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# Effects of a static magnetic field on biological samples

Peter Lazarakis  
University of Wollongong

Lazarakis, Peter, Effects of a static magnetic field on biological samples, Master of Science (Research) Physics thesis, School of Engineering Physics - Faculty of Engineering, University of Wollongong, 2009. <http://ro.uow.edu.au/theses/3033>

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**Effects of a Static Magnetic Field on Biological Samples**

**by**

**Peter Lazarakis**

**A thesis submitted in partial fulfilment of the requirements for  
the award of the degree of**

**Master of Science (Research)  
Physics**

**from**

**UNIVERSITY OF WOLLONGONG  
SCHOOL OF ENGINEERING PHYSICS**

**September 1, 2009**

## Acknowledgements

I would like to thank my supervisor George Takacs for all the help and advice throughout this time. Also the many people that helped with organising the use of various equipment and facilities - Lisa Seymour in the Biology department for teaching me all about growing cells, Martin Carolan and Peter Metcalfe who helped get me access to the hospital facilities and gave advice whenever needed and also in particular Rodney Vickers who was always willing to help with problems with the Bomem - which occurred unfortunately often.

## **Abstract**

FTIR spectroscopy uses the absorbed light in an IR beam to determine the composition of a sample. This study was done using FTIR techniques to determine the damage done or alterations caused when a magnetic field was applied to a biological sample (cell cultures).

The effects of magnetic fields on biological samples is an area that is not very well understood with little reliable data available.

Various experiments investigating the influence of a magnetic field on cell growth, the chemical bonds in cells and the effects during irradiation were performed. Consistently it was seen that the largest changes to the cell were found in hydrogen bonds, most commonly in water. Though perhaps this may not normally create any significant biological impact when a biological sample is irradiated, as in radiotherapy, the chemical and physical structure of water is quite important.

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## Notations and Abbreviations

Deoxyribonucleic Acid	[DNA]
Double Strand Break	[DSB]
Fourier Transform Infrared	[FTIR]
Hydrogen	[H]
Linear Quadratic No Threshold	[LQ]
Oxygen Enhancement Ratio	[OER]
Relative Biological Effectiveness	[RBE]
Single Strand Break	[SSB]

# Chapter 1

## Introduction

The biological effects of radiation are a phenomenon that has been widely studied but is still not completely understood. Different techniques have been developed and applied to the problem, including FTIR methods. The potential of these techniques is still being explored and their ability to provide new data on the problem is undoubted.

### 1.1 Biological Effects of Radiation

Though radiation can be very harmful it can also have beneficial uses, as in radiotherapy: gamma, beta, proton and ion radiation are all used in the treatment of cancer and other diseases. Also the environment we live in is full of radiation, so it is important to understand the potential effects, both beneficial and detrimental, of radiation.

The aim of this thesis is to investigate the effect of a magnetic field on biological samples exposed to radiation. It has been shown in previous studies [32, 33] that there is a significant change to the amount of damage done to cells when irradiated in the presence of a magnetic field compared to when there is no magnetic field present, as seen in figure 1.1, however it is not known why such a change is seen. If this could be understood then the

application of magnetic fields may be of use in radiotherapy and/or radiation protection.

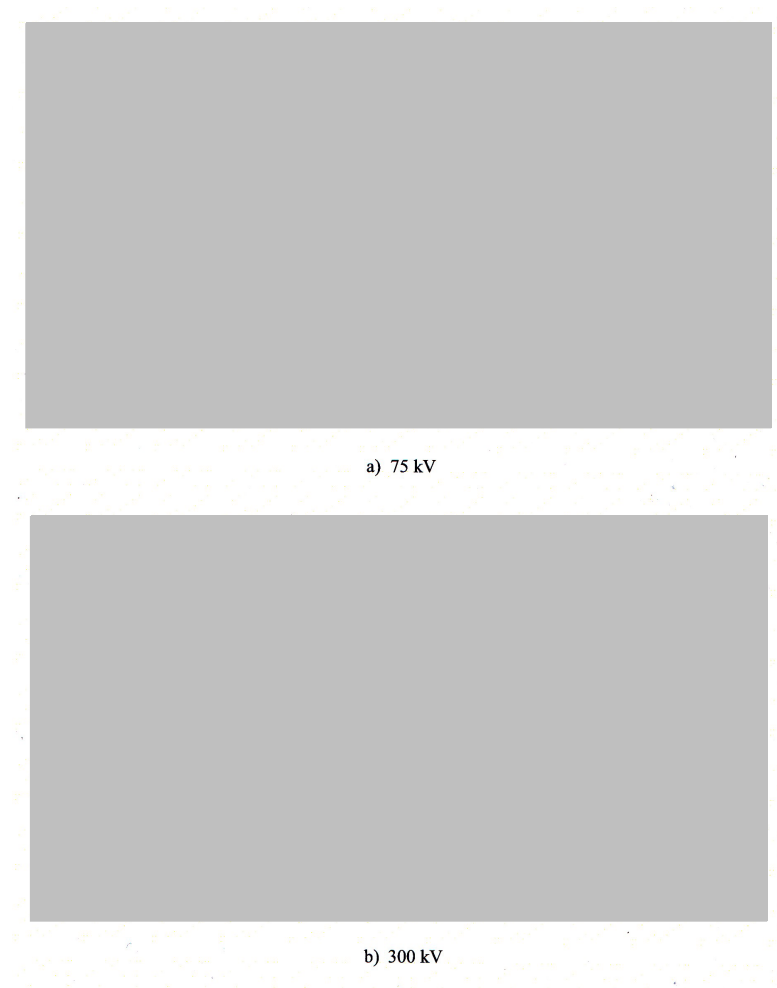


Figure 1.1: Comparison of clonogenic survival of NALM6 cells following irradiation in the presence of a 1.3 T magnetic field and without a magnetic field at x-ray beam energies of a) 75 kV and b) 300 kV. Figure from [33] by C. Abdipranoto.

