600MHz	Used by Telstra Used by Optus Used by Telstra

In this research project the commercial and custom-made TEM cells were used to irradiate the selected yeast cells and enzymes with low powers microwaves at the frequencies of 500MHz, 900MHz, 968MHz, 1.8GHz, 2.1GHz and 2.3GHz. Chapter 3 presents and describes in great details two exposure systems (commercial and custom-made TEM cells) used in this study and model systems being irradiated by low level microwave radiation.

3.1 Transverse Electro-Magnetic (TEM) cell

The Transverse Electro-Magnetic (TEM) cell is an enclosed box made of the conductor material, with its dimension varies depending on the operating frequency used. There is a layer of conductor sheet at the center of the TEM cell to place the yeast cell, called septum. One end of the box will be connected to a signal generator, as the source of microwave radiation, while the other end will be connected to the resistive load. The real advantage of using TEM transmission cells for making susceptibility or emissions measurements is the elimination of background interference without the introduction of measurement problems associated with shielded or anechoic enclosures. Furthermore, no electromagnetic (EM) fields are generated external to the cell and the cells produce uniform and readily determined fields. Shielded enclosures, on the other hand, reflect the emitted energy from their walls in such a complicated manner that prediction of the enhancement or interference of the desired signal is extremely difficult. Measurements using the TEM cell are simple to make and require a minimum of detection equipment, e.g., no additional antennas are required [1].

Since 1974 and the introduction of TEM cells [1], there have been many improvements and different variations of transmission-line cells for electromagnetic interference (EMI) and electromagnetic compatibility (EMC) testing. GTEM, WTEM and EUROTEM are examples of available TEM cells. They come in different sizes

and shapes depending on a desired carrier frequency; however all these devices are used for establishing standard predictable EM field in a shielded environment (please refer to Chapter 2).

In this project, TC-5062AUHF TEM cell (100kHz–3GHz) from TESCOM Ltd, Unitechvill, Goyang, Korea (shown in Figure 4) was used a source of microwave radiation to irradiate the selected yeast cells and enzymes.

Figure 4. Commercial TC-5062A TEM cell [2]

Through the input port, an external signal is applied to generate a predictable field inside the TEM Cell. The GTEM was calibrated using a broadband electric field probe to determine the electric field produced at the sample position inside the camera for a given input power. We scaled the field values provided for 10dBm (10mW) input to

the power levels applied, using the equation [2]:

$$E_1 = E_0 \sqrt{\frac{P_1}{10mW}} \qquad (3.1.1)$$

where E_1 is the exposure field of the sample, E_0 is the calibration field we found using a test power of 10mW, and P_1 is the test power we used in our exposure. The calibration test showed that the estimated uncertainty in the generated test field is ±1–3% depending on the input signal frequency. The calibration test was performed by TESCOM laboratory (Tescom Company Limited, Goyang, Korea).

The operational principle of TC-5062A exposure camera [2] is essentially the same as other TEM Cell: with the applied RF voltage on one port of the cell, while the other port is terminated with a 50Ω resistor. The TEM Cell maintains 50Ω characteristic impedance along the cell. The E–H field inside the test volume is proportional to the input voltage and inversely proportional to the cell height. Therefore, the electrical field generated at the test point inside the TC-5061A can be calculated as:

$$E = \frac{signal\ level\ [V]}{dis.\ from\ top\ [M]} \qquad (3.1.2)$$

The TC-5062 has a specific pyramidal geometry designed to extend the usable frequency range 100kHz – 3GHz (Figure 4). Typically, this can be achieved by replacing one port of a two-port TEM cell with a wideband non-tapered hybrid discrete wave absorber termination. The GTEM cell, described thoroughly by Nothofe

[3], is a similar pyramidal cell that produces a similar field configuration. Since the TEM Cell produces the TEM waves, there is an orthogonal H-field (A/m) proportional to the E-field inside the TEM Cell. The relationship between the H- and E-fields is defined by the equation [3]:

$$H = \frac{E}{Z_{free}} \tag{3.1.3}$$

E is Electrical field in V/m, Z_{free} =377Ω and represent free space wave impedance.

where 377Ω is the free space wave impedance.

The electrical field inside the TEM cell can be calculated using the equation:

$$E = \frac{V}{L} \tag{3.1.4}$$

E is Electrical field in V/m, V is Voltage in [V] and L is the length of the camera in [m].

For the model TC-5060 UHF TEM, the length (L) is 0.22m [2]. It is a wide band TEM Cell with absorber termination. The large absorber wall eliminates potential resonance inside the cell and produces a wide band of operation. If the RF test signal from the signal generator is injected into the TEM Cell input port, the predictable TEM mode field is generated at the test position. In the case of a direct cable connection, the percentage of the leaked RF power compared to the signal is very small. The TC-5062A is an accurate, broad band RF coupler with a high quality shielding wall. In

addition, the voltage standing wave ratio (VSWR) of the TEM cell was tested with the sample holder (no sample) using microwaves within the interval of 300MHz–3GHz by ‘‘Measuring Instruments for Wireless Communications’’. It was reported that the VSWR has a maximal value of 1.7 for 3GHz. Therefore, the power propagating through the TEM cell can be calculated as follows [3]:

$$P_p = P_g\left(1-\frac{VSWR-1}{VSWR+1}\right) \tag{3.1.5}$$

where *Pp* is the power propagating through the cell, and *Pg* is the generator power. For VSWR = 1.7, the ratio Pp=Pg>0.93.

The signal generator used in this study was a Rhode & Schwartz (100kHz–1000MHz) SMX generator (Munich, Germany). All experiments with the selected yeast cells and enzymes were conducted at the room temperature 25°C, which was monitored by Temperature controller (Quantum Northwest) during experimentation.

3.2 Custom-made TEM cell design, fabrication and testing

3.2.1 TEM cell (exposure camera) design and simulation

Despite the availability of commercial TEM cells on the market, the fabrication of the custom-made TEM cell is essential for this project. The custom made TEM cell is more economical, its material and dimensions can be chosen to suit the project. The custom-built TEM cell is more suitable for the project as it was of particular importance to study the effects of MW frequency used in 2G mobile network (900-1800MHz) due to the previously published studies reporting the effects of low level radiofrequency radiation in this range on different biological media (please refer to Chapter 2).

Hence, the dimensions were selected to suit the aim of this PhD project, which is the exposure of the selected model systems to low power microwave radiation with a frequency around 900MHz. In designing a TEM cell, there are several considerations should be taken into account in order for the exposure camera to give a better performance for the intended purpose. For example, when higher frequency is being used, the dimension of the cell has to be smaller - for minimizing interference from the higher order modes.

For the design of the exposure camera, the electromagnetic High Frequency Structure

Simulator (HFSS) software was used to predict the homogeneity and uniformity of the field generation. Since a performance of electronic devices is dependent on its electromagnetic (EM) behavior, it is important to have a fast, accurate account of how the design will behave in real-world implementations — long before any prototype is built.

ANSYS HFSS simulation package delivers the most accurate answer possible with the least amount of user involvement. As the reference-standard simulation tool for 3-D full-wave electromagnetic- field simulation, HFSS is essential for designing high-frequency and/or high-speed components used in modern electronics devices. HFSS is a high performance full wave EM field simulator for arbitrary 3D volumetric passive device modeling that takes advantage of the familiar Microsoft Windows graphical user interface. It integrates simulation, visualization, solid modeling, and automation in an easy to learn environment where solutions to the 3D EM problems are quickly and accurate obtained. HFSS employs the Finite Element Method (FEM), adaptive meshing, and high quality graphics to enable unparalleled performance and insight to 3D EM problems.

HFSS is an interactive simulation system, whose basic mesh element is a tetrahedron. This allows a user to solve any arbitrary 3D geometry, especially those with complex curves and shapes, in a fraction of the time it would take using other techniques. The name HFSS stands for High Frequency Structure Simulator. Ansoft pioneered the use

of the Finite Element Method (FEM) for EM simulation by developing / implementing technologies such as tangential vector finite elements, adaptive meshing, and Adaptive Lancozos - pade Sweep (ALPS).

HFSS, part of the ANSYS high-frequency electromagnetic design portfolio, is integrated with ANSYS Workbench for coupling EM effects into complex analyses, such as temperature and deformation. ANSYS HFSS software is the industry standard for simulating 3-D full-wave electromagnetic fields. Its gold-standard accuracy, advanced solver and high-performance computer technology have made it an essential tool for engineers doing accurate and rapid design of high-frequency and high-speed electronic components. ANSYS HFSS is the industry-standard EM simulation package for RF and microwave as well as signal integrity design. It is ideal for analyzing any physical structure that relies on electromagnetic fields, currents or voltages for operation. Using a 3D full-wave frequency domain EM-field solver based on the finite element method, HFSS extracts scattering parameters, visualizes 3D EM fields, generates far-field radiation plots and provides ANSYS Full-Wave SPICE models.

In this study, HFSS simulation package was used to design a custom-made TEM cells that can generate a EMF of the desired characteristics, frequency of 900MHz and power of 10dBm (below the standard safety exposure limits). As was mentioned above, TEM cell is a device used to establish a standard predictable electromagnetic

field in a shielded environment. It is a box with the sides closed to prevent radiation of RF/MW energy into the environment and to provide electrical isolation. It consists of a coaxial transmission at each end to adapt to standard coaxial connectors. The coaxial transmission adapter is a line for high frequency signals and conductor for transmitting electrical, optical signals, electrical power and good for RF connectors. The electrical field is a vector field generated by electric charge and time raring magnetic field. HFSS has long been used by RF and microwave engineers to design high-frequency components found in communication systems, radar systems, satellites, smart phones and tablet devices. The technology addresses a wide range of RF and microwave engineering challenges, and these applications benefit greatly from the automated meshing feature and resulting high solution accuracy.

In designing the exposure chamber, the following criteria were taking into account: a new custom-made TEM cell should *be lightweight, portable and cost effective*. Hence, to determine the optimal parameters of the TEM Cell that can generate a homogeneous uniform electromagnetic field inside the exposure chamber at the frequency 900MHz and power 10dBm, we employed the ANSYS HFSS software that offers multiple solver technologies based on the proven finite element method. Using the HFSS, a 3D TEM cell was designed and shown in Figure 5.

The initial steps for the simulation were cited from previous work [1, 4]. Thereafter, a process of fine tuning of the design took place to find the most efficient design for

generating a field of the desired characteristics (Figures 6 - 8). Finite Element Method (FEM) was used for calculation.

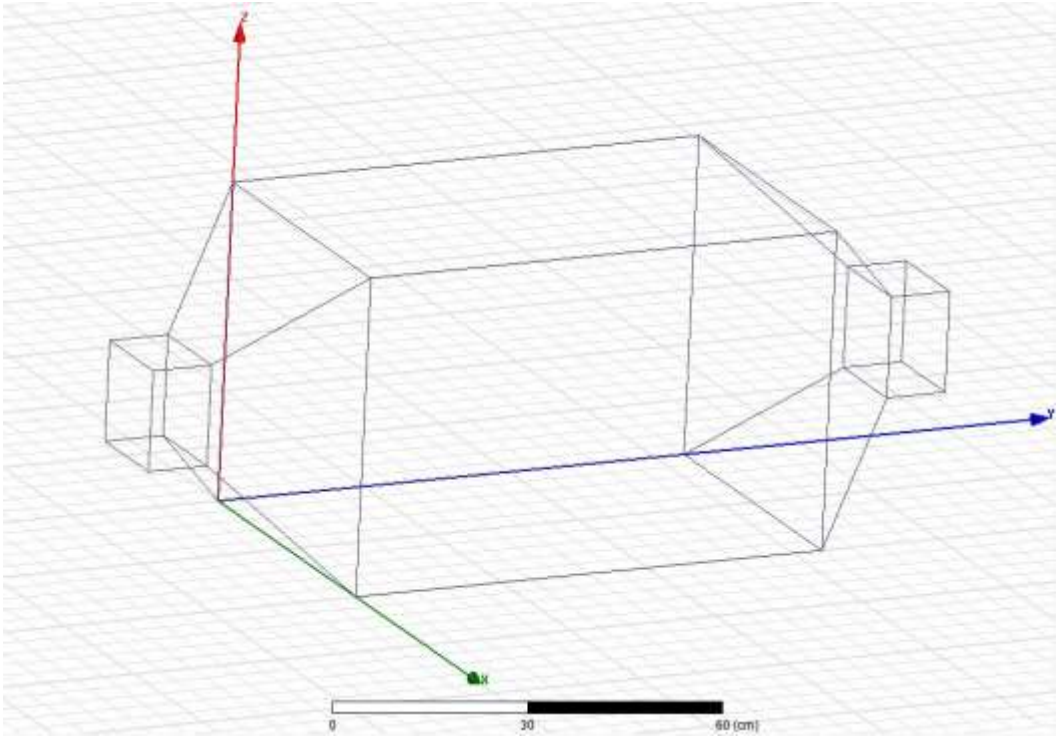

Figure 5. TEM Cell in 3D view

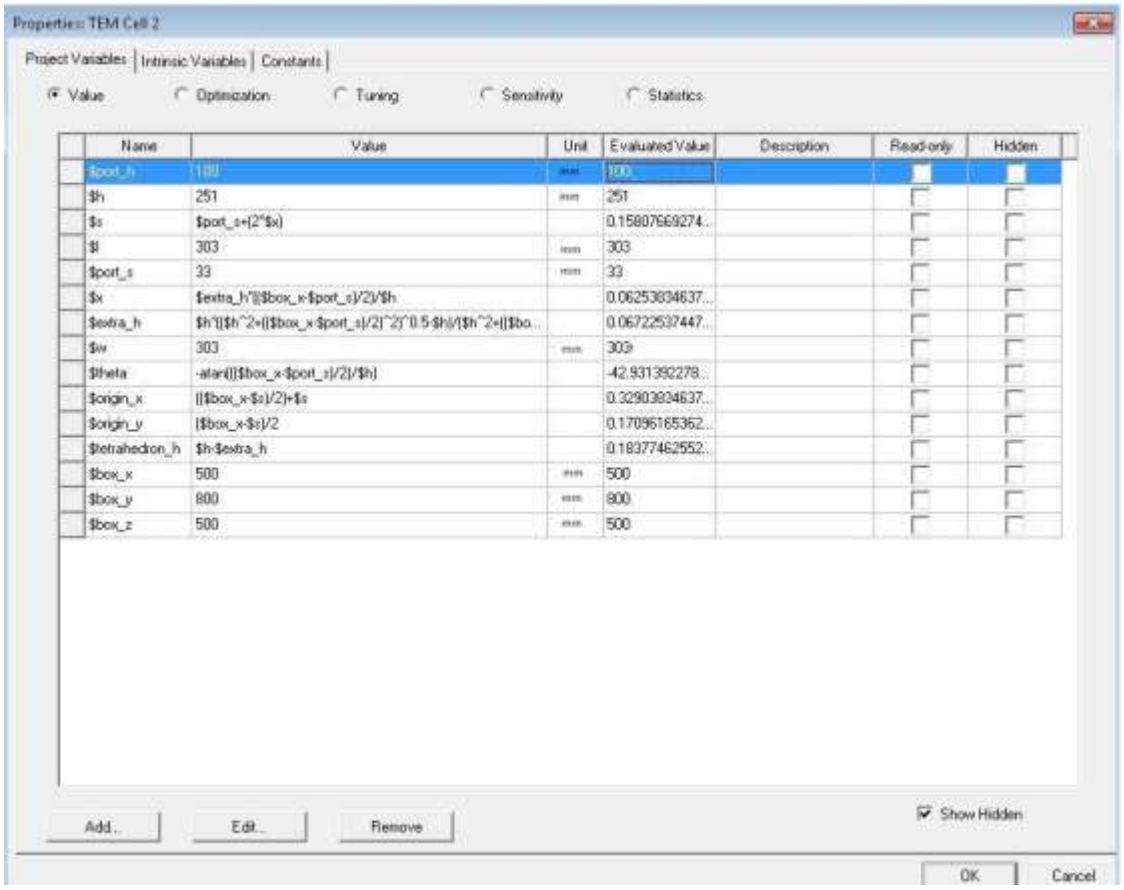

Figure 6. Calculated properties of the designed TEM cell

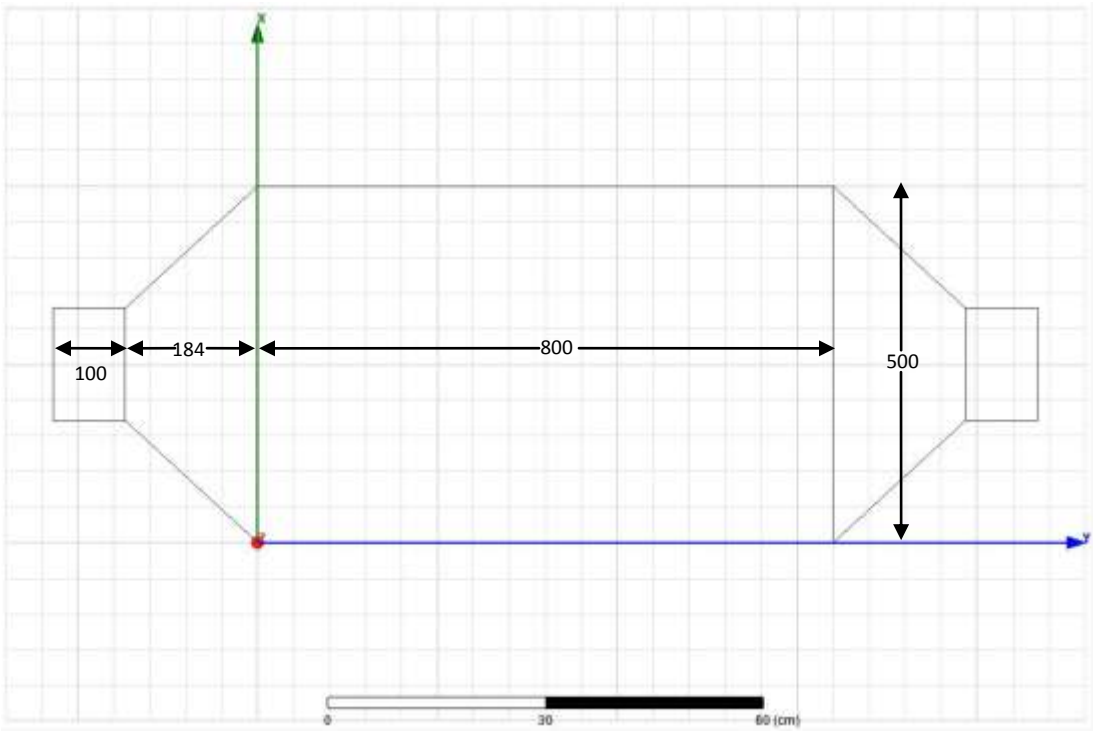

Figure 6. TEM Cell view from the side (all units are in mm)

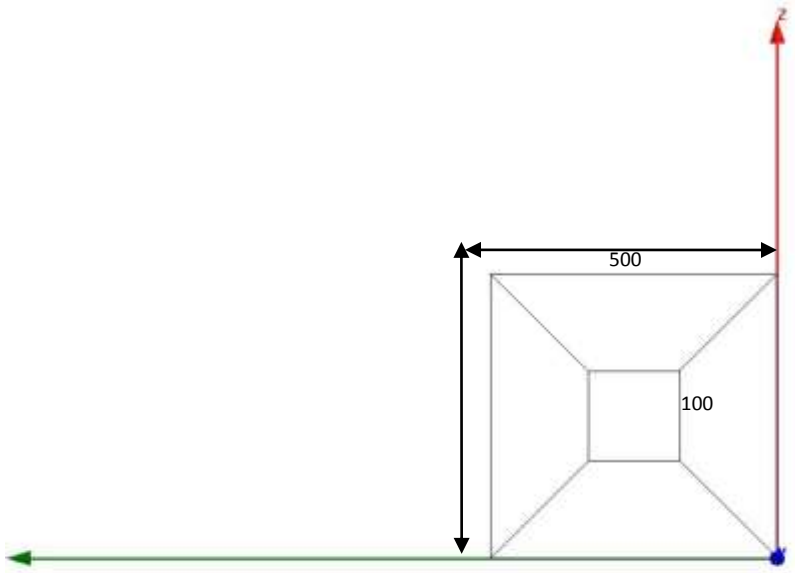

Figure 7. TEM Cell view from the front (all units are in mm)

The finite conductivity property of the Aluminum used in the design of the TEM Cell is shown in Figure 9.