The biological effects of radiation have been investigated using many methods, including FTIR. Though all the effects are not completely understood there are models that can predict many of the effects of radiation, though these tend to be less reliable at low doses. The most commonly used

model is the Linear Quadratic No Threshold model (LQ), which describes the damage done to biological material as proportional to the dose delivered for a wide range of doses. It also suggests that there is no lower limit for damage to occur, there is no absolutely safe level of radiation exposure; all doses bring some possibility of damage.

Extrapolation to low doses can be very difficult due, in part, to the large studies that would be needed to acquire any significant data - as discussed by Brenner et al [10] the number of individual data sets (samples) required for a statistically significant study will be approximately inversely proportional to the delivered dose at which the study is done, so for low doses the sample size is very large (several million for a 10 mSv dose [10]). This is because the dose delivered approaches the background radiation dose at low doses meaning that differentiating the effects of delivered dose to those derived from background becomes progressively more difficult. To measure the physical dose delivered in experiments GaF film was used in this thesis, for a review of GaF film refer to [34].

The basic LQ model can be defined by several parameters;

$S$  = cell survival probability (fraction of surviving cells, number from 0 to 1)

$D = \frac{E}{M}$ , absorbed dose in Gy (energy absorbed per unit mass - 1 Gy = 1 Joule/Kg)

And  $S$  is given by:

$$S = \exp(-(\alpha D + \beta D^2))$$

Where  $\alpha$  and  $\beta$  are characteristic of the type of tissue being irradiated. Also this basic model can be expanded to account for fractionated dose delivery, repopulation, reoxygenation and repair when dealing with radiotherapy.

Note that this equation for survival is for mammalian cells, when relating to bacteria like *E. Coli*  $\beta = 0$  giving

$$S = \exp(-\alpha D)$$

Also  $\alpha$  may be around 10 times smaller in bacteria compared to mammalian cells.

As different types of radiation can do differing amounts (and types) of damage for the same absorbed dose so the quantity of Relative Biological Effectiveness was introduced to compensate:

$$\text{RBE}_x = \frac{D(250\text{keV})}{D(x)}$$

Note that the doses above are defined for the same biological endpoint.

This is necessary as different types of radiation may have significantly different track structures when depositing energy in a medium, this can lead to different types of radiation delivering the same physical dose but the density of ionisations created along the track may be very different. Using RBE values different types of radiation can be scaled to compare biological endpoints.

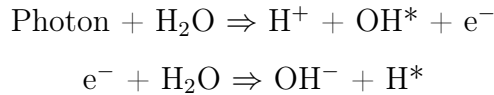
It is important to understand the different effects of different types of radiation as well as different radiation energies. High energy particles can have a significantly different linear energy transfer and so have different biological effects than lower energy particles. This difference is taken advantage of in medical radiation physics where Orthovoltage x-ray machines can deliver low energy photon beams and Linacs can deliver high energy beams of photons or electrons. Both types of beam are used in experiments in this thesis.

Ionising radiation interacts with biological materials by several processes. Photons can interact via scatter events (elastic - though this produces no biological effects, Compton), photo effect and pair formation [9]. Ions and heavy charged particles can interact via electron capture, collisions with atomic electrons and collisions with the nucleus of an atom [9]. Electrons can interact via collisions with atomic electrons, Bremsstrahlung and nuclear reactions. All of these interactions involve the deposition of energy in the material - they deliver a dose.

Note that non-charged particles like neutrons have not been mentioned, as they will not be dealt with in this thesis, though they too can deliver dose primarily via scatter interactions.

The effects of the delivered dose can be separated into two categories: deterministic effects and stochastic effects. These will result in damage to the system - most importantly to this study it can result in damage to DNA (SSB and DSB). This can occur via two processes: Direct damage where the quanta of radiation interacts with the DNA itself to cause SSBs or DSBs, or produces an electron (via scatter events) that interacts with the DNA, or Indirect damage where the radiation produces free radicals that then interact with DNA causing damage [9,11].

Indirect damage from, say, photon radiation:



Where \* indicates the atom has an unpaired electron in the valence shell resulting in the formation of two ion pairs ( $\text{H}^+$  and  $\text{OH}^-$ ) and two free radicals ( $\text{OH}^*$  and  $\text{H}^*$ ). The free radicals are highly reactive and may quickly reform with the ions to produce water or, if in close enough proximity, may bond with a DNA molecule leading to Indirect Damage.

The amount of damage done will depend primarily on the absorbed dose as well as tissue type and conditions.

Deterministic effects will only occur after some threshold of dose (at which point they definitely happen - the probability is unity, only the amount of interactions that occur increase with increasing dose), below that there will be no effect.

Stochastic effects happen at all doses with the probability of effects increasing with increasing dose. Note that there are several models that predict different levels of stochastic effects down to low doses, though the LQ model seems to be widely accepted as the most accurate.

Damage to a biological system can result in somatic or hereditary effects. These can cause cell death or mutation and lead to death of the individual, carcinogenesis, or other detrimental outcomes [9,10,16]. Note that there is also the potential for cells that are not directly hit by radiation to still be damaged - this is called the bystander effect [14, 15], though it should be noted that this is not necessarily a widely accepted phenomenon and even if the theory is correct the extent of the spread of damage and the mechanisms behind the spread are as yet unclear.

Note that the cell samples used in this study are stored after measurements are taken but not incubated further, so a prolonged study on the workings of cellular repair mechanisms after different delivered doses is not studied. Current models are based on data from high dose cases and assume that cellular repair mechanisms will work with the same effectiveness at low doses as they do at high doses, however if this assumption is not correct, and cellular (particularly DNA) repair is not as efficient at low doses, then the prediction of long term survival rates of low dose irradiated cells may be significantly affected. Whether or not the presence of a magnetic field during

or after irradiation may change the repair abilities of the cells is also not known. This, however, is not a concern of the current study as the primary interest of this paper is the change to the type and rate of initial damage caused by irradiation of cells in a magnetic field and the mechanisms behind this change.

## **1.2 Possible Effects of Magnetic Fields on Biological Samples under Irradiation**

Magnetic fields may potentially affect the damage caused to cells under irradiation in several ways:

1. Charged particles may be deflected (charged particle in a magnetic field moves in a helical path, where the field direction is perpendicular to the initial path of the particle), this could affect not only the primary beam (whatever the source, if charged particles) but the secondary particles as well, causing particles to be concentrated into a smaller volume - potentially causing an increase in LET and thus an effective increase in RBE. As the magnetic field will add a curvature to the path of charged particles so they may have a higher probability of damaging DNA or of creating free radicals close enough to cause indirect damage.
2. The application of a magnetic field could possibly alter the conformation of molecules, thus potentially changing their functionality. For example water molecules may have altered interaction probabilities as well as different bonding characteristics (Hydrogen bonding can be directly affected); this could easily lead to an altered level of free radical production.
3. The application of a magnetic field could possibly alter any membranes in the cell, as well as the cell wall, and in particular the ion channels embedded in these membranes. This could affect the rate of ion exchange

between the ICF and ECF (Intra- and Extra- Cellular Fluid, respectively) which would likely have a very significant effect on nearly all cellular functions. [18]

4. Magnetic fields could alter H bonds; if the hydrogen bonds in molecules are strengthened then the probability of DNA strand breaks and free radical creation may be reduced. On the other hand if the bonds were weakened then this could lead to extended lifetimes of free radicals and a correspondingly higher rate of indirect damage to DNA. The application of a magnetic field may cause the distribution of charges (electrons and protons in this case) to be altered [19] (as a molecule in a magnetic field with a magnetic moment may be forced to align with the external field, or the distribution of charges and molecules may be altered to align with the external field) or may cause changes to the internal energy structure of molecules via the Zeeman effect and the splitting of energy levels. Either physical alteration could lead to the strengthening or weakening of H bonds as described above, which in turn could lead to significant biological changes to the system. Quantitatively the extent of this change is still under investigation. [19, 29]

## 1.3 DNA

There are several important types of molecules in mammalian cells, however probably the most important is DNA: it is the storage site of all the information needed to build any cell component - the blueprints of the cell. If the DNA of a cell is destroyed then the cell will die. If the DNA is damaged then incorrect repair can lead to carcinogenesis or, again, cell death. Thus this thesis aims to focus particularly on the changes, if any, to DNA.

DNA (Deoxyribonucleic acid) is formed from two chains of nucleotides, forming a helix, with bases (Thymine, Adenine, Cytosine and Guanine) at-

tached to them, each base binds to another in the opposite chain such that the two molecules seem as one. The specific sequence of the bases along the chain represents the stored information.

One problem when using FTIR methods to investigate cellular changes is that practically every significant band related to DNA in the acquired spectrum will overlap with other, often larger, bands caused by other molecules, in particular proteins. However these other molecules will typically have their own unique bands which are unrelated to DNA, which can be compared to those non-unique bands to determine what the change in absorption between two spectra, solely due to DNA, is. By comparing spectra and determining the change in intensity and shift in wavenumber of individual bands any changes to the conformation of DNA (as well as proteins and lipids) should be detectable.

## 1.4 Bacteria and Mammalian Cells

Although the aim of this thesis is to investigate the use of magnetic fields in radiotherapy for humans, bacterial cells were used instead of mammalian cells, for reasons explained later (refer to chapter 2) and so a brief comparison of bacteria and mammalian cells, in general, is given here. The differences between bacteria and mammalian cells are significant as can be seen in table 1.1. Mammalian cells are typically larger than bacterial cells as well as having a slightly greater percentage of dry cell weight made up of DNA and RNA. They can also have significantly less carbohydrates and are more complicated and compartmentalised than bacteria. This greater complexity leads to a longer gene sequence.

These differences in cell structure lead to differing general reactions to



Table 1.1: Rough guide to make up of bacteria and mammalian cells by Naumann [7]

radiation. Bacteria are typically much more resistant to radiation than mammalian cells, with potentially tens of times the dose required when irradiating bacteria to achieve the same biological endpoint as with mammalian cells irradiated with a smaller dose. This high radioresistance is probably due to the relatively simple nature of the cells in question.

Whilst mammalian cells are composed of many organelles, each with their own membranes - Mitochondria, Golgi apparatus, Rough and Smooth Endoplasmic Reticulum - bacteria are much less compartmentalised with no nucleus or membrane bound organelles. Also the DNA of bacteria is a double strand closed circle, rather than the linear double strand of mammalian cells as seen in figures 1.2 and 1.3.

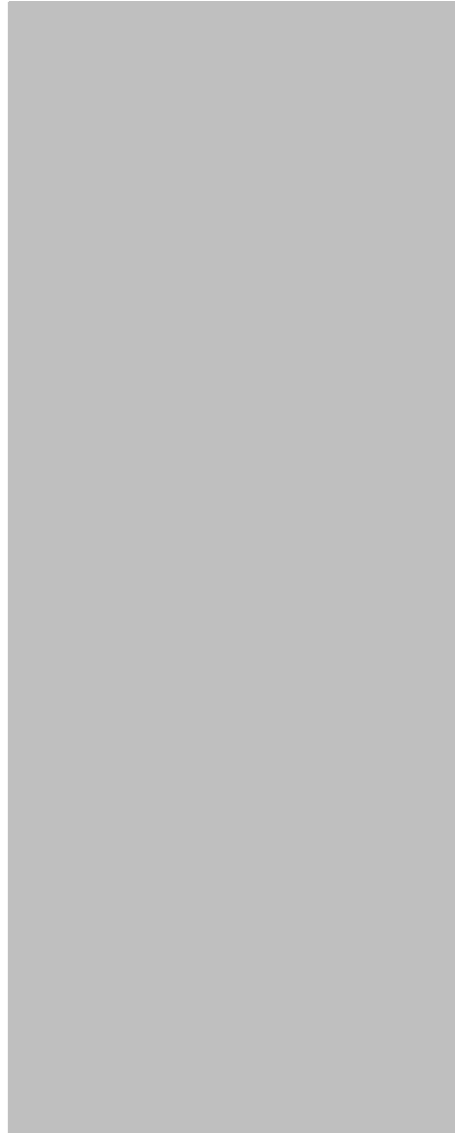


Figure 1.2: Mammalian DNA [17]



Figure 1.3: Bacterial DNA [17]

The type of bacteria used in this study was JM109 which is a non-pathogenic strain of *Escherichia Coli* typically used in biology labs. *E. Coli* has been studied in detail and is considered a model cell. Much research has been done into DNA repair and replication using *E. Coli* resulting in a greater understanding of how these similar mechanisms (and in many cases the same mechanisms) work in other types of cells, including mammalian cells.