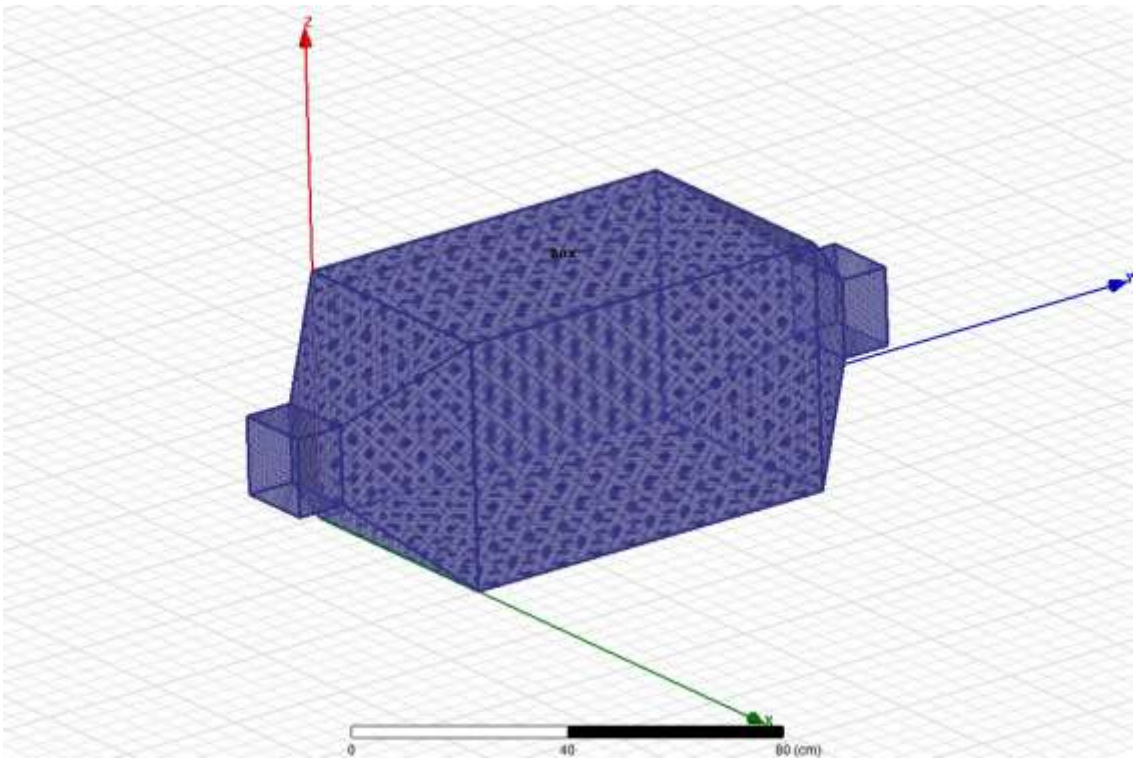

Figure 8. TEM Cell with its conductive boundary Aluminum

To meet the design requirements, the TEM cell model is fabricated from Aluminum with the pre-calculated dimensions presented below. Aluminum was chosen as a material for the exposure camera due to its excellent characteristics. The main advantage is that aluminum is not rusting and has *excellent corrosion resistance property* as opposed to iron. Other advantages include *low weight, high strength, superior malleability, easy machining,* and *good conductivity of heat and electricity*. In relation to its conducting property, aluminum is almost twice as good as copper and also very easy to be recycled Figures 10 and 11.

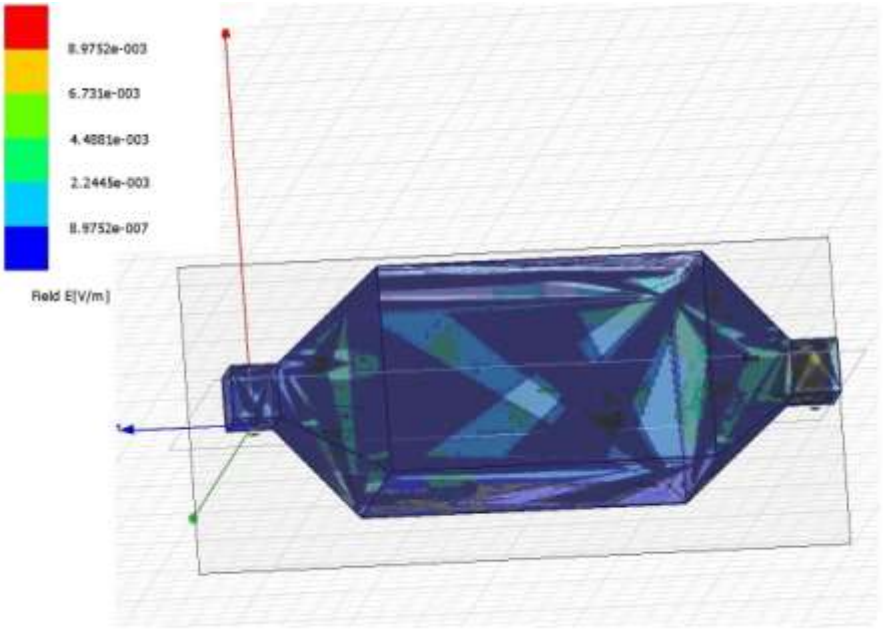

Figure 9. Magnitude of E-field inside the TEM Cell with its conductive boundary made from Aluminum

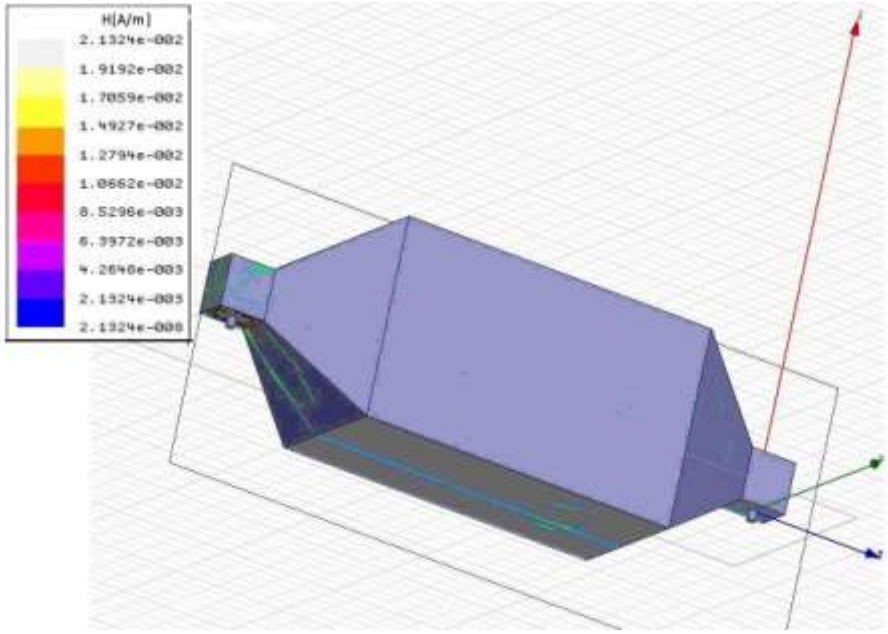

Figure 10. Magnitude of H-field inside TEM Cell with its conductive boundary made from Aluminum

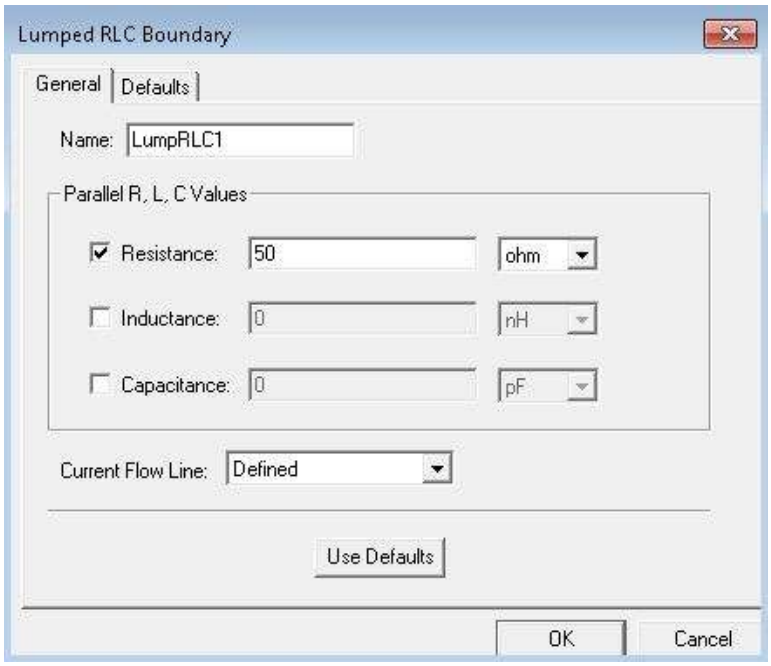

Figure 11. 50Ω load

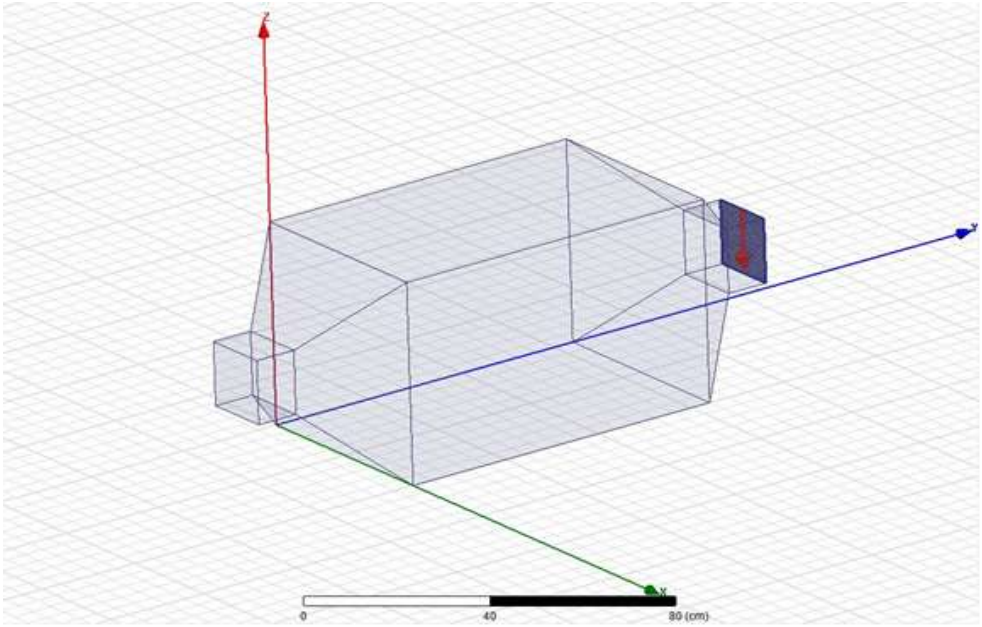

Figure 12. The load 50Ω at one end of the TEM Cell

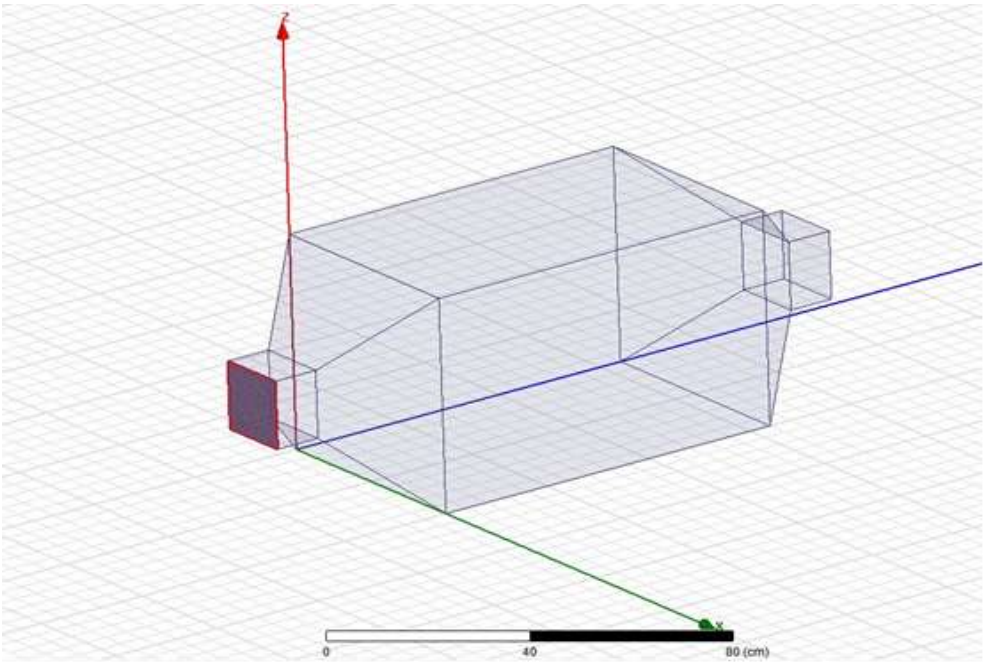

Figure 13. Electric field source at the other end of the TEM Cell

One end of the TEM cell will connect to a signal generator while the other one is the stopping load of 50Ω (Figure 13-14).

3.2.2 Software simulation of the generated field in the TEM cell

The simulation results are presented below, where it is possible to observe a 3D electric field pattern generated inside the TEM cell. In these simulations, a 3D electric field pattern is generated inside the custom made TEM cell that is shown in Figures 15, 16, 17 and 18. Figures 19, 20, 21, 22 and 23 show the horizontal and vertical views and electric filed patterns of the generated electric field inside the simulated TEM cell.

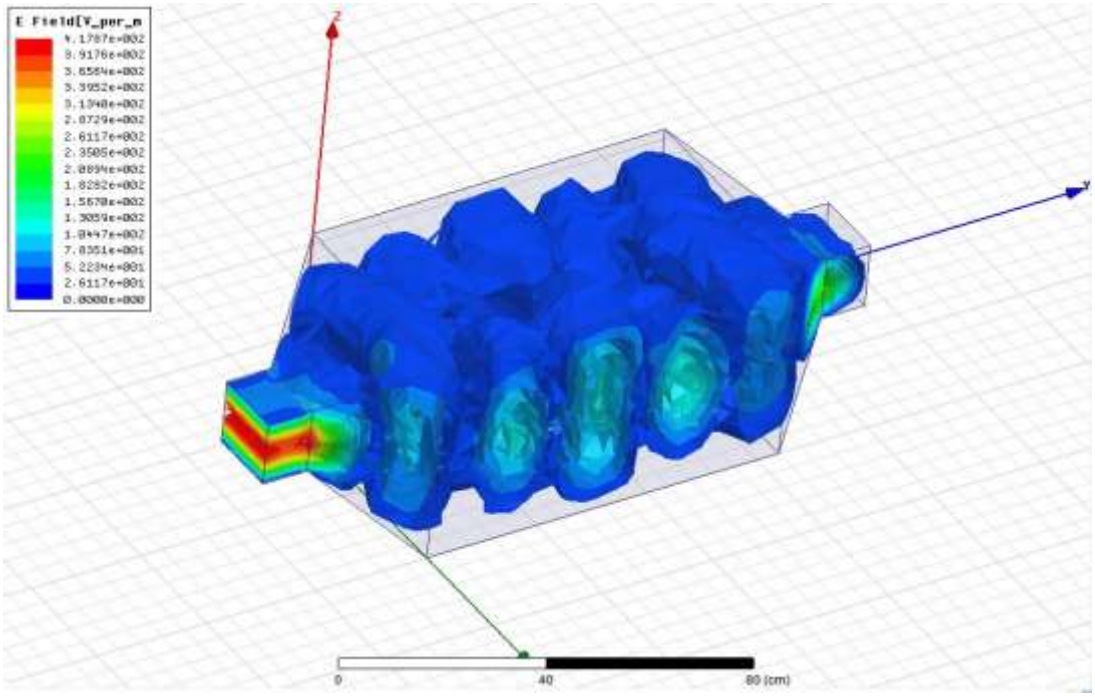

Figure 14. 3D Electric field pattern inside the box

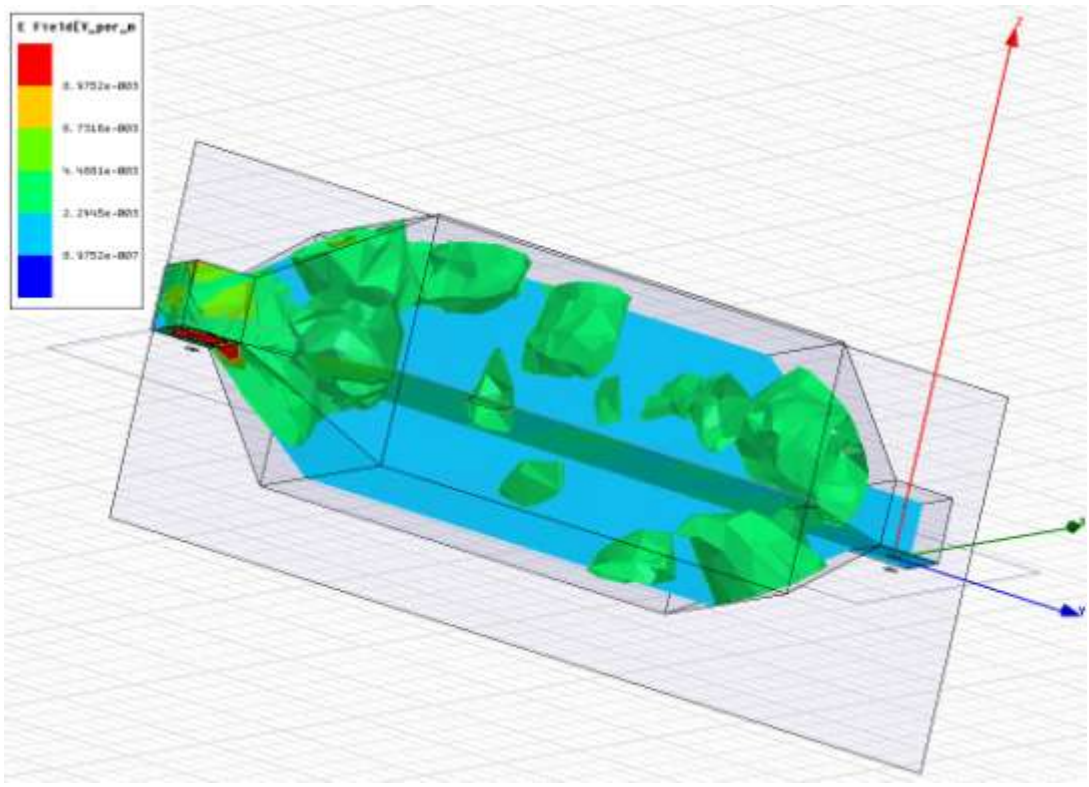

Figure 15. 3D Electric field pattern inside the box

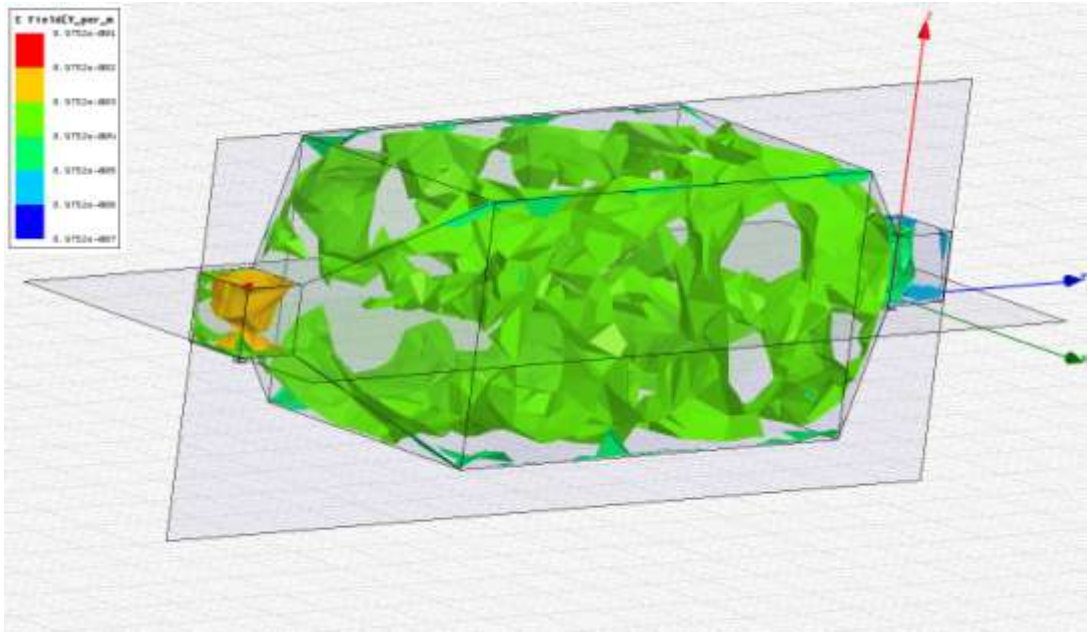

Figure 16. 3D Electric field pattern inside the box

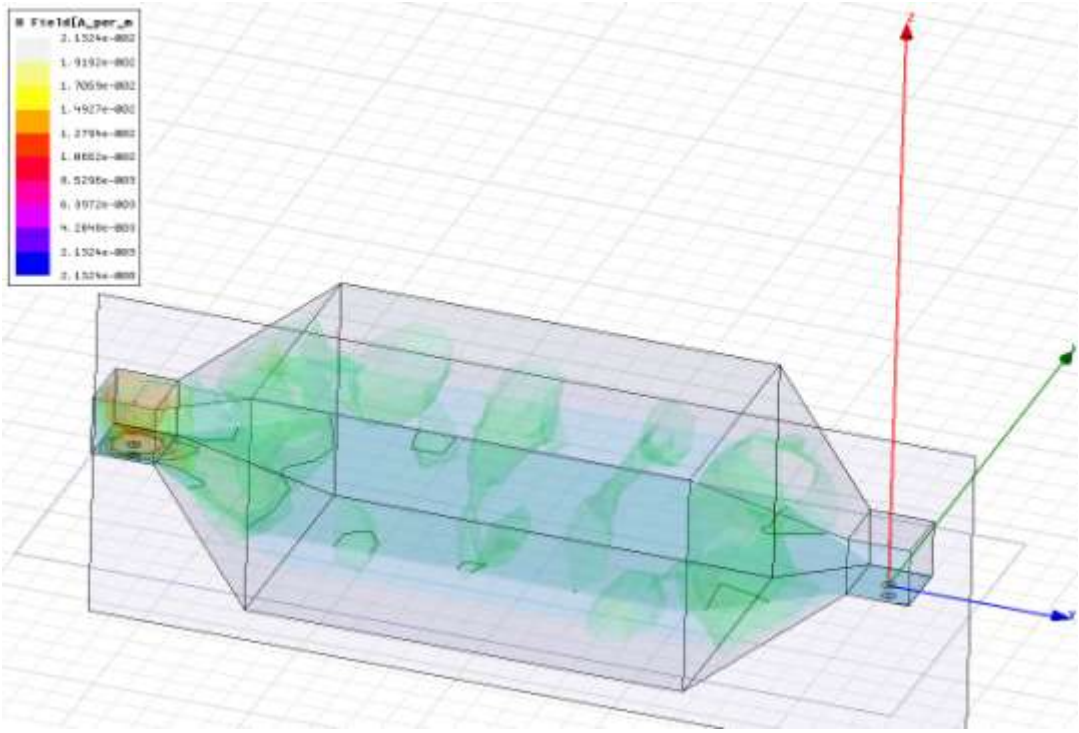

Figure 17. 3D Electric field pattern inside the box (vector- H1)