It has also been found that carcinogenesis, as well as mutagenesis in general, in *E. Coli* is very similar to that in human cells. Though not identical it is close enough that studies using *E. Coli* can be related to mammalian cells in general and used to predict effects in human cells. For example carcinogenesis in both mammalian and bacterial cells (in particular *E. Coli*)

will be the result of the creation of pyrimidine dimers in the DNA sequence that are not repaired and result in changes to the cell that do not preclude further replication [25, 31].

And of course, although the overall conformation of DNA molecules in mammalian cells is significantly different to that of bacteria the composition is the same; a series of bases (Guanine, Thymine, Cytosine and Adenine as well as Uracil instead of Thymine in RNA) connected in a specific pattern to create genes. This means that the chemical bonds are roughly the same, in number, type and strength for any given length of DNA as can be seen in figure 1.4. This is, largely, what allows the comparison between mammalian cells and bacteria. Though it should be noted that there will be differences in the IR spectra of these two different types of DNA as the difference in overall structure, as noted previously, will lead to different bond strengths, and possibly even different bonds.



Figure 1.4: DNA Chemical Structure [17]

So while all external influences, be they mutagenic, carcinogenic or the various causes of cell death, may not induce exactly the same response in bacterial cells as they would in mammalian cells, the measured responses can still be useful in determining the response to the same stimulus in a variety of other cells or cell types in general.

It should also be noted that PK15 cells, which are porcine kidney epithelial cells, were also used infrequently (as availability was limited) to demonstrate the effect of a magnetic field on mammalian cells and to give a brief comparison to bacteria. Also U937, a human leukemic cell line, was used initially for some of the experimental verification and testing of equipment only as it was easily available at the time (early on in the testing phase), however later proved to be limited in supply.

## Chapter 2

# Experimental Methods

In this project cell samples were grown, irradiated under several different conditions and the resulting changes determined using biological (survival curves) and physical (FTIR Spectroscopy) methods.

FTIR spectroscopy was used because it could provide a wealth of data and the equipment was readily available. Though other types of vibrational spectroscopy can also provide complementary data. For example Raman spectroscopy can provide information about areas of molecules that FTIR can not.

When determining what type of cells to grow the specific requirements of each part of the project must be considered. As the irradiations were done at Wollongong Hospital ICCG and booked in advance a reliable source of cells must be ensured. Furthermore these cells must be able to provide information relevant to carcinogenesis and cell death in humans. Also the cell culture must be easily grown to a relatively high density for FTIR measurements to be taken, typically  $10^6$  cells/mL or higher is desirable - cultures of lower density may not provide an adequate beam absorption and result in readings that are essentially background. Also desirable, though not essential, is the ability to either continue growing the samples after irradiation or at least to

be able to store them in a state where they are not undergoing any significant change.

These requirements essentially ruled out using mammalian cells as the process of growing them is quite involved and they can be quite difficult to keep alive for anyone who is not experienced at the process. Also the problem of a relatively short lifetime of approximately less than a day once out of the incubator means that because some of the measurement processes can be quite time consuming even while data is being collected a significant amount of cell death may be occurring simply due to the environment the cultures are in.

Bacteria however are much easier and reliable to grow, and also easy to grow to high enough densities. Although they do grow best under specific conditions they can survive a much larger range of environments than mammalian cells typically can. Taking bacterial cell cultures out of the incubator for several hours should not result in any significant cell death simply due to typical environmental factors ('typical environmental factors' being temperature variations away from 37°C, growth medium being altered in composition - for example mammalian cells need a 5% CO<sub>2</sub> gas mixture to remain viable) that may change outside the confines of the laboratory. They can be easily stored in a cool room at around 0°C and be essentially in stasis with no significant cell activity.

However while they are easy to grow they will have a different reaction to radiation exposure than mammalian cells. Mammalian cell survival curves typically have a 'shoulder' at the start and then decrease at a faster rate, while bacterial survival curves are linear, (when on a Log<sub>10</sub> survival scale) as can be seen below in figure 2.1.