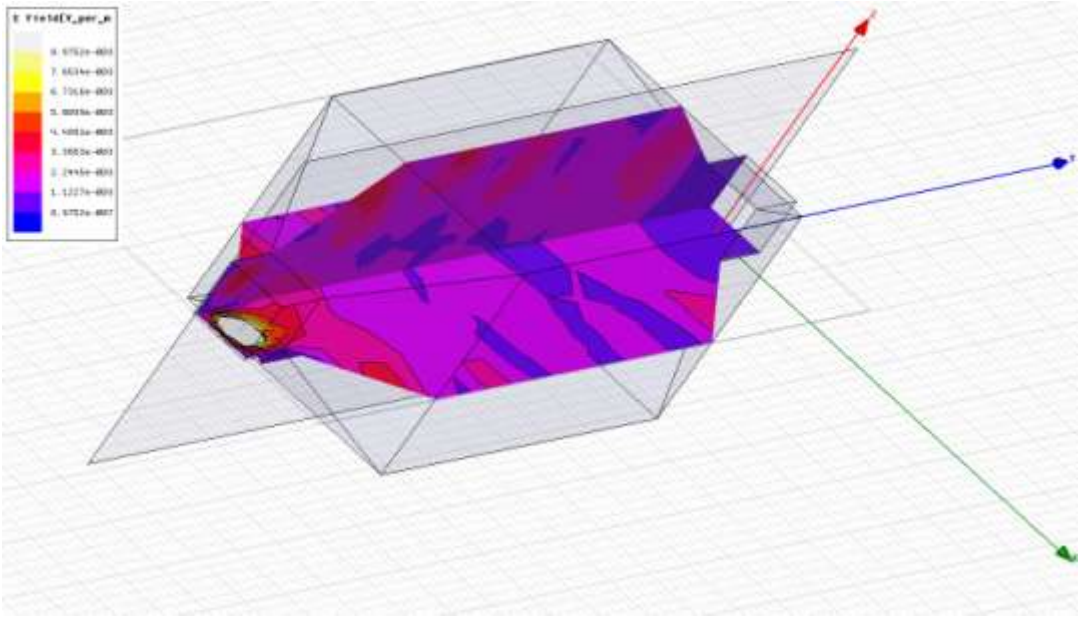

Figure 18. Electric field vertical and horizontal

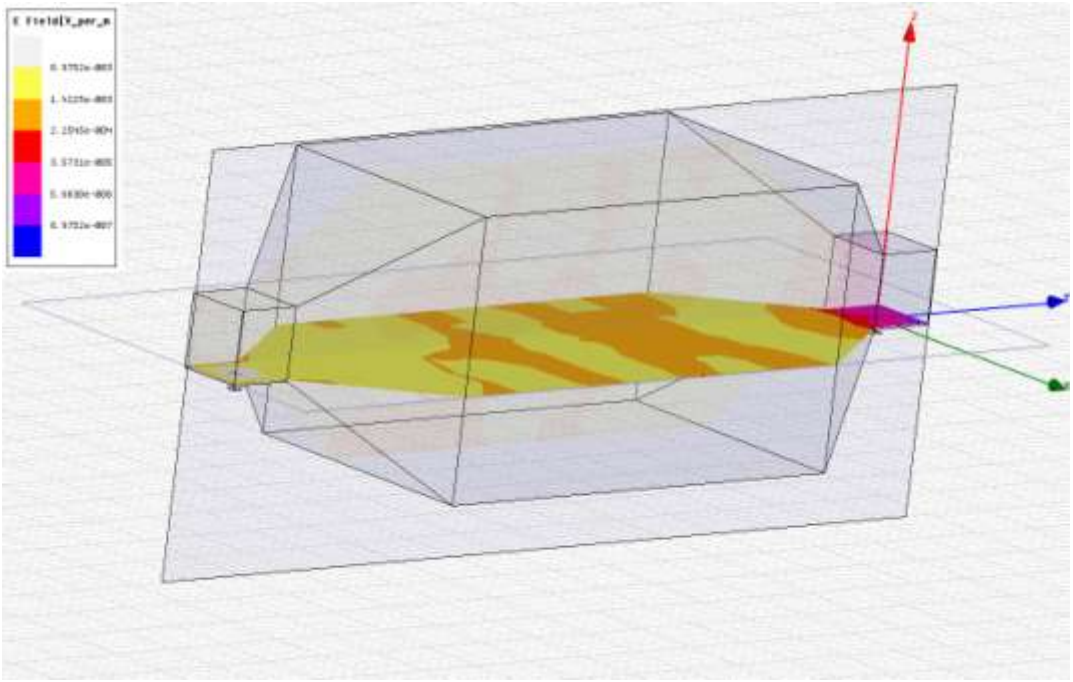

Figure 19. Electric field horizontal plane

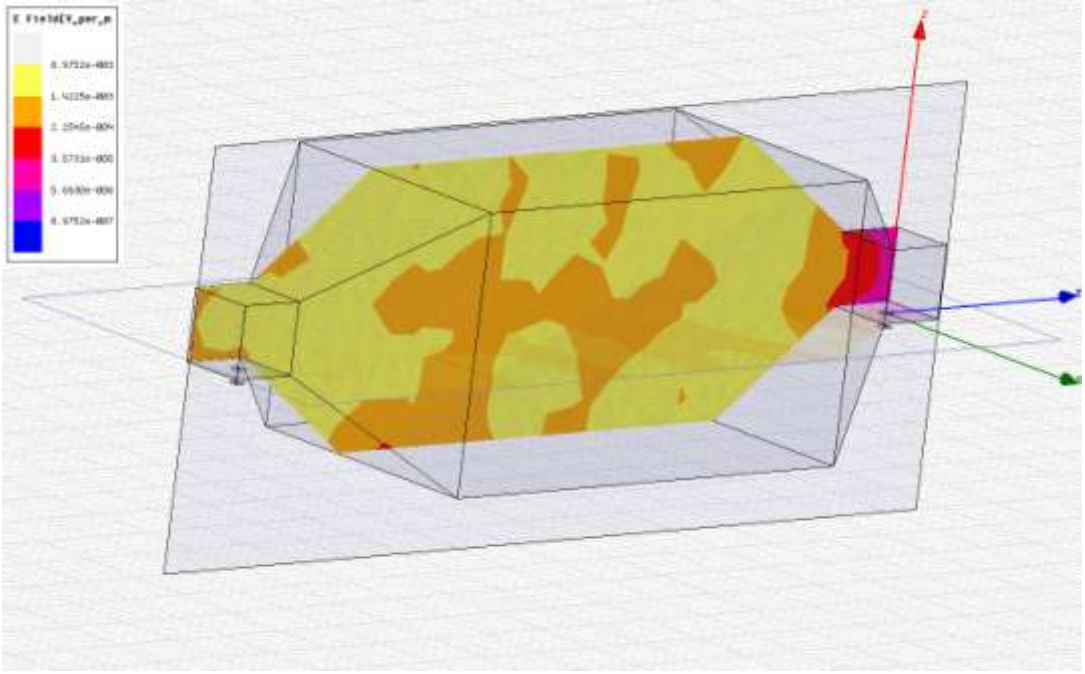

Figure 20. Electric field vertical plane

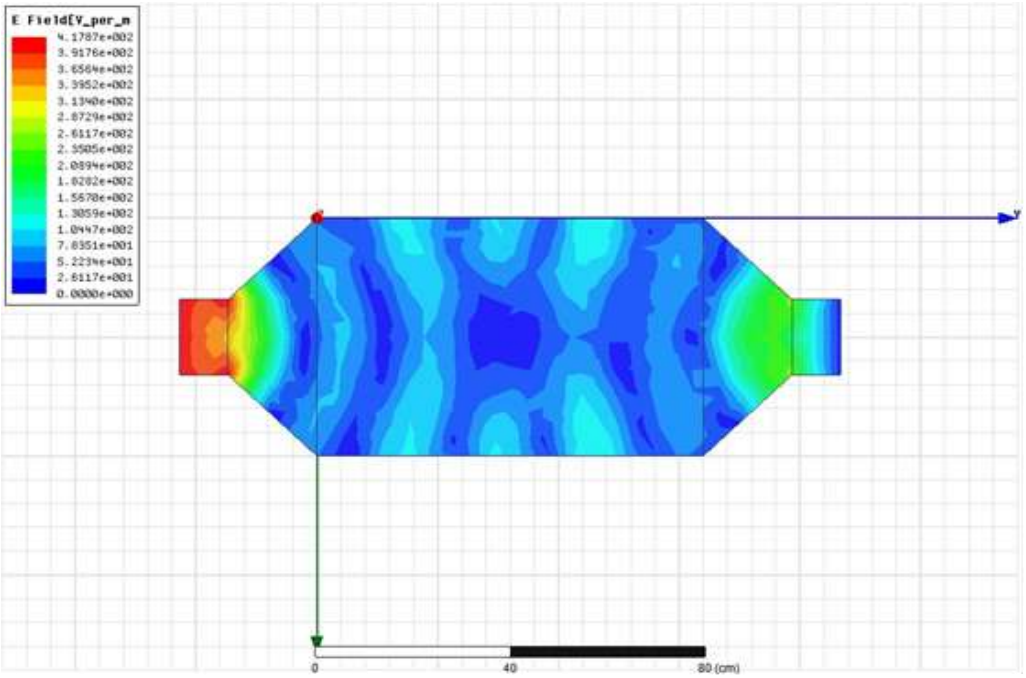

Figure 21. Top view of the electric field pattern inside the box

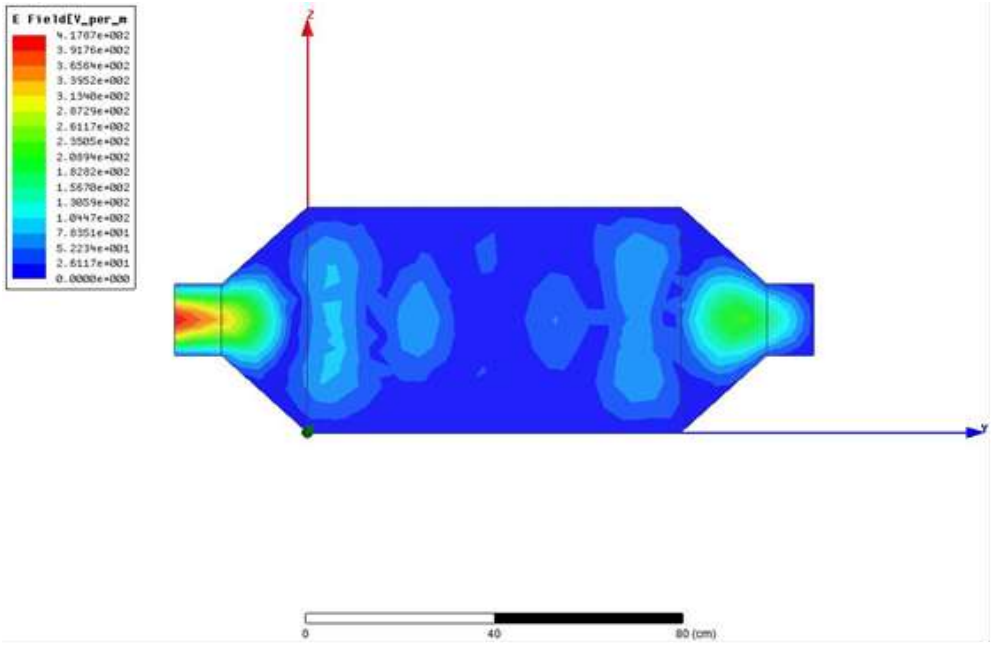

Figure 22. Top view of the electric field pattern inside the box

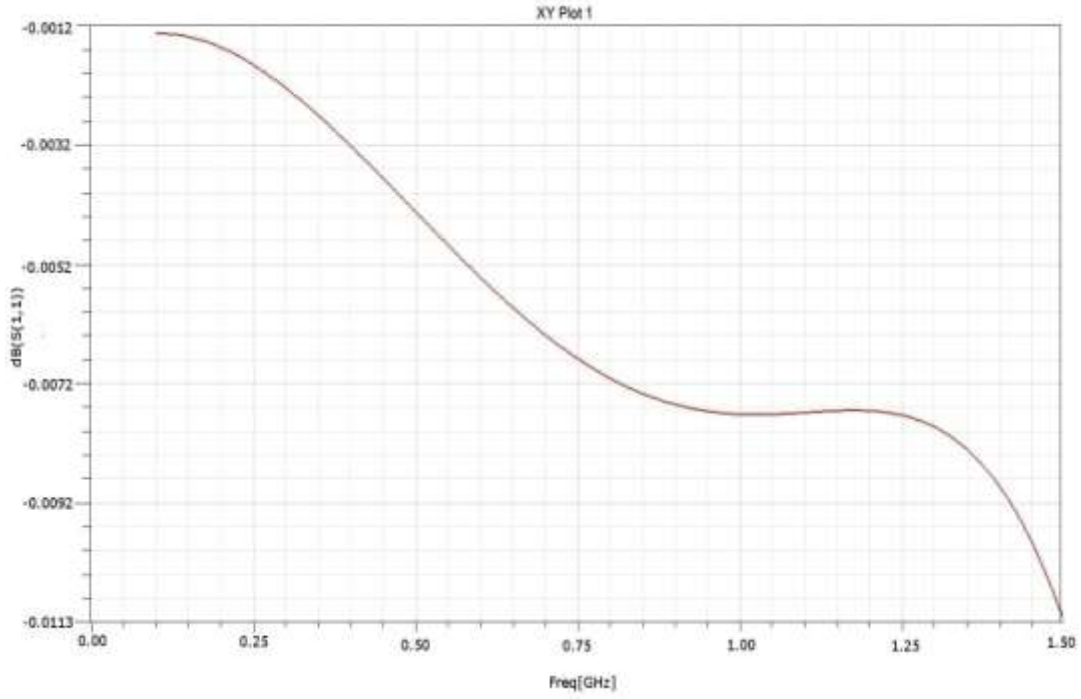

Figure 23. dB(S(1,1)) frequency response -parameters image

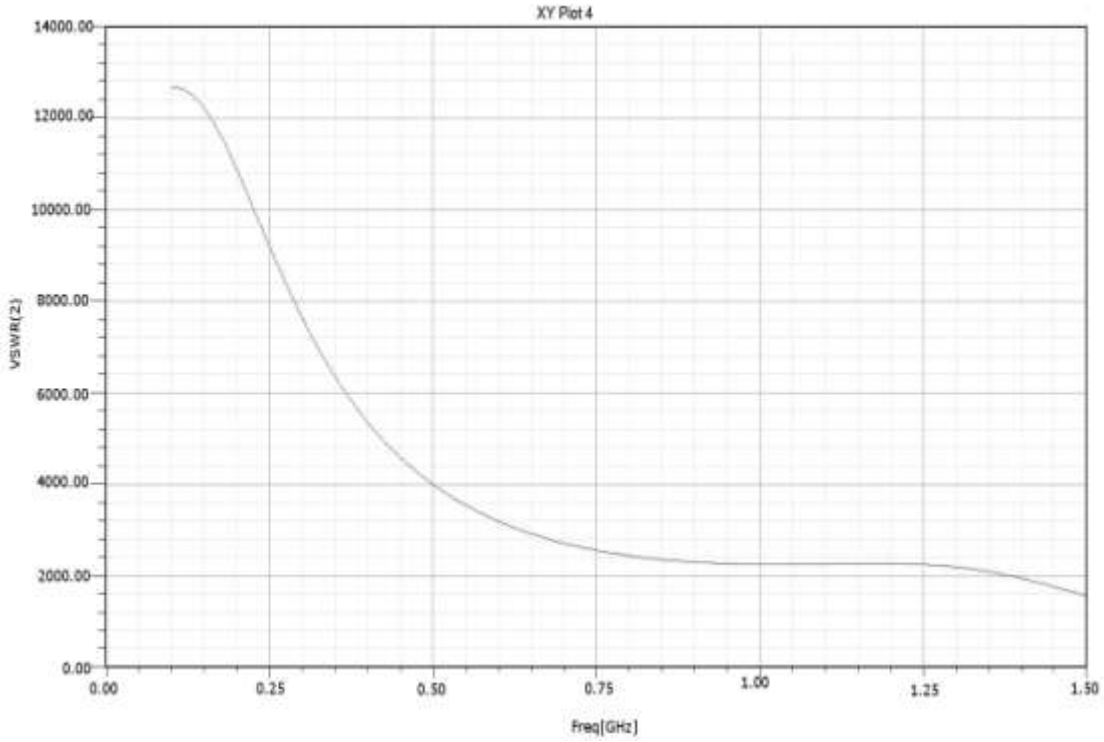

Figure 24. Voltage Standing Wave Ratio (VSWR) frequency response

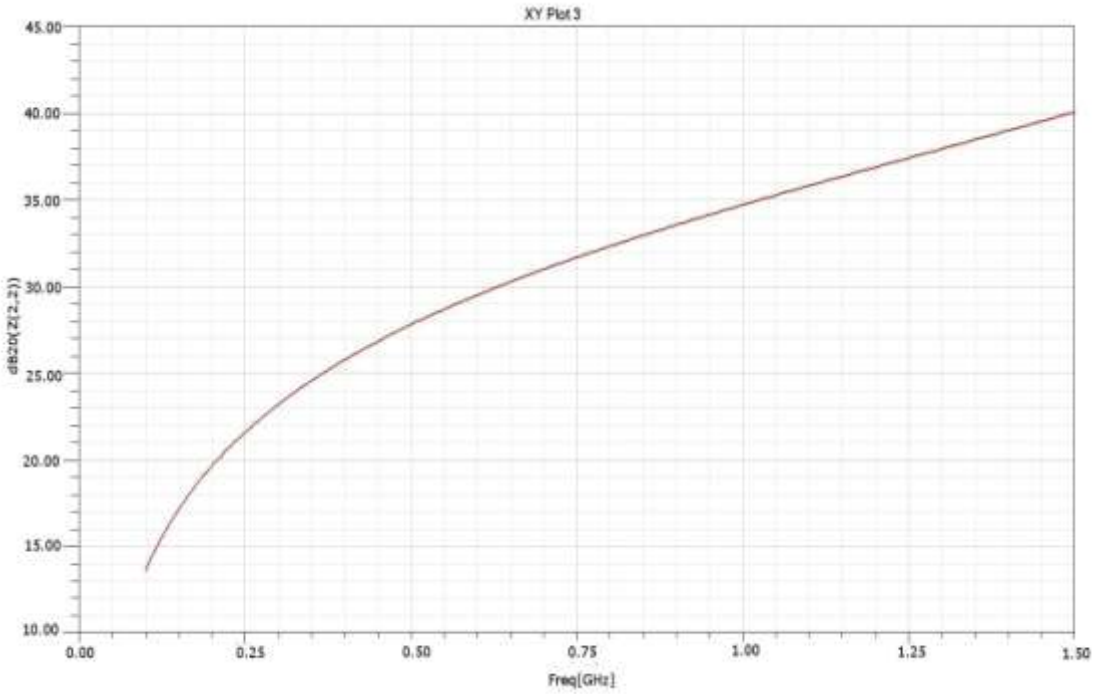

Figure 26. The dB20(Z(2,2)) frequency response

3.2.3 Fabrication and testing of the TEM cell characteristics using Network Analyser

The custom-made TEM cell was fabricated from Aluminium with the dimensions obtained from the simulation analysis (Figure 27).

The camera dimensions are presented in Table 2.

Figure 25. Fabricated custom-made TEM Cell

Table 1. Dimensions of the TEM cell

Variable	Value
port_h	100 mm
h	251 mm
s	158 mm
l	303 mm
port_s	33 mm
x	62.54 mm
extra_h	67.23 mm
w	303 mm
theta	-42.93°
origin_x	329 mm
origin_y	170.96 mm
tetrahedron_h	183.77 mm
box_x	500 mm
box_y	800 mm
box_z	500 mm

To test the characteristics of the EMF generated insight the TEM cell, Network Analyzer was used. As can be seen from the images below, the fabricated prototype met the design requirements. The maximum peak *frequency 968MHz and power 10dBm* of the generated field was confirmed through the testing by Network Analyser and are shown in Figures 28 and 29.

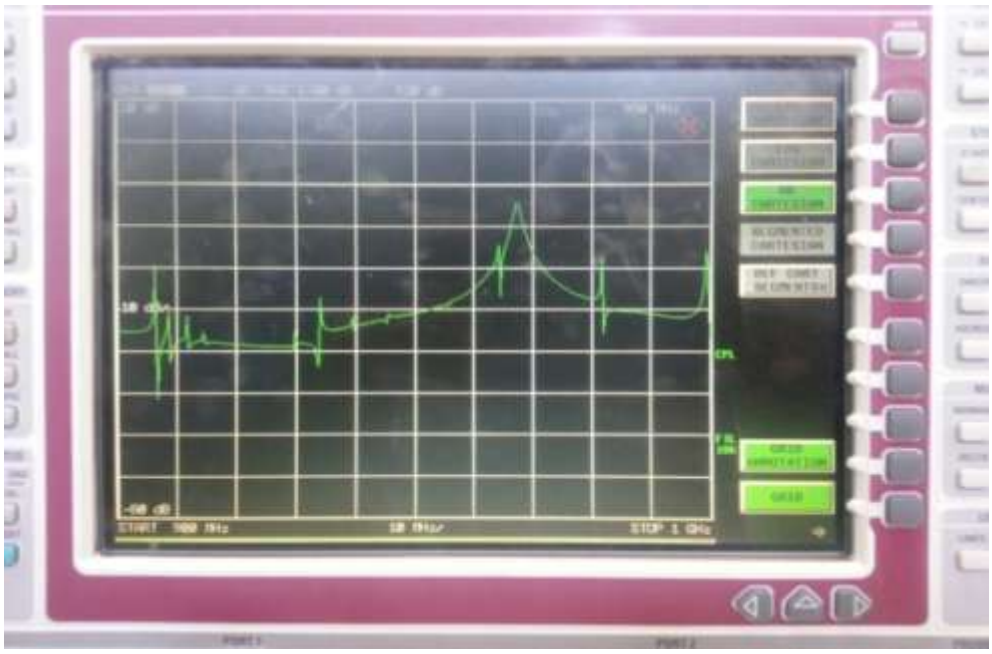

Figure 26. Ratios of waves' quantities in ch2 between b2, a1

Figure 27. The frequency marked 968MHz in ch3 between b1 and port 1

The constructed exposure camera was used in this project for experimentation with the selected model systems aiming to study and evaluate non-thermal effects of the

applied MW exposures. Figures 28 & 29 show the graphical presentation of transfer characteristics of the constructed TEM cell. The results of the experimental studies using two exposure cameras are presented in Chapter 4.

3.3 Model systems investigated in the project

3.3.1 Yeast Cells

Yeast cells are good representatives of the biological cells, including the eukaryotes. They are easy to obtain, similar to human cells but grow a lot faster, incredibly flexible and do not require sophisticated experimental conditions. In addition, yeast cells are safe, so there is no contamination problem. It is well-documented that yeast cells are representatives of eukaryotes, including human cells, in many aspects of fundamental cellular processes [5]. Furthermore, the yeast *Saccharomyces cerevisiae* was reported to be used as a model organism in several experiments, which can be performed under biologically and technically well-controlled conditions after exposure to microwave radiation [6].

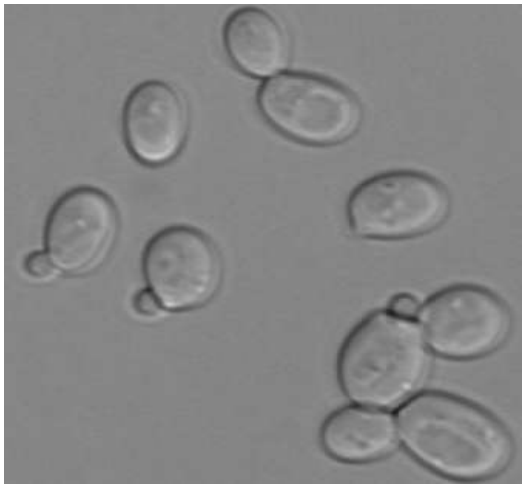

Figure 28. Saccharomyces cerevisiae [7]

Yeast is a general term, covering a wide range of very different single-celled fungi. In the molecular biology laboratory, two species are commonly employed as models for biomedical research: the budding or brewer's yeast *Saccharomyces cerevisiae*, and the fission yeast *Schizosaccharomyces pombe* [8, 9]. In culture, yeast cells follow a very predictable pattern of growth that can easily be divided into four phases: lag, log, deceleration and stationary phases. During the lag phase no growth occurs as newly pitched yeast mature and acclimate to the environment. This is followed by the log phase, where cells are rapidly growing and dividing. Nutrients are in excess relative to cell number and waste is being sufficiently diluted as to be insignificant. The growth rate in this phase will follow first order kinetics, as cell-number increases, cell growth begins to slow. A number of parameters each with saturation effects become significant. Eventually the yeast cells reach the stationary phase, where no growth occurs due to high waste concentration or complete substrate consumption [8, 9].

Likewise, in most growing eukaryotic cells, the cell cycle is divided into two basic parts: mitosis and inter-phase (three phases). The four phases proceed successively, taking from 10–20 hours depending on cell type and developmental state. Inter-phase comprises the G_1, S, and G_2 phases. G_1 phase (gap 1) corresponds to the interval (gap) between mitosis and initiation of DNA replication. During G_1, the cell is metabolically active and continuously grows but does not replicate its DNA. G_1 is followed by Sphase (synthesis), during which DNA replication takes place. The completion of DNA synthesis is followed by the G_2 phase (gap 2), during which cell growth

continues and proteins are synthesized in preparation for mitosis. When the genetic material is evenly partitioned and the cell divides. Non-dividing cells exit the normal cycle, entering the quiescent G_0 state [10].

Although yeast cells are single cells, they are true eukaryotes, and share fundamental cell features with metazoan systems. This offers unique tools to the cell biologist, providing complementary approaches and insights into functions of larger eukaryotes [11]. Both yeast species are harmless, having tractable genetic systems, and easily manipulated in the laboratory using superb molecular tools [10, 11].

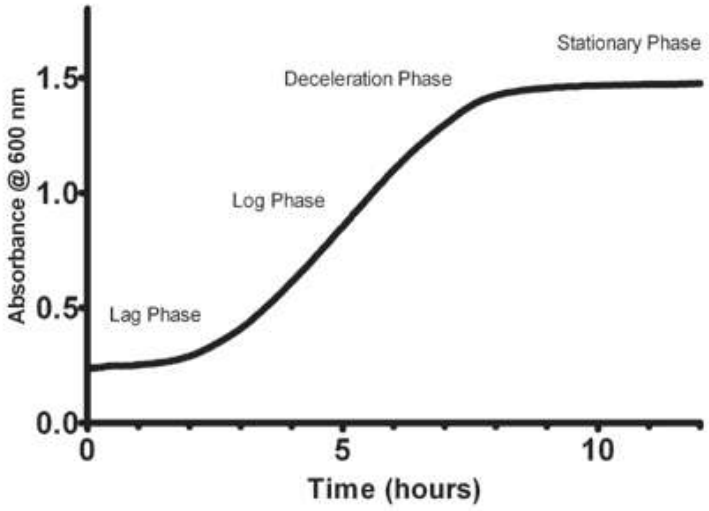

Figure 29 Typical yeast growth curve. Saccharomyces cerevisiae grown in YPD media at 30°C for 12 hours with data measurements every 2 minutes [7]

In the laboratory, both the budding yeast, *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*, are typically maintained as haploid cells. Cells are tolerant of cold and can be stored frozen at –70°C. Generation time varies with media and temperature, but is generally in the 2-4 hour range. In response to nutrient limitations, yeast cells exit the cell cycle and enter stationary phase; it is a period of dormancy, more severe than the G_0 phase in mammalian cells. A particular strength of both systems is that they also have a diploid sexual cycle: haploid cells of opposite mating types can mate, resulting in cell and nuclear fusion.

Diploids can be maintained in the laboratory or induced to enter meiosis and sporulate. The four spores packaged in the yeast ascus are the fungal equivalent of human gametes. Furthermore, both cell types have highly organized internal structures with the membrane-limited compartments typical of eukaryotic cells, including a nucleus, mitochondria, Golgi and other structures. Thus, the entire life cycle of yeast cells provides a simple model for events occurring in human cells [5]. *Saccharomyces cerevisiae* was adopted as a model system for laboratory study in the 1930s, as investigators developed genetic tools to understand its life cycle and differentiation [7]. With potent genetic tools and a typical eukaryotic cell organization, budding yeast became a favorite system to study cell biology questions. It was the first eukaryote to be sequenced, which has sparked a whole new era developing genomics tools [8, 11].

In this research project, *Saccharomyces cerevisiae* yeast cells were selected as a model

of the eukaryote cells to investigate the effects of low intensity microwave radiation on its growth and proliferation. The experiments were conducted by placing the yeast cells inside of the TEM cell and irradiating them at different MW frequencies and powers. Two different exposure cameras were used for exposures, i.e. the commercial and custom-made TEM cells.

In essence, the simple growth requirements and rapid division time of yeast cells make them convenient for laboratory research as a model organism because it scores favorably on a number of these criteria:

- As a single-cell organism, *S. cerevisiae* is small with a short generation time (doubling time 1.25–2 hours at 30^{o}C or 86F) and can be easily cultured. These are all positive characteristics in that they allow for the swift production and maintenance of multiple specimen lines at low cost;
- *S. cerevisiae* divides with meiosis, allowing it to be a candidate for sexual genetics research;
- *S. cerevisiae* can be transformed allowing for either the addition of new genes or deletion through homologous recombination. Furthermore, the ability to grow *S. cerevisiae* as a haploid simplifies the creation of gene knockouts strains;
- As a eukaryote, *S. cerevisiae* shares the complex internal cell structure of plants and animals without the high percentage of non-coding DNA that can

confound research in higher eukaryotes;

- *S. cerevisiae* research is a strong economic driver, at least initially, as a result of its established use in industry.

3.3.2 Enzymes model systems

In this experimental evaluation, the following proteins were selected to study the effects of applied low power microwave radiation of enzyme kinetics: L-Lactic Dehydrogenase and Glutathione Peroxidase. These enzymes are specific proteins, whose role is critical in accelerating metabolic reactions in living organisms.

L-Lactate Dehydrogenase (also known as L-Lactic Dehydrogenase)

A lactate dehydrogenase (LDH or LD) is an enzyme found in animals, plants, and prokaryotes. Lactate dehydrogenase is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD^+ and back. Lactate dehydrogenases exist in four distinct enzyme classes. Each one acts on either D-lactate (D-lactate dehydrogenase (cytochrome)) or L-lactate (L-lactate dehydrogenase (cytochrome)). Two are cytochrome c-dependent enzymes. Two are NAD(P)-dependent enzymes. This article

is about the NAD(P)-dependent L-lactate dehydrogenase.

LDH is a protein that normally appears throughout the body in small amounts. Many cancers can raise LDH levels, so LDH may be used as a tumor marker, but at the same time, it is not useful in identifying a specific kind of cancer. Measuring LDH levels can be helpful in monitoring treatment for cancer. Non-cancerous conditions that can raise LDH levels include heart failure, hypothyroidism, anemia, and lung or liver disease. LDH is involved in tumor initiation and metabolism. Cancer cells rely on anaerobic respiration for the conversion of glucose to lactate even under oxygen-sufficient conditions (a process known as the Warburg effect) [12]. This state of fermentative glycolysis is catalyzed by the A form of LDH. This mechanism allows tumorous cells to convert the majority of their glucose stores into lactate regardless of oxygen availability, shifting use of glucose metabolites from simple energy production to the promotion of accelerated cell growth and replication [12]. For this reason, LDH A and the possibility of inhibiting its activity has been identified as a promising target in cancer treatments focused on preventing carcinogenic cells from proliferating.

In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show false-positively high levels of LDH due to erythrocyte damage. It can also be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH peak at 3–4 days and remain

elevated for up to 10 days. In this way, elevated levels of LDH (where the level of LDH1 is higher than that of LDH2) can be useful for determining whether a patient has had a myocardial infarction if they come to doctors several days after an episode of chest pain [13].

The dehydrogenases are enzymes that catalyze a variety of oxidation-reduction reactions within a cell. L-Lactic Dehydrogenase (LDH) from rabbit muscle, EC1.1.1.27 (Worthington Assay), has been selected as a protein example for this study. The LDH catalyzes the reversible reduction of pyruvate to L-lactate using NADH (Nicotinamide Adenine Dinucleotide, Reduced form) as a coenzyme. The NAD+ is not optically active at 340nm and the oxidation of NADH is directly proportional to the reduction of pyruvate. Therefore the LDH activity can be calculated from the rate of decrease in absorbance at 340nm.

L-Lactate Dehydrogenase

Pyruvate+ β-NADH <=> L-Lactate + β-NAD

Where β-NADH - β-Nicotinamide Adenine Dinucleotide, Reduced Form; and β-NAD - β-Nicotinamide Adenine Dinucleotide, Oxidized Form.

Glutathione Peroxidase

Glutathione Peroxidase (GPx) is the general name of an enzyme family with peroxidase activity, whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Several isozymes are encoded by different genes, which vary in cellular location and substrate specificity.

Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels. Glutathione peroxidase 2 is an intestinal and extracellular enzyme, while glutathione peroxidase 3 is extracellular, especially abundant in plasma. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans [14].

Mice genetically engineered to lack glutathione peroxidase 1 (Gpx1 knockout mice) are grossly phenotypically normal and have normal lifespans, indicating this enzyme is not critical for life. However, Gpx1 / mice develop cataracts at an early age and exhibit defects in muscle satellite cell proliferation. However, glutathione peroxidase 4 knockout mice die during early embryonic development. Some evidence, though,

indicates reduced levels of glutathione peroxidase 4 can increase life expectancy in mice. No information is available on knockouts of the other isozymes. The *bovine* erythrocyte enzyme has a molecular weight of 84kDa. It was discovered in 1957 by Gordon C. Mills [15].

Glutathione peroxidase (GPx, EC 1.11.1.9) – a second protein example studied here - provides a mechanism for removing of peroxides in living cells. It plays a crucial role in protecting cells from damage by free radicals, which are formed by peroxide decomposition. The reaction is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (b-Nicotinamide Adenine Dinucleotide Phosphate, Reduced). The decrease in NADPH absorbance measured at 340nm during the oxidation of NADPH to NADP+ is indicative of GPx activity, since GPx is the rate limiting factor of the coupled reactions (Sigma-Aldrich Assay).

$$\text{R-OOH} + 2\,\text{GS} \xrightarrow{\text{GPx}} \text{R-OH} + \text{GSSG} + \text{H2O}$$

$$\text{GSSG} + \text{NADPH} + \text{H+} \xrightarrow{\text{GR}} 2\,\text{GSH} + \text{NADP}$$

Where GPx is glutathione peroxidase, GR is glutathione reductase, and R-OOH is

organic peroxide. The reaction is performed at 25°C and pH 8.0. The biological activity of the analyzed enzymes was measured using the standard, well accepted procedure, i.e. Spectrophotometric Rate Determination.

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CHAPTER 4

Experimental Evaluation of Low Level Microwave Radiation on Selected Biological Systems

In Chapter 4 experimental evaluation of the effects of low level microwave radiation of different frequencies and powers were investigated at the cellular and molecular levels using the commercial and custom-made exposure cameras. The findings of these studies are presented below.

4.1 Study 1 - Non-thermal effect of the microwave exposures at 900MHz and selected low powers on the proliferation rate of Saccharomyces Cerevisiae Yeast

Overview: This study evaluates the effect of non-thermal weak radiofrequency microwave (RF/MW) radiation on the proliferation response of the yeast Saccharomyces cerevisiae. S. Cerevisiae strains type II (Sigma-Aldrich) were exposed to the microwaves at 900MHz and the selected powers of 13dBm, 3dBm and -7dBm using the commercial Transverse Electro-Magnetic (TEM) cell. The average specific absorption rate (SAR) for a single cell was 0.12W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells.

In these experiments, yeast cells were continuously exposed to the MW radiation. Changes in yeast culture growth were monitored using the Spectrophotometry method. Measurements of the yeast cells' growth in control (sham-exposed) *vs.* irradiated samples were performed. The results revealed that the rate of yeast growth was increased at 13dBm and 3dBm of applied MW exposures.

Experimental studies conducted in the millimeter band at very low microwave energy flux densities (no more than a few milli watts per square centimeter) induced highly specific effects of applied radiation. The results revealed: (a) the effect of irradiation depends strongly on the frequency of the microwaves; (b) in certain microwave power ranges, the effect of exposure depends on variation of the power through several orders of magnitude; (c) the observed effects are significantly dependent on duration of exposure. A resonant effect of microwaves on the division rate of both the cell cultures and yeast was observed [1].

Two types of effects can be ascribed to microwaves, i.e. thermal and non-thermal. The heating effect of microwaves is already well known and documented [2], however, doubts remain on the existence of non-thermal biological effects, which are of interest to this research study. Saccharomyces cerevisiae is the most commonly used strain in scientific research, baking and fermentation and has become synonymous with the term yeast. Yeast has been used for thousands of years to ferment alcohol. It is well-documented that yeast cells are representatives of eukaryotes, including human cells, in many aspects of fundamental cellular processes [3]. Furthermore, the yeast *Saccharomyces cerevisiae* was reported to be used as a model organism in several experiments, which can be performed under biologically and technically well-controlled conditions after exposure to microwave radiation [4]. Yeast cells are single celled eukaryotic fungi organisms that reproduce asexually by budding or division. While yeast can vary in size, they typically measure 3-8μm in diameter [5].

When cultured for the fermentation of beer, yeast cells in culture follow a very predictable pattern of growth that can easily be divided into four phases: (1) lag; (2) log; (3) deceleration; and (4) stationary. During the lag phase, no growth occurs as newly pitched yeast cells mature and acclimatise to the environment. This is followed by the log phase, where cells are rapidly growing and dividing. Nutrients are in excess relative to cell number and waste is being sufficiently diluted as to be insignificant. The growth rate in this phase will follow first order kinetics. As cell numbers increase, cell growth begins to slow as a number of parameters (e. g. substrate and waste), each with saturation effects, become significant. Eventually yeast cells reach the stationary phase, where no growth occurs due to high waste concentration or complete substrate consumption.

In this study we applied microwave exposures at the 900MHz and the powers of 13dBm, 3dBm and -7dBm to irradiate S. cerevisiae yeast for evaluating the hypothesis that the external microwave radiation of the specific frequency and power can affect the growth and proliferation response in yeast. The experimental set up of the exposure and assessment system is shown in Figure 32.

The S. cerevisiae yeast powder was purchased from Sigma, Australia. The experimental solution was prepared as follows: 50g/l of YPD broth (Sigma,

Australia); 20g/l of S. yeast, and ionized water. The solution was incubated at 24° C for 72 hrs. Then the solution was kept at 4°C.

The yeast samples were prepared by diluting the experimental solution as 1ml in 100ml of the ionized water (1:100). The yeast samples were placed in the 20ml bottles. The bottles (external dimensions are h=50mm; d=25mm, V=20ml) were filled with the aliquot (liquid's column height of 45mm) and was kept at 22cm distance from the top of the TEM cell (Figure 33).

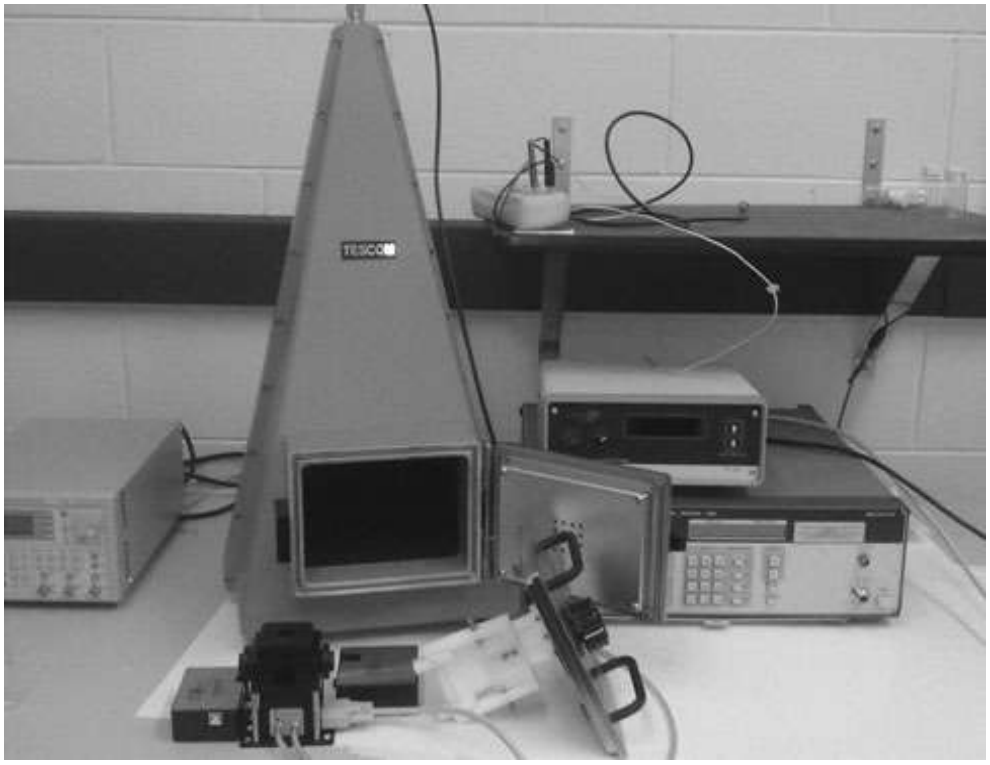


Figure 30. Experimental set up showing exposure camera, signal generator, temperature controller, cuvet holder and spectrophotometer

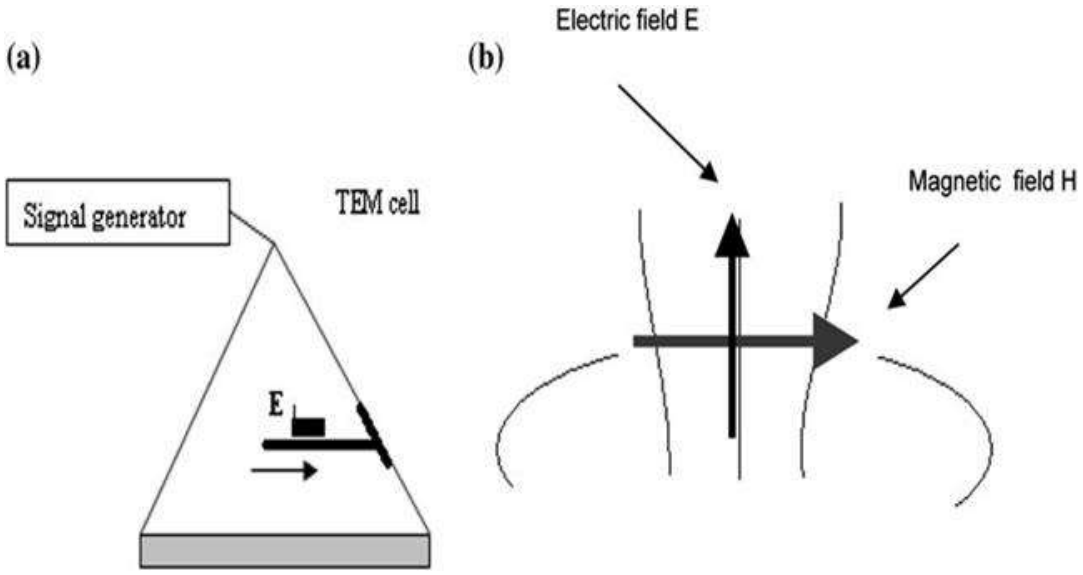


Figure 31. Experimental set up of exposure system.

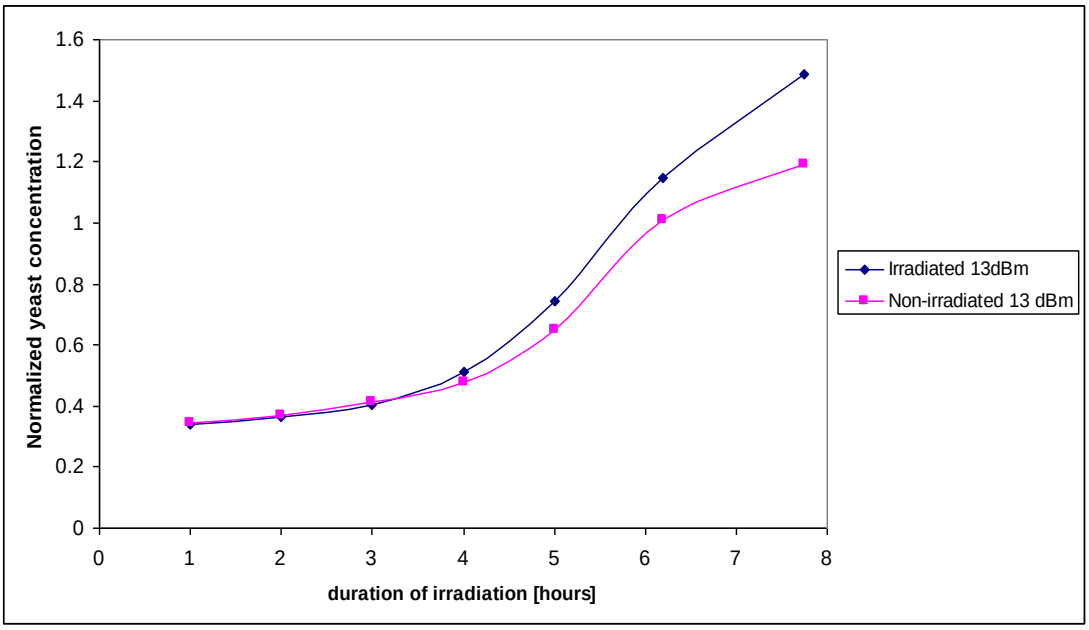
In these experiments, 3 yeast samples were irradiated continuously for 6 hrs and other 3 yeast samples were sham-exposed (no radiation) for the same time duration. Yeast cells solutions were exposed at **900MHz and powers of 13dBm, 3dBm and -7dBm.** Changes in yeast culture growth were monitored using the spectrophotometry method. Spectrophotometric analysis is based on turbidity and allows for indirect measurement of a number of yeast cells. The absorption coefficients of the yeast samples were measured using an Ocean Optics USB2000 spectrometer. The absorption characteristics of each (3 exposed and 3 non-exposed) samples were measured every 1 hr. The experimental data were collected and are presented in Figure 34.

The results obtained show the changes in normalized concentration of yeast cells

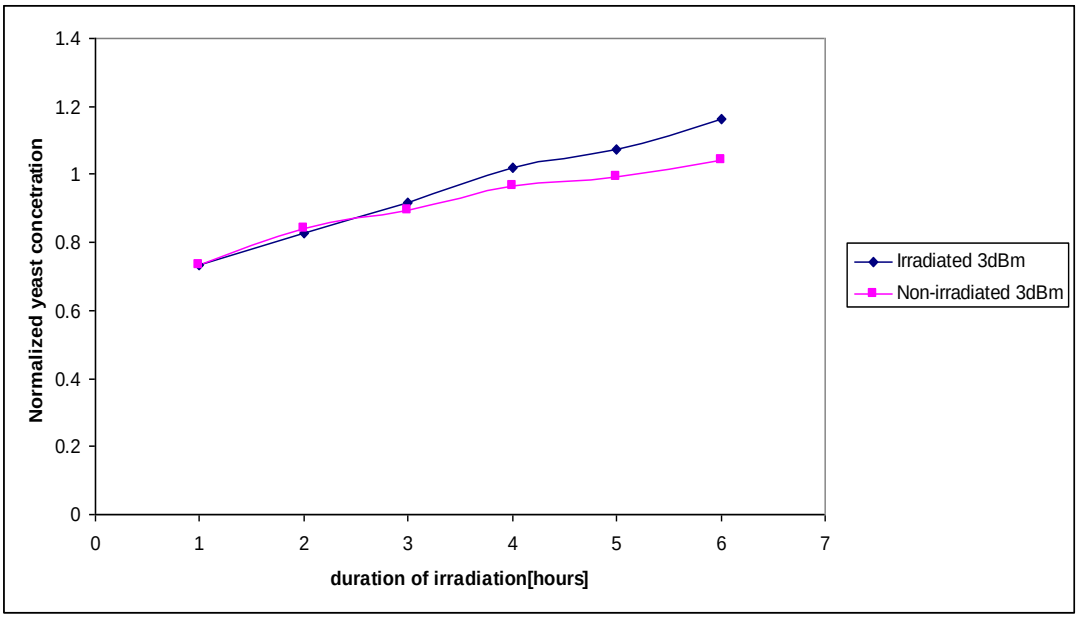
in time. Table 3 presents data on relative change (%) in proliferation response of yeast cells. The results obtained indicate that MW radiation at 900MHz and powers of 13dB and 3dB affect significantly (14% and 11% respectively) the concentration of yeast cells. Thus, it is concluded that the external radiation can modulate the proliferation of the exposed yeast cells.

Table 2. Relative change in the rate of the proliferation of yeast cells during the MW exposure using different powers in [%].

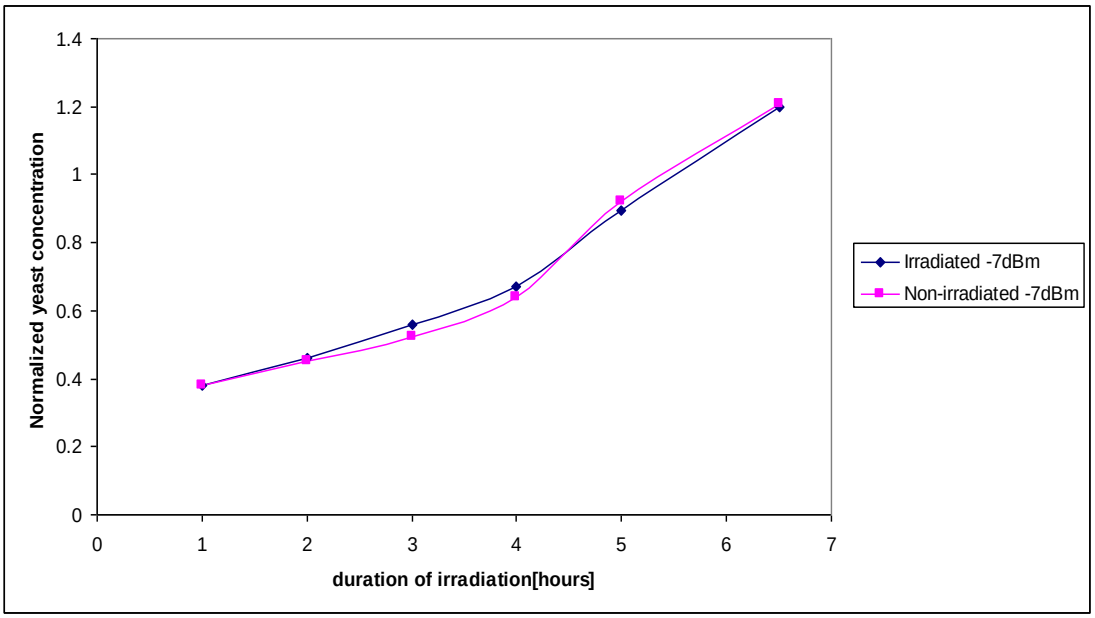
Microwave power	**13dBm**	**3dBm**	**-7dBm**
Relative change in the rate of proliferation (irradiated vs. non-irradiated sample)	+14%	+11%	-1%


Normalized yeast concentration
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
duration of irradiation [hours]
Irradiated 13dBm
Non-irradiated 13 dBm

(a)


Normalized yeast concetration
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
duration of irradiation[hours]
Irradiated 3dBm
Non-irradiated 3dBm

(b)



(c)

Figure 32. Changes in normalized concentration of yeast cells in time (non-irradiated vs. irradiated) at 900 MHz and the powers of: a) 13dBm, b) 3dBm and c) -7dBm

Final Remarks:

This experimental evaluation was aimed to test the hypothesis that the external low power MW radiation can affect the biological activity (growth and proliferation rate) of the yeast cells. The yeast samples were exposed and sham-exposed for 6 hours. The results obtained show that MW radiation at 900MHz and power of -7dBm induced no effect on yeast growth. However, microwaves at the same frequency of 900MHz and powers of 13dB and 3dB affected significantly (14% and 11% respectively) the concentration of yeast cells.

These findings imply that applied MW exposures induce modulating (increasing or inhibiting) effects on the studied yeast cells suggesting that these effects are power-dependent. These results indicated that further investigation is required to evaluate the effects of MW exposures at the frequency of 900MHz and other different powers. In addition to studying the MW exposures using the commercial TEM cell, the custom-made TEM cell was designed and constructed for further experimentation with biological examples. The details of the design, simulation and construction of the custom-made exposure camera are presented in the Chapter 3.

4.2 Study 2 - Effects of non-thermal microwave exposures at 500MHz and 900MHz and selected powers on the proliferation rate of Saccharomyces cerevisiae Yeast

As was mentioned earlier, yeast cells are single cells, they are true eukaryotes, and share fundamental cell features with metazoan systems [5, 6]. Both yeast cells, Saccharomyces cerevisiae and Schizosaccharomyces pombe, are typically maintained as haploid cells; they also have a diploid sexual cycle, e.g. haploid cells of opposite mating types can mate, resulting in cell and nuclear fusion. Thus, the entire life cycle of yeast cells provides a simple model for events occurring in human cells [6]. As was presented in the literature review section (Chapter 2), a number of studies were carried out to investigate responses of yeast cell to applied microwave radiation [7, 8]. The important findings are summarised below.

1) In [9], a diploid wild strain of S. cerevisiae cells were irradiated at 40-60GHz and powers of up to 50mW transmitted by a waveguide. It was shown that applied exposures alter the growth rate in the studied yeast cells. Study [10] also reported that yeast cell growth was affected by low power microwave radiation at the frequencies of 41.640 - 41.835GHz. Interestingly, the observed effects were shown to be strongly frequency-dependent and not correlated with the microwave powers used [10].

2) In other studies, Saccharomyces cerevisiae yeast cells were exposed to both static and alternative magnetic fields (homogeneous and non-homogeneous) of varying powers and durations [11]. Yeast cells were dispersed in a reagent and then exposed to a strong magnetic field using a magnetic circuit with the strength of 2.93T. Interestingly, the authors observed the differences in the budding angles of the yeast cells between the homogeneous and the non-homogeneous MFs, which were caused by alteration of the budding mechanism [11].