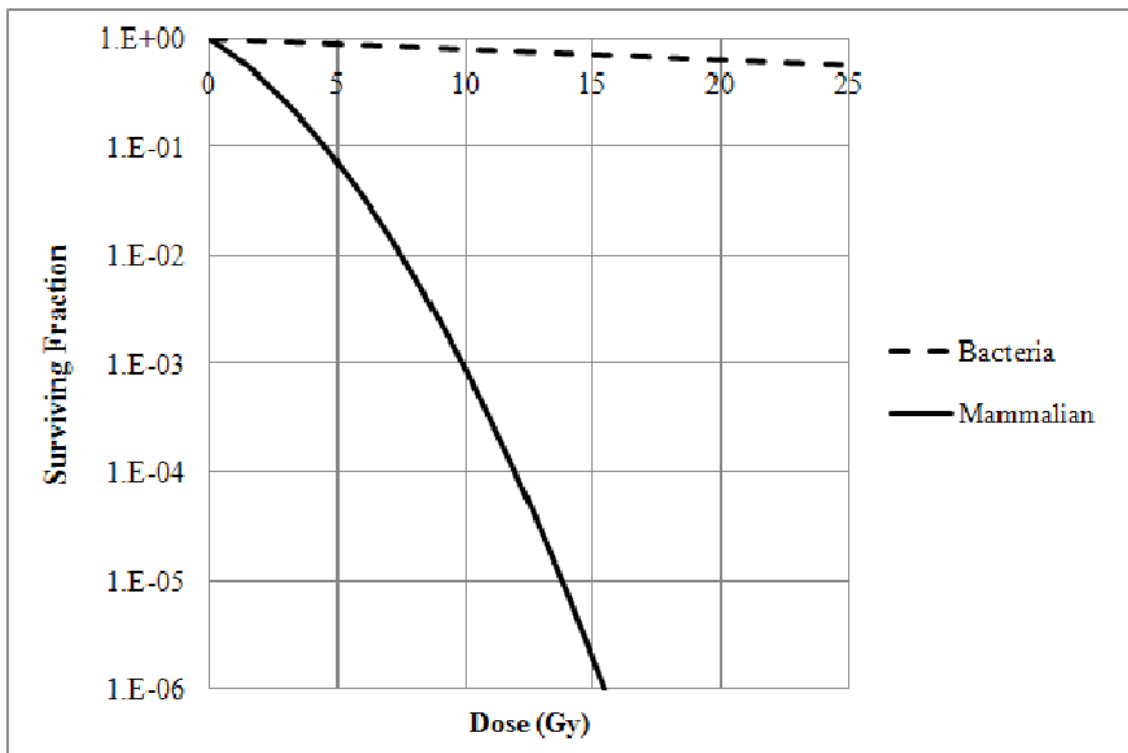


Figure 2.1: Typical survival curves of mammalian and bacterial cells

However this does not mean that any data taken from them will be entirely irrelevant, only that in some cases it will require interpretation. As mentioned previously it has been found that carcinogenesis in *E. Coli* is similar to that in mammalian cells and the effects of a magnetic field on DNA and other biologically relevant molecules should be similar if not identical.

For the above reasons *E. Coli*, in particular JM109, was chosen to be the primary cell culture used.

## 2.1 FTIR Spectroscopy / Vibrational Spectroscopy

Fourier Transform Infrared Spectroscopy is a technique that utilises the interaction of infrared radiation with matter to identify specific molecules. The radiation interacts with the molecular bonds, where some energy may be

absorbed by the bond causing the molecule to vibrate - hence vibrational spectroscopy - and resulting in a decreased intensity detected at that particular wavenumber. There are several different types of FTIR devices; the one used in this experiment was a rapid scan device using a globar source, KBr beam splitter and electronic detector.

### 2.1.1 Specifications of Spectrometer Used

Globar: a silicon carbide rod that is heated by running a current (approximately 55 amps) through it which causes it to emit a light spectrum approximating that of a black body radiator. It emits light in a range of about 200 - 10000  $\text{cm}^{-1}$ .

Beam Splitter: the beam splitter used was made of KBr which transmitted light in a range of 450 - 4000  $\text{cm}^{-1}$ . Unfortunately KBr is hygroscopic and so must be constantly kept in a vacuum.

Detector: a mercury cadmium telluride electronic detector was used, cooled with liquid nitrogen this had a range of 800 - 5000  $\text{cm}^{-1}$ .

Sample Holder: a simple but effective set up was used to hold samples within the sample chamber of the spectrometer. Basically two windows separated by an O-ring and held together by two washers clamped together with the sample between the windows so as to be protected from the vacuum in the chamber. The window material used was 80 $\mu\text{m}$  thick sheets of polypropylene. The spectrum of the empty sample holder can be seen in figure 2.2.

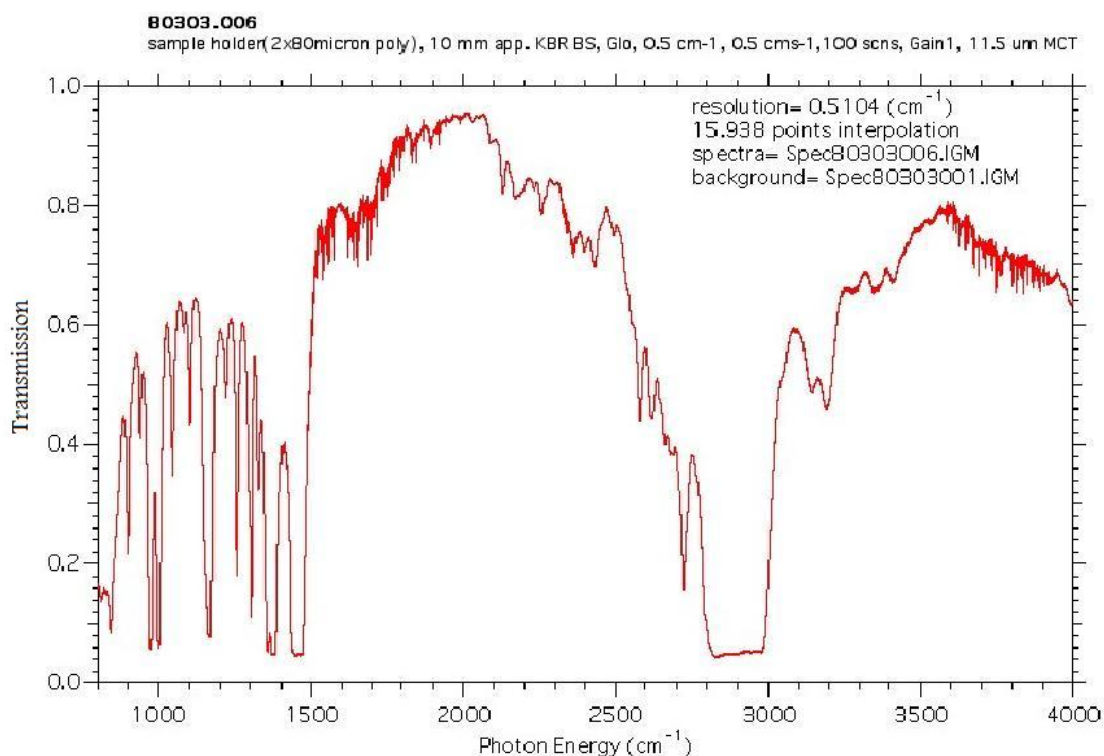


Figure 2.2: Spectrum of sample holder

### 2.1.2 Applications

One application that has enjoyed widespread success is that of identifying molecules and revealing something of their structure [1]. This can be done by recording the IR spectra of a sample and analysing the structure of it - the individual peaks can be identified using non-linear least square curve fitting and the resolution of the spectrum can be enhanced using convolution methods [1] while wavelet transformations can enhance the SNR [2], though other mathematical techniques can be used as an alternative. Each peak will correspond to a substance - whether protein, DNA, fat or water etc. enabling the identification and quantification of the components of the object being studied.