3) In [12], Saccharomyces cerevisiae yeast cells were preserved on malt extract agar slopes at room temperature and then exposed to microwaves (quasi-optical setup for more accurate result) ranging from 192GHz to 341GHz with the selected powers in mW range. The tests were performed in three different groups and different time exposures of 30 to 150min. A statistically significant difference in yeast growth was observed between the control and exposed groups. That was apparent for all of the exposure durations except for that at 150 min of irradiation. The results suggest that exposure duration has the greatest impact on cells at an early growth stage [12].

Noteworthy, published research shows that effects of microwave radiation on the mammalian cells are controversial and no conclusion has been reached. The results of various studies on mammalian cells could not demonstrate microwave-induced DNA damage and cell proliferation. In contrast, other studies have reported that modulated

microwave radiation is capable of causing DNA lesions and inhibition of cell proliferation [13-17].

This research project is aimed to study and evaluate if the biological *effects induced by different MW frequencies and low powers on yeast cells and enzymes are frequency- and power-dependent*. As was shown in Study 1, microwave exposures at 900MHz and powers of 13dBm, 3dBm and -7dBm induced modulating effects in the irradiated yeast cells that lead to significant changes in their growth rate. Therefore, it was of interest to study if other MW frequencies can induce similar effects in the exposed yeast cells. Hence, in Study 2 the experimental testing was conducted on the same yeast cells of Saccharomyces cerevisiae strain, which were exposed to microwave radiation at two frequencies **500MHz and 900MHz and powers of -17dBm, -13dBm, -10dBm, 0dBm, 10dBm, 13dBm and 17dBm**.

Similar to Study 1, TC-5062AUHF TEM cell (100kHz–3GHz) from TESCOM Ltd (Unitechvill, Goyang, Korea) was used to irradiate yeast cells here. The average specific absorption rate (SAR) for a single cell was 0.12W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells. The S. cerevisiae yeast powder was purchased from Sigma (Australia). The experimental solution was prepared as follows: 50g/l of YPD broth (Sigma, Australia); 20g/l of S. cerevisiae yeast, and ionized water. The solution was incubated at 240°C for 72 hrs. Then the solution was kept at 40°C. The yeast

samples were prepared by diluting the experimental solution as 1ml in 100ml of the ionized water (1:100). The yeast samples were placed in the 2ml cuvettes (GMBH+coKG post fach 1155). The cuvettes (dimensions are: 12.5x12.5x45mm) were filled with the aliquot.

Three yeast samples were irradiated continuously for 6 hours and other three control samples were sham-exposed for the same time duration. The absorption characteristics of each (3 exposed and 3 non-exposed) samples were measured every 1 hour. The control group (non-exposed yeast cells) was kept at the same experimental conditions. Changes in yeast culture growth were monitored using the spectrophotometry method. The intensity of the yeast samples absorbance was measured using an Ocean Optics USB2000 spectrometer. The experimental set up of the MW exposure and assessment of yeast cells is shown in Study 1, Figure 33. The results of this study, presented in Figures 35 and 36 clearly show that, in comparison with the control group of yeast cells (100%), the microwave exposures at 500MHz and 900MHz and different powers can induce changes in yeast cell growth/proliferation.

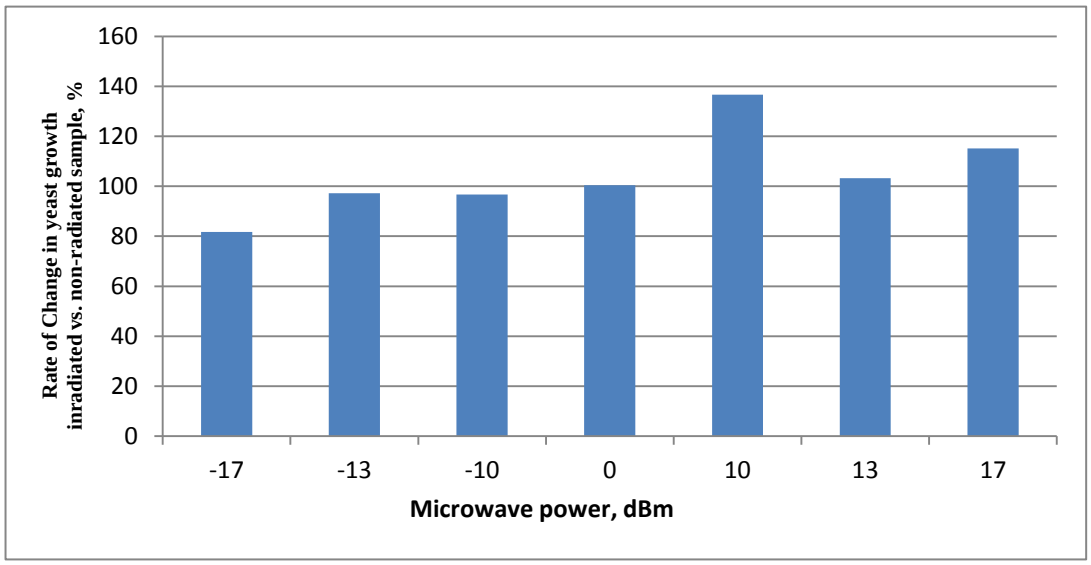


Figure 33. Changes in yeast growth upon microwave exposures at 500MHz and different powers

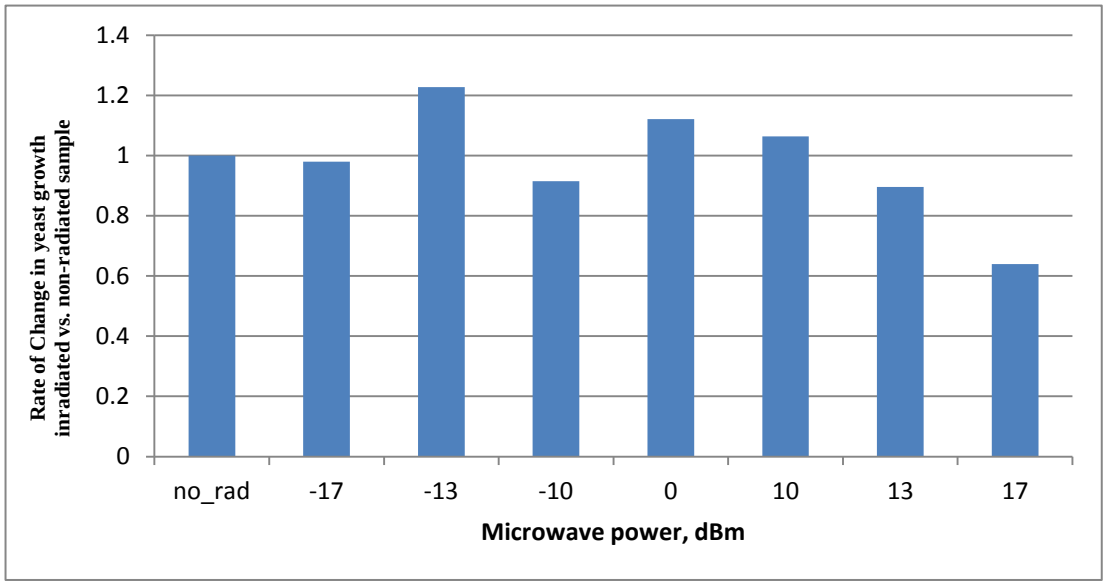


Figure 34. Changes in yeast growth upon microwave exposures at 900MHz and different powers

Interestingly, the observed effects are power-dependent for both studied frequencies. From Figure 35 it can be seen that at the frequency of 500MHz and power of -17dBm

the decrease of 20% in yeast cell growth is achieved. At the powers of -13dBm and -10dBm we can see only a slight decrease in cells growth. We also can observe no effect on cells at the power of 0dBm. In contrast, a slight increase is seen at the power of 13dBm, and more significant increase of 18% is achieved at 17dBm. The maximum increase of 38% in yeast cell growth rate is achieved with the power exposure of 10dBm.

Figure 36 shows that at the frequency of 900MHz and powers of -13dBm, 0dBm and 10dBm the yeast cell proliferation rates are increased (21%, 12% and 6% respectively). In contrast, at the same frequency of 900MHz and the powers of -17dBm, -10dBm, 13dBm and 17dBm the opposite effect is observed. The exposures at these particular parameters decrease the growth and proliferation of yeast cells with the different degree of suppression. The maximum decrease in yeast cells growth (38%) is seen with the power exposure of 17dBm. Hence, the findings of this sub-study are in accord with the results of Study 1 and reveal that applied microwaves at 500MHz and 900MHz and selected powers can modulate yeast cells growth and these effects are power-dependent.

Final remarks:

The results obtained show that applied MW radiation at 500MHz and 900MHz and different powers produce modulating effects on the growth of yeast samples. However, the observed effects vary significantly. The findings reveal that the growth

pattern of yeast cells can be increased or inhibited by exposures of particular frequencies and powers, thus confirming the hypothesis that non-thermal effects of low power MW are strongly power-dependent, which was also clearly shown by the results obtained in Study 1.

4.3 Study 3 - Effects of Low Power Microwave Radiation on Biological Activity of L-Lactate Dehydrogenase Enzyme and Growth Rate of S. Cerevisiae Yeast

Overview: In this study we experimentally evaluated non-thermal effects of low power microwave exposures on kinetics of L-Lactate Dehydrogenase enzyme and growth rate of yeast Saccharomyces Cerevisiae strains type II. The selected model systems were continuously exposed to microwave radiation at the frequency of **968MHz and power of 10dBm** using the designed and constructed custom-made TEM cell. The findings reveal that microwave radiation at 968MHz and power of 10dBm inhibits L-Lactate enzymatic activity by 26% and increases significantly by 15% the proliferation rate of yeast cells.

Some effects of RF/MW radiation on biological processes can be reversible and thus, do not impact human health [18]. However, the significance of investigating non-thermal RF/MW biological effects at the molecular level should not be underestimated. The effects of RF radiation on global gene and protein expression in different biological systems have been investigated and most studies focused on the mobile phone frequencies (800MHz – 2000MHz) at a relatively low exposure density (average SAR near 2.0W/Kg). Based on current literature, it can be summarized that RF exposures can change gene and/or protein expression in certain types of cells, even at powers lower than the standard recommended exposure levels. However, the biological consequences of most of the changed genes/proteins are still unclear, and

need to be further explored. To date, a limited number of studies of non-thermal effects of RF radiation on cells, tissue, bacteria and molecules report often conflicting results (please refer to Chapter 2).

One approach to the problem is studying the effects of low power RF radiation on protein activity (molecular level of interaction). Proteins are macromolecules found in a living cell and play a crucial role in almost every biological process. Of interest are enzymes, a specific group of proteins, whose role is critical in accelerating metabolic reactions in living organisms. Isolated aqueous enzyme solutions were studied previously as model systems to determine if external radiation could influence the selected biological processes [19-21].

In Study 3, the selected L-Lactate dehydrogenase enzyme and Saccharomyces Cerevisiae yeast cells were continuously exposed to microwave radiation at the frequency of **968MHz and power of 10dBm** using the custom-made TEM cell. The design, simulation and construction of the TEM cell were presented in details in Chapter 3. The generated electromagnetic field inside the TEM cell was simulated using ANSYS HFSS software prior to fabrication of the exposure camera's prototype in order to monitor homogeneity of the generated field at the location of the samples inside the TEM cell. As mentioned previously, Aluminium was selected as a suitable material for construction of the TEM cell operational prototype due to its excellent corrosion resistance as opposed to iron. USB2000 spectrophotometer (Ocean Optics)

was used to measure the changes in absorption characteristics of L-Lactate dehydrogenase enzyme and growth rate of yeast cells induced by applied MW exposures.

The samples were irradiated by MW at the frequency 968MHz and power 10dBm, and the results of exposures being compared with the control group (non-irradiated samples). The experimental set up of the exposure and assessment of biological samples is shown in Figure 37.

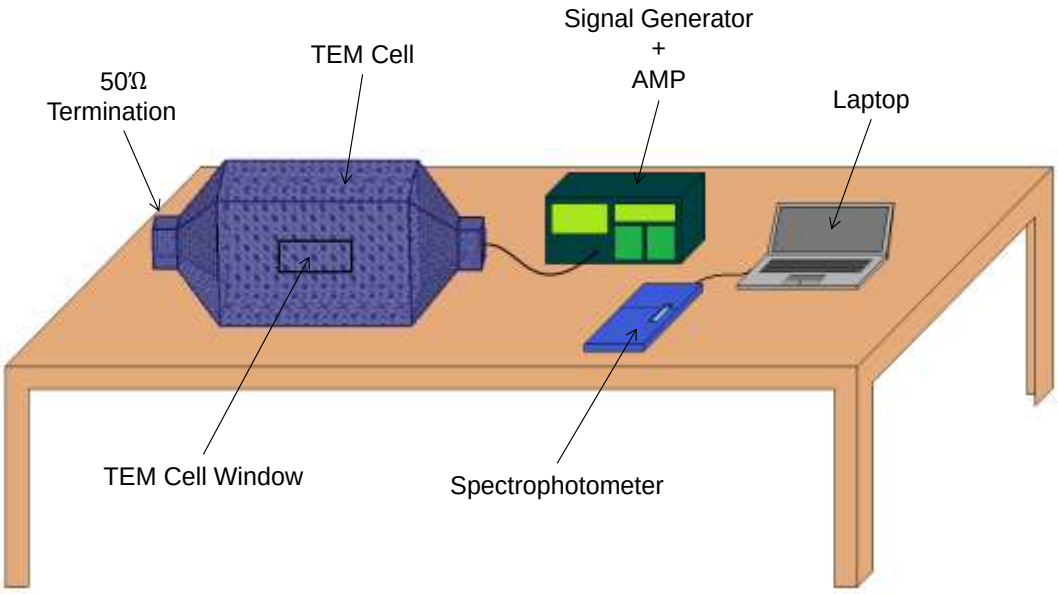


Figure 35. Experimental set up of exposure and assessment of irradiated biological samples

Irradiation of L-Lactate dehydrogenase enzyme

In the first sub-study, L-Lactate dehydrogenase (LDH) enzyme solutions were prepared according to the LDH enzyme Assay (Worthington Biochemical Corporation). The cuvettes (external dimensions are h=50mm; d=25mm, V=20ml) were filled with enzyme-substrate solutions. Activity of L-Lactate dehydrogenase enzyme was measured by calculating a rate of change of absorption of enzyme-substrate solution at 340nm. Five test samples with enzyme-substrate solutions were placed in the custom-made exposure camera (968MHz, 10dBm). An additional sample (pyruvate and identical buffer but without LDH enzyme) was also exposed to MW radiation. As controls, we used three non-irradiated samples with enzyme and one without enzyme. The control samples were kept under the same experimental conditions except the MW exposure.

Enzyme-substrate solution samples were irradiated in three continuous periods of exposure (180 sec each). After 180 sec of irradiation, an enzyme sample was taken out of the TEM cell. The irradiated enzyme was then added to the experimental solution and changes in NADH concentrations were measured. Spectrophotometer was set to record absorption spectra of the enzyme-substrate solution at 340nm every 5 sec. Thus, the total measurement time for 5 enzyme samples was 25sec with 5 recordings taken in 5sec intervals. Activity of the experimental solution was calculated

as a rate of change of its absorption; hence the experimental data are presented as absorption *vs.* time plot.

After each measurement, the samples were returned to the same positions inside the camera. The positions of the quvettes inside the quvette holder as well as the order of measurement of absorption characteristics were kept unchanged during the whole experimental procedure. This set up made it possible to measure the absorption characteristics of each quvette separately. It was important to monitor the changes in enzyme kinetics after different periods of irradiation to enable to draw the conclusion about its kinetics (absorption) changes in time dependent manner. Experimental data were collected and are shown in Table 4. Figure 38 show our findings on changes in absorption of experimental solutions (changes in concentration of NADH) under the influence of irradiated LDH enzyme.

Reagents: 6.6mM NADH 0.2 M Tris·HCl buffer at pH 7.3; 30mM Sodium pyruvate in 0.2 Tris·HCl buffer, at pH 7.3.

Final aliquot consist was prepared as follows:

2ml of Tris + 80ul NADH solution + 80ul Sodium pyruvate solution + 80uL LDH solution

Table 3. Changes in absorption of experimental solution (changes in concentration of NADH)

	Radiated enzyme sample	**Non-irradiated enzyme sample**
1	-0.0026	-0.0042
2	-0.0031	-0.0038
3	-0.0037	-0.0045
4	-0.0033	
5	-0.0027	
Average	-0.0031	-0.0042
Standard deviation (SD)	0.0005	0.0004

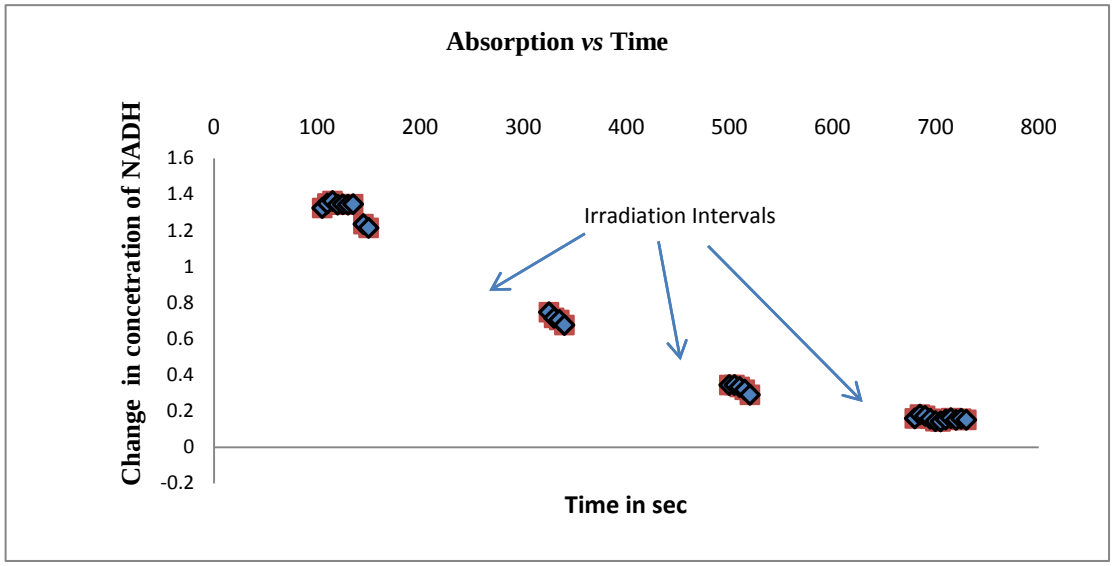


Figure 36. Time-dependent changes in concentration of NADH under the influence of irradiated LDH enzyme

The results for five (5) irradiated enzyme-substrate samples and three (3) non-irradiated are presented in Table 4 along with their average values and standard deviation errors. The results obtained clearly show that MW radiation at **968MHz and power 10dBm** modulated the activity of experimental solutions. The findings reveal that the absorption characteristics of experimental solutions were decreased by **26%** (Figure 38).

Irradiation of S. cerevisiae yeast cells

In the second experimental sub-study, yeast cells were continuously exposed to MW radiation in the custom- made TEM cell at the frequency 968MHz and power 10dBm. S. cerevisiae yeast powder was purchased from Sigma, Australia. The experiments with yeast cells were conducted in a similar manner as outlined previously in Studies 1-3. The experimental solution was prepared as follows: 50 g/l of YPD broth (Sigma, Australia); 20 g/l of S. yeast, and ionized water. The solution was incubated at 24°C for 72 hrs. Then the solution was kept at 4°C. Yeast samples were prepared by diluting the experimental solution as 1 ml in 100ml of the ionized water (1:100).