As Jung [1] has shown this can lead to the accurate identification of proteins, ligands and even individual side chains giving insight to the physical structure of the molecules investigated. As demonstrated the instrumental set-up is not overly complicated, requiring in its simplest form an interferometer, IR source and detector and control electronics, though this can become more complicated when accessories are used for other techniques like FTIR-ATR spectroscopy (Attenuated Total Reflection, note this paper will be primarily concerned with transmittance FTIR methods). Jung [1] provides an overview of both instrumentation and methods of analysis with a dual focus on the physical principles and the mathematics behind the technique. The author demonstrates how information provided by FTIR studies, in conjunction with mathematical analysis, can lead to the identification of recognition phenomena between different proteins and molecules in the human body and provide information on both the primary and secondary structure of proteins.

This is possible because of the structure of the molecule. As each type of molecule has its own structure it will produce individual peaks in the spectrum, each peak corresponding to a vibrational mode, though single peaks may not be unique to a specific molecule the combination of peaks for each type of molecule will be unique, thus allowing for identification from IR spectra. This is shown by Stehler et al. [3] where the vibrational modes of a molecule are described in terms of Hooke's law and the Franck-Condon principle. These provide a mathematical framework for the description of the interaction of IR radiation with a molecule. By comparing the simple two-body harmonic oscillator with a diatomic molecule and using Hooke's law the vibrational frequency of each mode of the molecule may be calculated - though as the structure investigated becomes larger and more complicated the calculations also increase in complexity. The authors also use

the Franck-Condon principle to explain the transitions and combinations between vibration levels that can lead to slightly altered or more complicated spectra. Note that interaction between the molecule and the IR radiation can only take place if the molecule has a vibrational mode that will allow an oscillating dipole moment - the dipole moment can change (though for the case of diatomic molecules there must be a non-zero dipole moment).

The main advantages of FTIR systems over regular IR spectroscopy is that FTIR devices can record data from all wavenumbers simultaneously rather than one channel at a time and also have an increased signal to noise ratio as all of the beam energy is directed at the detector all of the time rather than only one particular wavenumber/element of the beam as in a single channel spectrometer, this is called the multiplex advantage.

Another significant advantage of FTIR systems over other methods is the wide variety of sample types that can be used; typically anything from aqueous solutions to solids can be studied. Though sampling concerns are not as stringent as with some other methods problems may still arise.

There are several different methods for preparing samples for study by FTIR methods. The choice of which to use depends mostly on what type of material you are studying, whether it is a solid, aqueous solution, water content etc.

In this study liquid samples were chosen for use, this way the change to samples by the samples preparation process would be minimised: when using solid samples the process of creating a pellet or powder inevitably involves significantly altering the sample, in the case of cell cultures which already have a very complicated spectrum this could make analysing the spectra very difficult. Also by using living cell cultures in their natural form any effects on the medium they grow in may also be accounted for.

### 2.1.3 Aqueous Solutions

These can sometimes be easily and simply prepared by placing a drop of the sample on a window, placing another window on top of the drop and placing the resulting plate in the beam [6].

Issues can arise if there is too much absorption of the beam by the window so materials like KBr or CaF<sub>2</sub> can be used as windows. Both KBr and CaF<sub>2</sub> are transparent to a scope of wavelengths in the IR range (about 0.15  $\mu\text{m}$  [66600  $\text{cm}^{-1}$ ] to 9  $\mu\text{m}$  [1100  $\text{cm}^{-1}$ ] for CaF<sub>2</sub>) and so are useful materials that should have little effect on the measured spectrum.

KBr, however, is hygroscopic so it must not be exposed to enough water to damage it or alter the spectrum seen - this can even mean limiting its exposure to normal atmosphere. When using samples containing water it must be remembered that KBr is water soluble so desiccating the sample and mixing it with a gel or oil solution may be necessary, as long as this process does not damage or alter the sample significantly.

If there is too much water in the sample and the measured beam intensity is too low as a result then a smaller amount of sample may be used, or you can even try using D<sub>2</sub>O as a solvent instead of water (desiccate and re-hydrate with D<sub>2</sub>O) which will cause a small shift to lower frequencies in some of the bands, but not reduce the intensity [1]. These methods have been quite successful in producing a spectrum with good resolution (4 - 6  $\text{cm}^{-1}$ ) and high enough SNR to adequately analyse the data [7], so as long as you ensure that the preparation process used is readily reproducible it should be possible to do a quantitative as well as qualitative analysis on any sample.

When considering sample preparation the most important element is reproducibility. As long as you can keep the preparation technique constant most other elements (water or other vapour in the spectrum, spectral resolu-

tion, scanning time etc.) will have a minimal effect on the output. Particularly when working with grown cell cultures, the microbiological parameters (environment of incubation) will be of much greater importance than the sample preparation when trying to generate reproducible results [7].

Though a wide variety of samples can be used, as mentioned previously problems related to the sample type or method used can still arise. If the sample is too thick then very little of the incident energy will be transmitted, if particle size is too large then scattering of the beam may occur producing a sloping baseline [4], also shifting of some peaks in the resulting spectrum may arise as a result of re-hydration with another agent such as D<sub>2</sub>O [1]. All of these can have a significant influence on the final spectrum, and if they are not known and accounted for may result in incorrect analysis of the acquired data.

Though this is not always the case; sometimes these problems may result in changes that simply make taking a spectrum difficult. As discussed by Griffiths [8], if the transmittance of incident energy falls too low then a significant amount of noise may be observed in the spectrum. This can particularly be a problem with samples containing water, which has very strong absorbing bands around 3400 cm<sup>-1</sup> and 1640 cm<sup>-1</sup> and a weaker one at about 2120 cm<sup>-1</sup>, which are in the same region of the spectrum where several protein characteristic peaks are found, such as the Protein Amide I band. Though this will probably not become a problem until transmittance drops to very low levels - a transmittance of 1% can be sufficient to record a spectrum, though around this level and below extra noise may be observed. For this reason it would seem desirable to use very thin samples to produce a large transmittance, however there must also be enough of the sample such that absorbance is high enough to be measured by the detector [4], so a

middle ground for sample thickness must be found, with path-lengths of less than 15 mm through water desirable [8] and a thickness greater than 1 mm is suitable to ensure absorbance is high enough.

Of course once an adequate spectrum has been obtained it must be analysed. As mentioned previously this can be done using several different methods to increase SNR and resolution and then curve analysis methods such as factor analysis, hierarchical clustering, linear discriminant analysis and artificial neural networks can potentially be used for complex spectra in a diagnostic capacity [7,13].

These methods have been used successfully to study biological samples [1,3,7,12,13] as well as many other types of materials.

## 2.2 Growing Cell Cultures

The cells were grown in the Biology departments Bacterial Culture lab at Wollongong University using the following process.

LB (NaCL 10g/L, Yeast Extract 5 g/L, Tryptin 10g/L added to purified water) and Agar (NaCL 10g/L, Yeast Extract 5 g/L, Tryptin 10g/L, Agar 15g/L added to purified water) was mixed up and autoclaved (to ensure that they were sterile). Then the Agar was poured into petri dishes and stored in a cool room at approximately 0°C. Once the Agar had hardened a sample of JM109 was obtained from the Biology departments freezer and streaked onto two of the Agar plates (plating) which were then placed into the incubator at 37°C overnight. This was done in bacterial culture hoods in the Bacterial Culture lab which is a PC2 lab, so all material in potential contact with the bacteria were sterilised before and after use. One of these plates was used in the future to seed cultures and the other kept as a backup to use if the primary source became contaminated or in some way unusable. Whenever

they were not in use they were also stored in the cool room.

Seeding a culture is done by pouring a known amount (typically 20 mLs) of LB into a falcon tube and using a sterile toothpick to pick off one colony from the original Agar plates and placing it in the falcon, sealing it and leaving it in the incubator overnight (for a known amount of time - typically approximately 20 hrs). The following day the falcon can be removed and the relative optical density (OD) measured to determine the cell density.

Note that all work with JM109 was done in a PC2 lab under a bacterial culture hood to protect both the user and the culture. When transporting the cultures the 'Guidelines for the Transport of GMOs version 2.1 OGTR' were followed (though JM109 is not a GMO still the extra precautions against spills were easy to enact and so deemed suitable). This primarily entailed any samples being transported in both a primary and secondary air tight unbreakable labeled container.

## 2.3 Cell Irradiations

All irradiations were done at the ICCC at Wollongong Hospital.

Initial cell cultures were irradiated using the Orthovoltage X-Ray machine. The cultures were irradiated with a 250 keV beam at 12 mA and 68 MU/min = 68 cGy/min. The cultures were kept in small 5 mL sealable tubes. The sample setup varied according to what kind of magnet setup was used as the size of some of the magnets prohibited the samples being surrounded with water or other material to account for scatter.

These irradiations were done at 250 keV because that is the energy that is used to define RBE, which means that data from this may be more easily and reliably related to data from other sources.

Later cell cultures were irradiated using the Linac. The cultures were

irradiated with 6MV Gamma rays at  $600 \text{ MU/min} = 600 \text{ cGy/min}$ . The cultures were kept in the same small 5 mL sealable tubes as used previously. The samples were setup in a water tank and placed at a depth of 10 cm which meant they did not receive the maximum dose as indicated by the dose planning computer, but instead 66.1 % of that dose, as indicated by the depth dose curve below. Unfortunately this was not accounted for immediately and incorrect doses were put into the planning computer. As a result of this instead of irradiating samples to 10, 20 and 30 Gy as intended the samples were irradiated only to 6.61, 13.22 and 19.83 Gy.

Note that these irradiations were not done at 250 keV because a higher dose rate was desired which could not be achieved at such a low energy. The higher dose rate was used to test if there was any significant effect on the repair of cells when compared to low energy, low dose rate irradiations in a magnetic field.

In both cases permanent magnets were used to apply a static homogeneous magnetic field over some of the cell cultures during irradiation, while a dummy setup with the same shape and dimensions of the permanent magnets was placed around the cell cultures that were irradiated without an applied field. This was done to ensure that the dose was the same to both sets of cells as the magnet setup may cause a significant change in the amount of radiation reaching the sample due to scatter. This is because of the different absorption and scatter cross sections of the magnet setup compared to water.

A schematic of the experimental setup used can be seen in figures 2.3 and 2.4 showing the magnet and small cylindrical 5 mL sealable containers the cell cultures were kept in. The magnet setup was then immersed in water and irradiated.

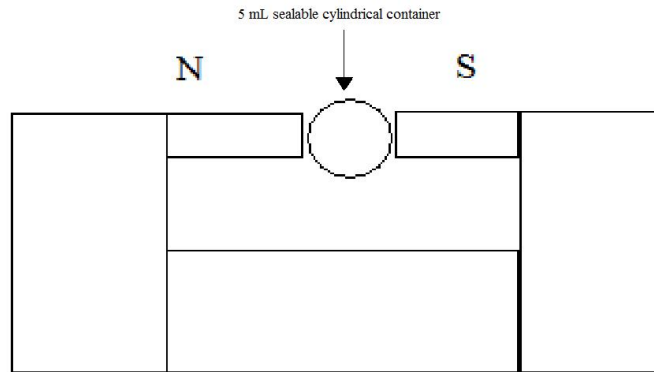


Figure 2.3: Schematic of experimental setup shown from side on view

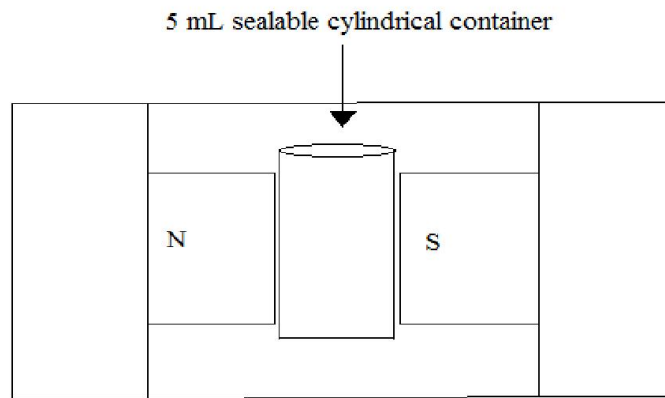


Figure 2.4: Schematic of experimental setup shown from top view looking down on setup