In these experiments, 3 yeast samples were irradiated continuously for 4 hrs and other 3 yeast samples were sham-exposed (non-irradiated) for the same time duration. The absorption characteristics of each (3 exposed and 3 non-exposed) samples were measured every 30min. Changes in yeast culture growth were monitored using the

spectrophotometry method. Spectrophotometric analysis is based on turbidity and allows for indirect measurement of a number of yeast cells. The absorption coefficients of the yeast samples were measured using an Ocean Optics USB2000 spectrometer. Measurements of the yeast cells' growth in control samples *vs*. irradiated samples were performed. Experimental data were collected and presented in Table 5. The average specific absorption rate (SAR) for a single cell was 0.12W/kg. SAR was calculated by averaging the individual parameters of the cell components in accordance with their volume fraction in live cells. The experimental data were collected and are shown in Figure 39.

Table 4. Yeast cells growth values of irradiated and non-irradiated samples for different time exposures

Time (h)	IR sample1	IR sample 2	IR sample 3	Non-IR sample 1	Non-IR sample 1	Non-IR sample 3
0.5	-0.00256	-0.00258	-0.00258	-0.00255	-0.00255	-0.00248
1	-0.00257	-0.00257	-0.00256	-0.00259	-0.00259	-0.00259
1.5	-0.00249	-0.00251	-0.00253	-0.00257	-0.00258	-0.00257
2	-0.00249	-0.00251	-0.0025	-0.00255	-0.00255	-0.00255
2.5	-0.00246	-0.00244	-0.00247	-0.00251	-0.00253	-0.00252
3	-0.00238	-0.00237	-0.00237	-0.00247	-0.0025	-0.00247
3.5	-0.00229	-0.00229	-0.00231	-0.00242	-0.00251	-0.00246

From Figure 39 we can observe changes in growth rate of S. cervisiae yeast cells in the first 3.5 hours: three samples exposed to MW radiation at 968MHz and power 10dBm and three others are the non-irradiated samples. The results obtained clearly

show the significant exponential increase in the yeast growth rate for all three samples exposed to MW radiation resulting in 15% increase in yeast growth rate upon microwaves exposures.

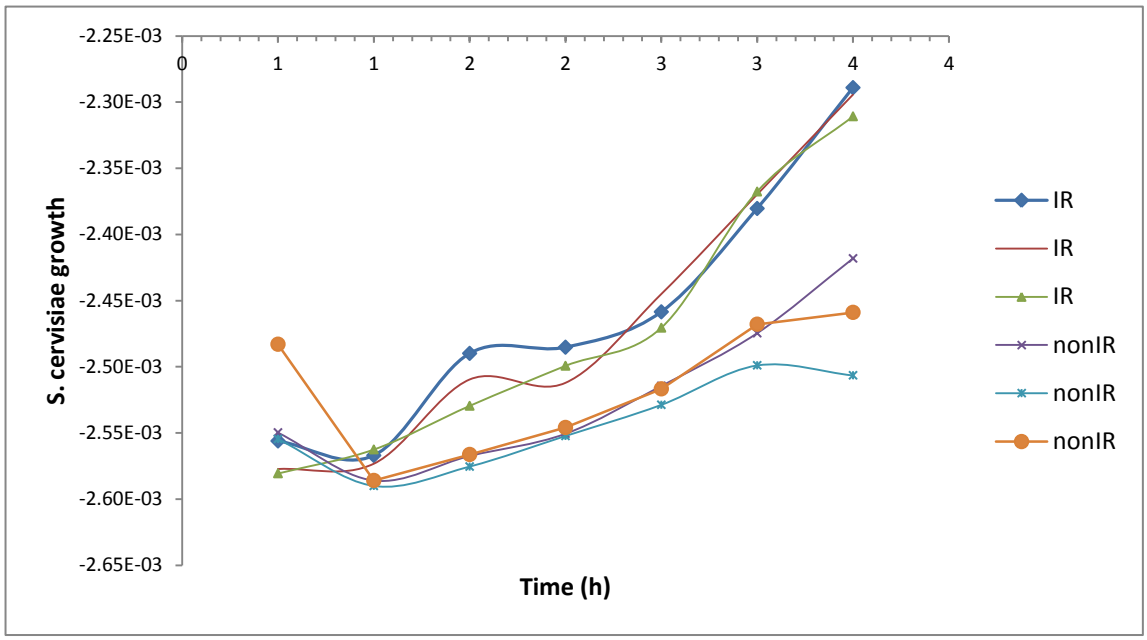


Figure 37. Change in yeast cells growth rate in time upon MW exposures

Final remarks:

In this experimental evaluation, LDH enzyme solutions and S. cervisiae yeast cells were exposed and shame-exposed to the MW radiation at 968MHz and power of 10dBM using the custom-made TEM cell. The results of irradiation of LDH enzyme samples show that MW radiation at 968MHz and power 10dBm modulated its kinetics, which led to changes in the absorption characteristics of the studied experimental solution. The applied MW exposures decreased the activity of the

irradiated enzyme which in turn decreased the concentration of NADH by 26%. In the second experiment, yeast samples were exposed and sham-exposed for 4 hours by MW radiation. The results obtained show that the proliferation rate of the exposed yeast cells increased by 15%. These findings imply that the external non-thermal MW radiation at the selected frequency and power can modulate the selected biological process, i.e. inhibits the activity of LDH enzyme and increases the growth rate of yeast cells.

4.4 Study 4 - Effects of Low Power Microwaves at 1.8GHz, 2.1GHz and 2.3GHz on L-Lactic dehydrogenase and Glutathione peroxidase enzymes

Overview: This study evaluates the effects of low power microwaves on kinetics of L-Lactate dehydrogenase and Glutathione peroxidase enzymes irradiated at the frequencies of 1.8GHz, 2.1GHz and 2.3GHz and power of 10dBm using the commercial Transverse Electro-Magnetic (TEM) cell. The selected frequencies are used frequently in G4 and G5 mobile networks. The findings reveal that microwaves at the studied parameters induce changes in the enzymes' kinetics which lead to modulation of rate of change in corresponding reactions these enzymes catalyse.

At present, there is no substantiated scientific evidence to indicate adverse health effects produced by RF/MW exposures at the levels below national and international safety standards. Studies on human subjects have shown consistently that there is no evidence that prolonged exposure to weak electric fields results in adverse health effects [22, 23]. However, the question of whether chronic exposure to weak magnetic fields is equally harmless remains open [23]. Laboratory studies on animals and cell cultures have shown that weak magnetic fields may have effects on several biological processes. For example, they may alter hormone and enzyme levels and the rate of movement of some chemicals through living tissue. Although these changes do not appear to constitute a health hazards, they need further investigation in other to

elucidate possible mechanisms of existing effects including the long term effects. Some effects of RF radiation on biological processes can be reversible and thus, do not impact human health. However, the significance of investigating non-thermal RF biological effects at the molecular level should not be underestimated.

By using only particular enzyme related to a specific biochemical reaction, many of the problems associated with whole body irradiation can be eliminated. As was shown in Studies 1-3, low level MW exposures at the particular frequencies and powers can modulate kinetics of L-Lactate dehydrogenase and ffect the growth rate of Saccharomyces cerevisiae yeast cells. Interestingly, the observed effects were frequency- and power-dependent, i.e. the activity of studied model examples could be inhibited or enhanced by the applied MW of the particular frequency/power characteristics. However, little is known about the actual mechanisms behind this phenomenon. One of the hypotheses is that the RF/MW radiation can induce dipole oscillations in a protein active site and thus, can alter protein/enzymatic function. This statement is in accordance with the increased permeability of aqueous protein solution measured using the dielectric spectroscopy at the frequency range of 10MHz and 1000MHz [24].

In Study 4, two enzymes-substrate solutions of L-Lactate dehydrogenase and Glutathione peroxidase enzymes were continuously exposed to the microwaves at the frequencies of 1.8GHz, 2.1GHz and 2.3GHz and power 10dBm. The commercial

TEM cell (TC-5062AUHF TEM cell (100kHz–3GHz) from TESCOM Ltd, Unitechvill, Goyang, Korea) was used to generate the electromagnetic field of the desired parameters. The signal generator employed in this study was a Rhode & Schwartz (100kHz–1000MHz) SMX generator (Munich, Germany). All experiments were conducted at the room temperature 25°C, which has been monitored during experimentation by Temperature controller, Quantum Northwest.

Changes in activity of irradiated enzyme-substrate solutions have been recorded by measuring absorption coefficient of the solution at 340nm using Ocean Optics USB2000 spectrophotometer. In order to measure the absorption coefficients with minimal disruption, spectrophotometer was positioned outside of the TEM cell. Samples were continuously irradiated at the selected frequencies and power. It should be noted, to measure absorption coefficients, each sample was removed from the TEM cell every 300sec, with its absorption coefficient being recorded for 30sec, and then a sample was returned to the TEM cell for the next irradiation interval of 300sec. A sampling rate for absorption coefficient at 340nm was set at 1sec. The results were compared with the control group that was kept under the same experimental conditions in the TEM cell with the signal generator being switched off (sham-exposed).

Inside the TEM cell, the supplied signal of 10dBm generated the electrical field of the intensity of 21V/m. Therefore, it is possible to estimate the approximate value of SAR using the following equation:

$$SAR=\sigma|E|^2/\rho$$

where E - electrical field intensity, σ – conductivity and ρ - density of an experimental solution.

It was assumed that enzyme-substrate solution is homogeneous. In our preliminary experiments, the conductivity, σ, of an enzyme-substrate solution for the L-Lactic dehydrogenase (LDH) assay was measured σ= 2.5 S/m. The density of the solution, ρ, was calculated based on the amounts of chemicals used for the preparation of the experimental solution (according to the LDH assay) ρ = 1.28 g/ml. Thus, the estimated value of SAR is 0.861 W/kg for irradiation of LDH enzyme-substrate solutions. In experimentation with Glutathione peroxidase enzymes-substrate solutions, its conductivity was measured σ = 2.5 S/m, calculated density ρ=1.31 g/ml, thus the SAR is estimated 0.841 W/kg. Hence, the values of SAR in both experiments are slightly lower than the maximum values of SAR limits for modern mobile phone handsets.

The dehydrogenases are enzymes that catalyze a variety of oxidation-reduction reactions within a cell. L-Lactic Dehydrogenase (LDH) from rabbit muscle, EC1.1.1.27 (Worthington Assay), has been selected as a protein example for

this study. The LDH catalyzes the reversible reduction of pyruvate to L-lactate using NADH (Nicotinamide Adenine Dinucleotide, Reduced form) as a coenzyme. The NAD+ is not optically active at 340nm and the oxidation of NADH is directly proportional to the reduction of pyruvate. Therefore the LDH activity can be calculated from the rate of decrease in absorbance at 340nm.

Glutathione peroxidase (GPx, EC 1.11.1.9) – a second protein example studied here - provides a mechanism for removing of peroxides in living cells. It plays a crucial role in protecting cells from damage by free radicals, which are formed by peroxide decomposition. The reaction is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (b-Nicotinamide Adenine Dinucleotide Phosphate, Reduced). The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPx activity, since GPx is the rate limiting factor of the coupled reactions (Sigma-Aldrich Assay).

MW exposures of L-Lactic dehydrogenase (LDH) enzyme

L-lactic dehydrogenase enzyme solutions were irradiated with the MW at three selected frequencies of 1.8GHz, 2.1GHz and 2.3GHz and power of 10dBm (power was the same for each studied frequency). Five enzyme solution samples were placed insight the TEM cell and exposed for 300sec. The measurement of absorption rate of NADH (corresponding to changes in concentrations of NADH and thus, to changes in the rate of speed of the reaction) was done four times with the intervals of about 100 sec. The samples were continuously exposed in the camera with 3 short intervals of 100sec, the absorption coefficients of NADH were measured using USB2000 spectrometer with the recording interval of 10sec. The rate of change in absorption coefficient was measured as gradient of the absorption vs. time. The gradient was measured between 20sec and a 100sec using linear regression (Figure 40). The reaction rate was determined by a decrease in absorbance at 340nm resulting from the oxidation of NADH. One unit causes the oxidation of 1uM of NADH per minute at 25°C and pH 7.3, under the specified conditions.

Reagents:

6.6mM NADH 0.2M Tris·HCl buffer at pH 7.3; 30mM Sodium pyruvate in 0.2 Tris·HCl buffer, at pH 7.3. Final aliquot consist was prepared as follow 2ml of Tris + 80ul NADH solution + 80ul Sodium pyruvate solution + 80uL LDH solution

Control samples (sham-exposed) were kept under the same experimental conditions (T=25°C). In control group, non-irradiated enzyme was added to the experimental solutions. The measurements of absorption rate of NADH were repeated five times for four different time intervals at each MW frequency 1.8GHz, 2.1GHz and 2.3GHz (as well as for control samples). The average rate of change of the absorption coefficients/concentrations of NADH have been calculated and presented in Table 6. The results obtained reveal that exposures at all three studied MW frequencies induced an increase in LDH activity that lead to changes in NADH absorption rate/changes in NADH concentration.

Table 5. Changes in rate of absorption of NADH with irradiated and non-irradiated LDH enzyme. The intervals (minimum, maximum and mean values recorded for five analyzed samples) for the rate of change of NADH

Non Radiation(10^{-3})	**1.8 GHz (10^{-3})**	**2.1 GHz (10^{-3})**	**2.3 GHz (10^{-3})**
4.28-4.56 (4.34)	5.17-5.32 (5.22)	6.45-6.72 (6.56)	6.20-6.38 (6.27)

In particular, in comparison with the sham-exposed (non-irradiated) samples, the MW exposures at 1.8GHz resulted in 20% increase, 2.1GHz – 51% increase, and 2.3GHz - 44% increase in NADH absorbance; with the highest increase observed at 2.1GHz and lowest at 1.8GHz.

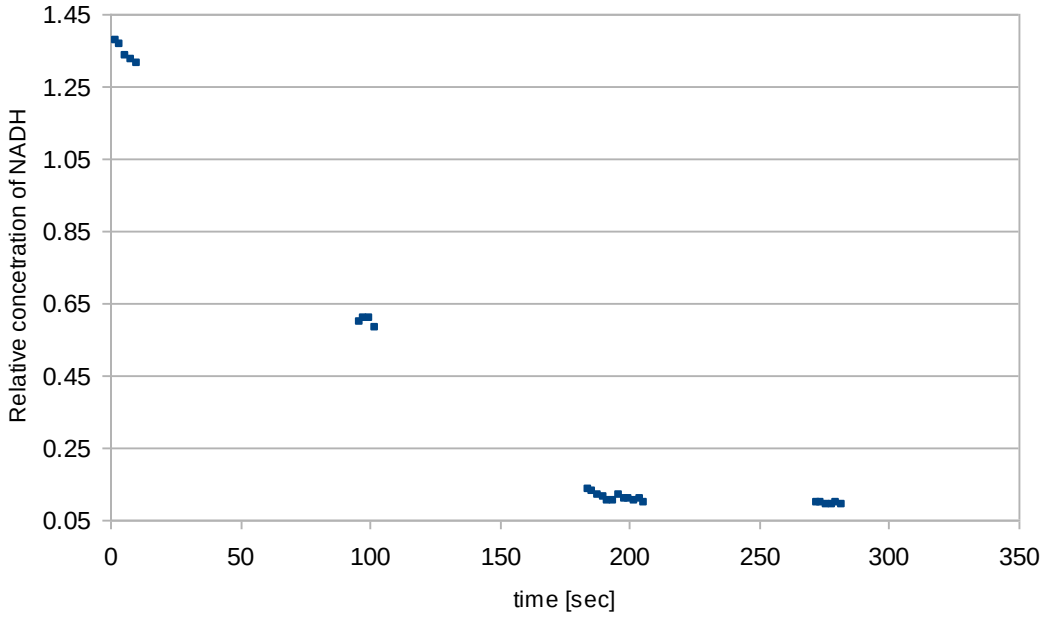


Figure 38. Changes in concentration of NADH in time due to the exposure of LDH at 1.8GHz and power of 10dBm.

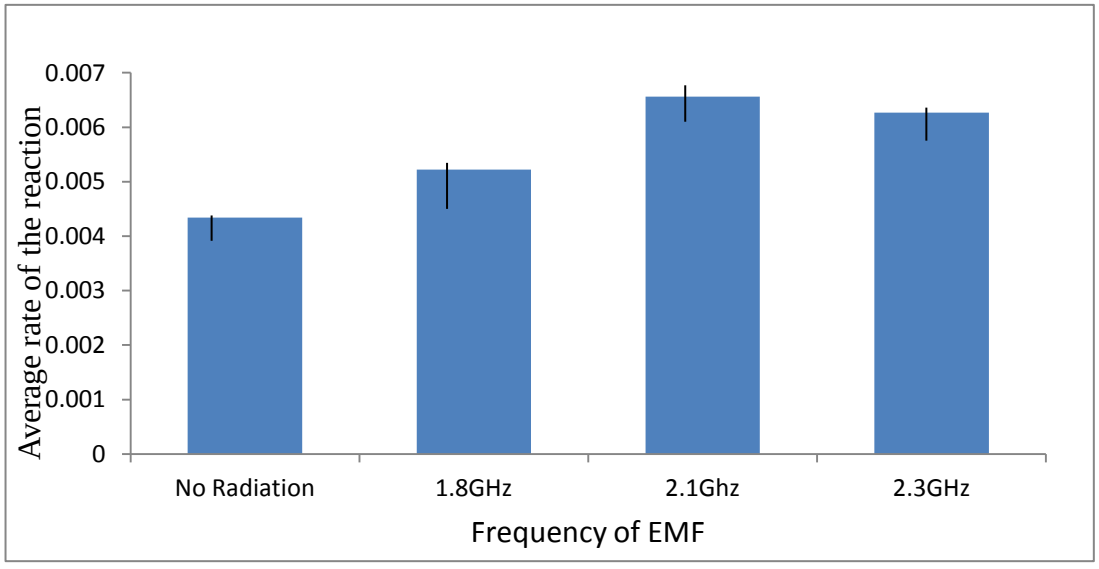


Figure 39. Rate of change of NADH concentration for non-irradiated and irradiated LDH enzymes

MW exposure of Glutathione peroxidase enzyme

The experimental evaluation of the effects of the selected MW exposures on kinetics of Glutathione peroxidase (EC.1.11.1.19) was conducted according to the Sigma-Alrdich assay. This chemical reaction is a two-step process. Firstly, Glutanione is changed from the reduced form to the oxidized form in the presence of Glutathione peroxidase enzyme using H_2O_2 as an oxidant. Then, the reverse reaction occurs: Glutanione, the oxidized form is changed to Glutanione, reduced form, using Gluthanione reductase enzyme. Of particular interest is to observe and quantify the conversion of β-NADPH into β-NADP, by monitoring the changes in its absorption at 340 nm. The concentrations of the substituent are calculated to achieve the recording time of approximately 30 minutes (Figure 42). The rate of change of concentration of NADPH was measured by calculating gradient of regression line in the interval of 20 - 400sec. Three experimental solution samples were placed insight the TEM cell and irradiated at two frequencies 1.8GHz and 2.1GHz and the power of 10dBm (power was the same for each studied frequency).

Experimental procedure:

1. Three irradiated and three non-irradiated samples were used for each MW exposure. Non-irradiated samples were kept under the same experimental conditions as the exposed samples.

2. Three irradiated samples were placed insight the TEM cell and have been mixed by rotation at the same speed. Off note, the absorbance of NADPH in non-irradiated experimental solutions was measured more frequently (Figure 42).

To avoid a frequent change in the intensity of the electromagnetic field, the absorption coefficients of NADPH in the irradiated experimental samples were measured only four times. Both irradiated and non-irradiated samples show very small standard deviation for each measurement < 0.5%. All calculations were done with test-blank data.

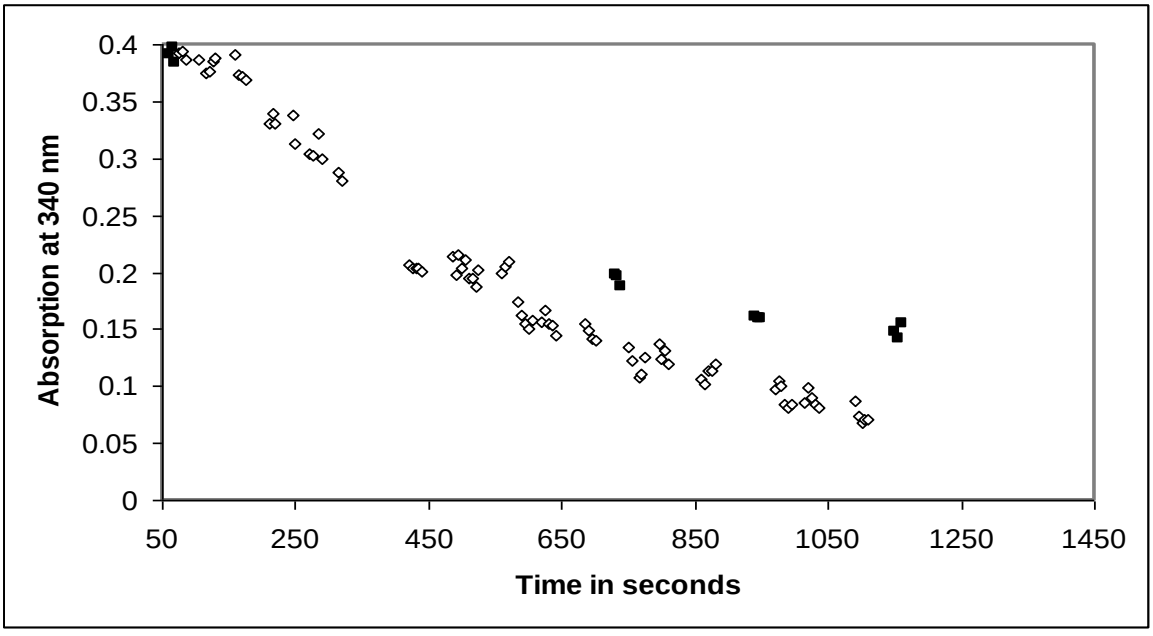


Figure 40. Change in absorption of NADPH at 340 nm due to the production of β-NADP from β-NADPH in the presence of Guthanione Reductase. ■ - correspond to the irradiated samples and □ - correspond to the non-irradiated samples

The results obtained show that the MW exposures at 1.8GHz and 2.1GHz and power of 10dBm induce the suppressive effect on the studied biochemical reaction. In particular, the MW radiation significantly decreased the rate of the reaction (speed) from 0.00021units/sec to 0.00018units/sec, which equals to 15% reduction in the speed of the reaction (Figure 43).

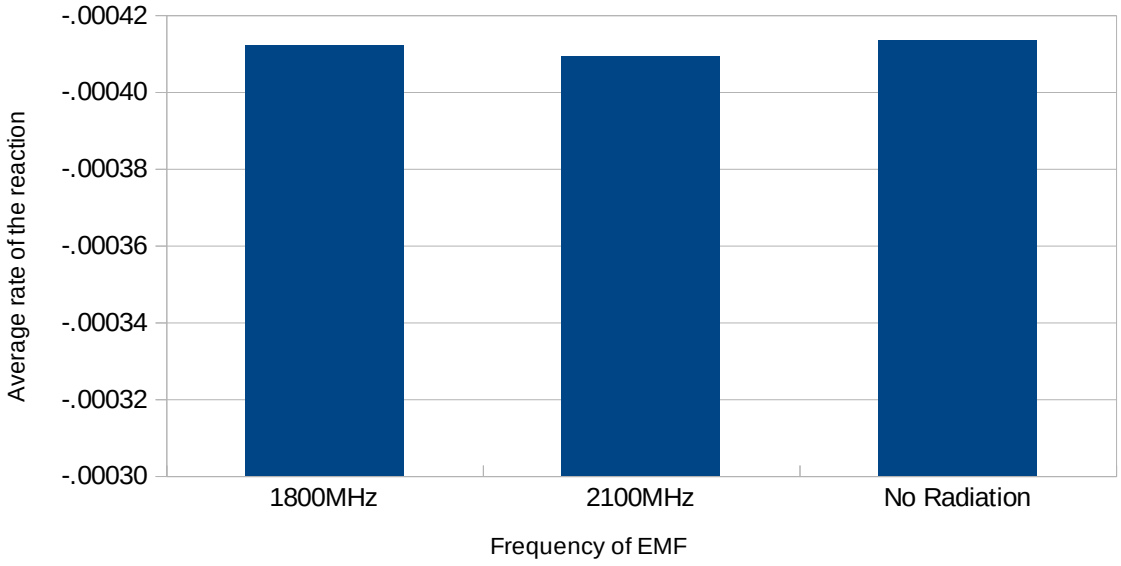


Figure 41. Rate of change of NADH concentration for non-irradiated and irradiated Gluthanion reductase/peroxidase enzymes

Final remarks:

This study was aimed to test the hypothesis that low power MW exposures at 1.8GHz, 2.1GHz and 2.3GHz and power of 10dBm can induce changes in the irradiated L-

Lactate dehydrogenase and Glutathione peroxidase enzyme-substrate solutions exposed and sham-exposed during experimentation.

The results obtained show that applied MW can affect the kinetics of the selected enzymes, which lead to modulation of the speed in the corresponding reactions catalyzed by these enzymes. In particular, the MW radiation of L-Lactate dehydrogenase enzyme resulted in the frequency-dependent effects on the observed biological reaction as follows: exposures at 1.8GHz and 10dBm resulted in 20% increase, 2.1GHz – 51% increase, and 2.3GHz - 44% increase in NADH absorbance.

For irradiation of Glutathione peroxidase enzyme, the findings reveal that MW exposures at 1.8GHz and 2.1GHz and power of 10dBm induced the inhibitory effects on the studied biochemical reaction that resulted in 15% reduction of the reaction speed. These findings imply that the external non-thermal MW radiation at the particular frequency and power can modulate the selected biological process, with the observed effects showing frequency- and power-dependency.

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CHAPTER 5

CONCLUSION

In the last few decades, the use of microwave radiation has greatly increased in radar and communication systems, food-processing technology, and other industrial applications. The development of consumer and medical microwave devices for clinical diagnosis and therapy also has prompted widespread interest and has stimulated much research into the mechanisms of interaction between microwave radiation and living matter. Experimental studies made in the millimeter band at very low microwave energy flux densities (no more than a few milli watts per square centimeter) induced highly specific effects of applied radiation.

The concept of non-thermal microwave effects has received considerable attention in recent years and is the subject of intense debate in the scientific community. Non-thermal microwave effects have been postulated to result from a direct stabilizing interaction of the electric field with specific (polar) molecules in the reaction medium that is not related to a macroscopic temperature effect. Non-thermal biological effects of microwaves depend on several physical parameters and biological variables [1], therefore, only results obtained under the same conditions of exposure should be compared in "replication" studies.

Important features of non-thermal MW effects include the following [1, 2]:

• Effects of resonance type within specific frequency windows.

• Dependence on type of signal, modulation, and polarization.

• Resonance effects are observed in specific intensity windows including super-low power densities (PDs) comparable with intensities from base stations/masts.

• With decrease in intensity, narrowing of the resonance windows occurs.

• The non-thermal MW effects are more sensitive to the duration of exposure than to the PD in the range of 10-17-10-6W/cm2. Decreasing of PD by orders of magnitude can be compensated by several-fold increasing of exposure time. Therefore, duration of exposure may have significantly larger role as compared to PD.

• Some of these features indicate quantum-mechanical mechanism for non-thermal MW effects.

• The effects depend on cell density. Radical scavengers/antioxidants have a potential to abolish MW effects. The effects depend on physiological conditions during exposure. Genomic differences influence response to MWs [3].

This research project was aimed at investigating the effects of low power microwave radiation on the selected cells and proteins with the specific focus on the frequencies emitted by mobile phones. The frequencies selected for this investigation are used frequently in G3, G4 and G5 mobile networks.

This PhD project included the following sub-studies that were successfully completed within the project:

1. **Custom-made microwave exposure camera design, fabrication and testing** (Chapter 3).
2. **Design and software simulation of the generated field inside the exposure camera** (Chapter 3)

3. Experimental evaluation of the effects of low level microwaves on selected biological systems (Chapter 4):

3.1 Experimental study 1 - Non-thermal effects of the microwave exposures at 900MHz and selected low powers on the proliferation rate of Saccharomyces Cerevisiae Yeast

3.2 Experimental study 2 - Effects of non-thermal microwave exposures at 500MHz and 900MHz and selected powers on the proliferation rate of Saccharomyces Cerevisiae Yeast

3.3 Experimental study 3 - Effects of Low Power Microwave Radiation on Biological Activity of L-Lactate Dehydrogenase Enzyme and Growth Rate of S. Cerevisiae Yeast

3.4 Experimental study 4 - Effects of Low Power Microwaves at 1.8GHz, 2.1GHz and 2.3GHz on L-Lactic dehydrogenase and Glutathione peroxidase enzymes.

The experimental evaluations were conducted using the commercial and custom-made exposure cameras (details are presented in Chapters 3&4). The experimental findings are summarized below:

Study 1

In Study 1 the microwave exposures at the 900MHz and the powers of 13dBm, 3dBm and -7dBm were applied to irradiate S. cerevisiae yeast sample aiming to evaluate the hypothesis that the *external microwave radiation of the specific frequency and power can affect the growth and proliferation response in yeast cells*. The yeast samples were exposed and sham-exposed for 6 hours. The results obtained show:

- MW radiation at **900MHz and power of -7dBm** induced no effect on yeast growth.
- MW radiation at the same frequency of **900MHz and powers of 13dB and 3dB** affected significantly (14% and 11% respectively) the concentration of yeast cells.

These findings imply that applied MW exposures induce modulating (increasing or inhibiting) effects on the studied yeast cells suggesting that these effects are *power-dependent*.

Study 2

In Study 2 the experimental testing was conducted on the same yeast cells of Saccharomyces cerevisiae strain, which were exposed to microwave radiation at two frequencies 500MHz and 900MHz and powers of -17dBm, -13dBm, -10dBm, 0dBm, 10dBm, 13dBm and 17dBm. Similar to Study 1, TC-5062AUHF TEM cell (100kHz–3GHz) from TESCOM Ltd (Unitechvill, Goyang, Korea) was used to irradiate yeast cells here.

The results showed:

1) MW radiation at the frequency of **500MHz** and power of **-17dBm** the decrease of **20%** in yeast cell growth is achieved. At the frequency of **500MHz** and **powers of -13dBm and -10dBm** we can see only a slight decrease in cells growth. We also can observe no effect on cells at the frequency of **500MHz and power of 0dBm**. In contrast, a slight increase is seen at the power of **13dBm**, and more significant increase of **18%** is achieved at **17dBm.** The maximum increase of **38%** in yeast cell growth rate is achieved at **the frequency of 500MHz the power exposure of 10dBm**.

2) MW radiation at the frequency of **900MHz and powers of -13dBm, 0dBm and 10dBm** affected the yeast cells sample. The findings revealed that the yeast cell proliferation rates are increased (21%, 12% and 6% respectively). In contrast, at the same frequency of **900MHz and the powers of -17dBm, -10dBm, 13dBm and 17dBm** the opposite effects are observed. The exposures at these particular parameters decreased the growth and proliferation of yeast cells with the different degree of suppression. The maximum decrease in yeast cells growth (38%) is recorded at the frequency of **900MHz and the power of 17dBm**.

Hence, the findings of Study 2 are in accord with the results of Study 1, and reveal that applied microwaves at 500MHz and 900MHz and selected powers can modulate yeast

cells growth and these *effects are power-dependent*. Interestingly, the observed effects vary significantly. The findings reveal that the growth pattern of yeast cells can be increased or inhibited by exposures of particular powers, thus confirming the hypothesis that non-thermal effects of low power MW are *strongly power-dependent*, which was also clearly shown in the results obtained in Study 1.

Study 3

In Study 3 we experimentally evaluated non-thermal effects of low power microwave exposures on kinetics of L-Lactate Dehydrogenase enzyme and growth rate of yeast Saccharomyces Cerevisiae strains type II. The selected model systems were continuously exposed to microwave radiation at the frequency of 968MHz and power of 10dBm using the designed and constructed custom-made TEM cell.

The findings of Study 3 reveal that microwave radiation at **968MHz and power of 10dBm** inhibits the enzymatic activity of L-Lactate dehydrogenase by 26% and increases significantly (15%) the proliferation rate of yeast cells. These findings imply that the external non-thermal MW radiation at the selected frequency and power can modulate the selected biological process, i.e. inhibits the activity of LDH enzyme and increases the growth rate of yeast cells.

Study 4

Study 4 evaluated the effects of low power microwaves on kinetics of L-Lactate dehydrogenase and Glutathione peroxidase enzymes irradiated at the frequencies of 1.8GHz, 2.1GHz and 2.3GHz and power of 10dBm using the commercial Transverse Electro-Magnetic (TEM) cell. The selected frequencies are used in G4 and G5 mobile networks in Australia.

The results obtained show that applied MW can affect the kinetics of the selected enzymes, which lead to modulation of the speed in the corresponding reactions catalysed by these enzymes.

In particular:

- The MW radiation of L-Lactate dehydrogenase enzyme resulted in the *frequency-dependent effects* on the observed biological reaction as follows exposures at **1.8GHz and 10dBm resulted in 20% increase, 2.1GHz – 51% increase, and 2.3GHz - 44% increase in NADH absorbance**.
- For irradiation of Glutathione peroxidase enzyme, the findings reveal that MW exposures at **1.8GHz and 2.1GHz and power of 10dBm induced the inhibitory effects on the studied biochemical reaction that resulted in 15% reduction of the reaction speed**.

These findings imply that the external non-thermal MW radiation at the particular frequency and power can modulate the selected biological process, with the observed

effects showing *frequency- and power-dependency*.

Table 7. Findings reported in research studies investigating the microwave radiation in the same frequency and power range as this PhD research project

Frequency	Power	Cells/Tissues	Effect
915 MHz	1mW/g	Human Neuroblasoma [1]*	A significant increase in the efflux of calcium ions
450 MHz	0.29 mW/g	Effects of weak amplitude-modulated microwave fields on calcium efflux from awake cat cerebral cortex [2]*	Increased end-tidal CO2 excretion
837 MHz to 1909.8 MHz	5mW/g	Chromatin in human cells[3]*	The microwave irradiation of human cells induces the significant increase of Heterocromatin granules quantityy parameter
905 MHz	0.5 mW/g	Sarcharomyces cerevisiae [4]*	significant reduction of colony growth compared to nonirradiated strains after all exposure times
1800MHz	0.06mW/g	Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats [5]*	Cronic microwave radiation exposure at low-level induces DNA damage
9.9MHz	1mW/g	Biochemical Changes in Rat Brain Radiation [6]*	Decrease activity of protein kinase

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Tabular representation of the experimental findings

Study 1 – Modulating Effects of Microwaves on Sarcharomyces cerevisiae yeast cells Growth

Frequency & Power	13dBm	3dBm	-7dBm
900MHz	14% increase in cells growth	11% increase in cells growth	No effect

Study 2 – Modulating Effects of Selected Microwave Exposures on Sarcharomyces cerevisiae Yeast Cells Growth

Frequency & Power	-17dBm	-13dBm	-10dBm	0dBm	10dBm	13dBm	17dBm
500MHz	20% increase	slight decrease	slight decrease	no effect	38% increase	slight increase	18% increase
900MHz	slight decrease	21% increase	slight decrease	12% increase	6% increase	slight decrease	38% decrease

Study 3 – Modulating Effects of Selected Microwave Exposures on Enzyme Activity

Frequency & Power	L-Lactate dehydrogenase	Yeast Cells
968MHz, 10dBm	26 decrease in enzyme activity	15% increase in cells growth

Study 4 – Modulating Effects of Selected Microwave Exposures on Enzyme Activity

Frequency & Power	L-Lactate Dehydrogenase	Glutathione Peroxidase
1.8GHz, 10dBm	20% increase	15% decrease
2.1GHz, 10dBm	51% increase	15% decrease
2.3GHz, 10dBm	44% increase	----------

Conclusive Remarks

The experimental findings of this research project clearly demonstrate that applied microwave exposures of selected frequencies and powers can induce modulating effects in the studied model systems. Moreover, the observed effects are frequency- and power-dependent, which provide an opportunity of altering the cellular and protein activities of the selected model systems. This, in turn, can lead to development of novel technologies in medical and food applications by employing the microwave radiation of the specific parameters able to affect/modify selected biological processes of interest.

CHAPTER 6

FUTURE WORK

It becomes extremely important to study the effects of long-term exposures of MW on human health. However, it is almost impossible to select a control unexposed groups because the whole population in industrial countries is exposed to wide range of MW signals from various sources such as base stations/masts, WILAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth) and wireless phones. Studies suggest that duration of exposure (must be at least 10 years of exposures for epidemiological cancer studies) may be more important for adverse health effects of non-thermal MWs than intensity of the exposure [1].

The adverse effects of "detrimental" MW signals can be diluted because people are exposed to various signals/frequencies including non-effective or even hypothetically beneficial. Available mechanistic studies indicate that at this point, the epidemiological studies can be either inconclusive, if negative, or underestimate significantly the hazard of using specific MW signals, if positive [1-3]. Therefore, *in vitro* molecular and cellular studies as well as *in vivo* studies on experimental animals shall be conducted to further address non-thermal effects of low power MW radiation and elucidate the mechanism behind this phenomenon.

Comments on possible future works:

- Up to date, within the ICNIRP and scientific community overall, there is no agreement on non-thermal effects of MWs and their complex

dependence on several physical and biological parameters. Published studies are sometimes showing the conflicting results. It is apparent that the important parameters of MW (frequency, intensity/power, exposure duration, modulation etc) are not properly controlled in "replication studies" of non-thermal effects of MWs and therefore, the data cannot be compared with the original results.

- The mechanisms behind the observed non-thermal effects are not yet elucidated. There is a need to establish a national program via collaborative inter-institutional research involving biochemists, molecular biologists, engineers and physicists for interdisciplinary mechanistic studies of non-thermal effects of MWs both from mobile phones and base stations.

- The types and frequency channels/bands for mobile communication, which do not affect humans, should be identified.

- Published *in vitro* studies indicate that the duration of exposure can be more important for non-thermal effects than intensity and therefore, effects of MWs from base stations on *primary human cells* should be studied [1-3].

- Because non-thermal MWs affect not only brain cells, but also blood cells and probably some other human cells including cells of reproductive organs, use of hands-free option cannot minimize all possible health effects. Therefore, there is a need for *in vitro* studies of low power non-thermal MW exposures on a variety of cell types.

- *In silico* studies should be undertaken to simulate the effects of applied MW of different frequencies and powers on various cells and proteins.

- Possibilities of minimizing the adverse effects of non-thermal MWs using different approaches should also be studied.

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PUBLICATIONS

Journal paper:

1. H.S. Alsuhaim, V. Vojisavljevic & E. Pirogova. Effects of low power microwaves at 1.8, 2.1, and 2.3 GHz on l-Lactic dehydrogenase and Glutathione peroxidase enzymes. *Journal of Electromagnetic Waves and Applications*. **2014**, DOI:10.1080/09205071.2014.934924

Refereed conference papers:

1. H.S. Alsuhaim, V. Vojisavljevic, & E. Pirogova, E. **2013** The effects of low power microwaves at 500 MHz and 900 MHz on yeast cells growth', in ***Proceedings of Progress In Electromagnetics Research Symposium (PIERS 2013),*** D. C. Chang (ed.), The Electromagnetics Academy, Cambridge, USA, 667-670.

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