The experimental setup was the same for all irradiations, the only difference being that for the zero applied magnetic field irradiations the magnet setup was replaced with a dummy setup of the same dimensions. Also it should be noted that the magnets used initially were not available for all experiments, however even though other magnets were used the experimental setup was the same. The magnetic field strength applied was measured using a Daley Electronics Teslameter model T-22A which could measure the

magnetic field strength down to the mT scale. The error associated with measurements from this device was taken as  $\pm 2$  mT based on the smallest increment that could be measured and on observed variations in measurements.

Also a small strip of GaF film was used during the Linac irradiation placed in the dummy setup to determine if the dose was significantly altered by the presence of the dummy. Gafchromic EBT films were used as they were the only type of dosimeter readily available at the time. GaF film is a reliable and easy to use dosimeter, for more details on GaF film refer to [34]

## 2.4 Biological Data

Once cells had been irradiated they were transported back to the Bacterial Culture Lab at Wollongong University where measurements of the survival rates were taken.

Survival curves were obtained by growing samples of the irradiated cultures at different densities and comparing the growth rates.

All work with cultures here was done under a PC2 bacterial culture hood.

Firstly 180  $\mu\text{l}$  of sterile PBS was mixed with 20  $\mu\text{l}$  of cell culture in a sterile container, then 20  $\mu\text{l}$  of this mixture was added to 180  $\mu\text{l}$  of sterile PBS, and 20  $\mu\text{l}$  of that mixture was added to another 180  $\mu\text{l}$  of sterile PBS and so on. This way the cell culture sample was diluted by a known amount - the first part contained a 1 in 10 cell to PBS solution, the second a 1 in 100 solution and so on until the final one contained a 1 in  $10^{12}$  solution.

20  $\mu\text{l}$  of each of these solutions was then plated out and incubated overnight. This was repeated three times for each solution so that each cell density solution had three sets of data to obtain an average from.

The following day the plates were removed and the number of colonies

that had grown in each solution were counted, note that here several of the high density solutions had colony counts too high to accurately measure and other low density solutions had no colonies growing at all, this meant that an appropriate solution density must be chosen to represent that cell culture sample. In this case all the different samples (samples from cells that had been irradiated to different doses and under different conditions) had appropriate colony counts at the same density, meaning that the doses delivered were insufficient to cause even one Log cell kill (where one Log cell kill means a 10 % survival rate). This was desirable as any large difference in cell density of the cultures would make taking IR spectra difficult as the path length of the sample holder would have to be varied according to variations in density of the samples being measured. This would result in differing amounts of water in the beam and would effectively make comparing spectra from two different samples very difficult.

Now:

$$\text{Surviving Fraction of cells} = \frac{\text{Number-of-Colonies-in-sample-X}}{\text{Number-of-Colonies-in-control-sample}}$$

This was a simple but accurate way of measuring the survival rate.

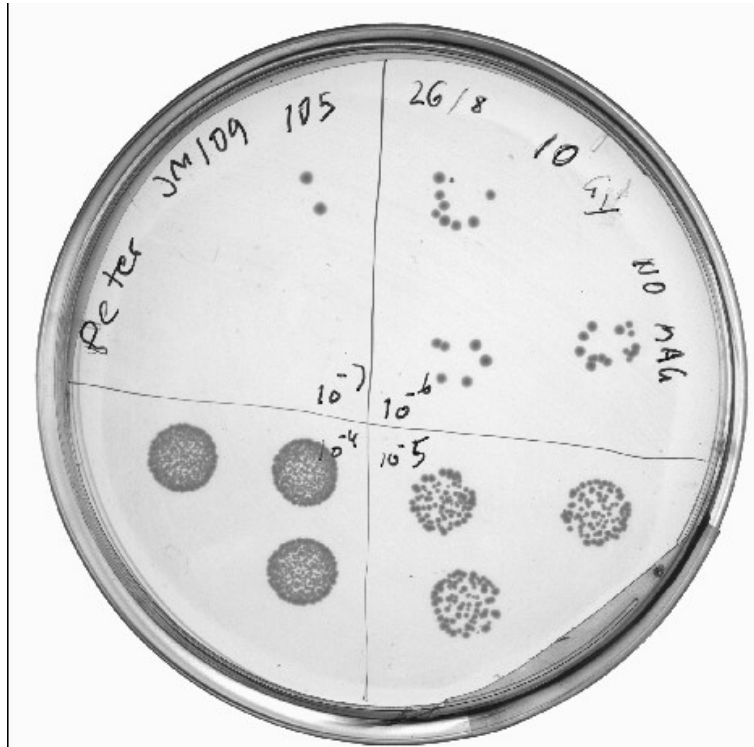


Figure 2.5: Plate of JM109 cell cultures grown from different densities to determine survival fraction

## 2.5 Physical Data

FTIR data was taken on the Physics Departments Bomem FTIR Spectrometer at Wollongong University.

First of all a sample holder had to be made as none were available, refer to figure 2.6. Spectra of potential window materials can be seen below in figures 2.7, 2.8, 2.9. The sample holder was essentially two windows separated by an O-ring and held together by two washers clamped together with the sample between the windows so as to be protected from the vacuum in the chamber. The window material used was  $80\mu\text{m}$  thick sheets of polypropylene. It was necessary to ensure the sample holder was air tight as the sample

compartment (as well as the rest of the spectrometer compartments) was kept under vacuum to ensure the beam was not absorbed significantly by air before it reached the detector.

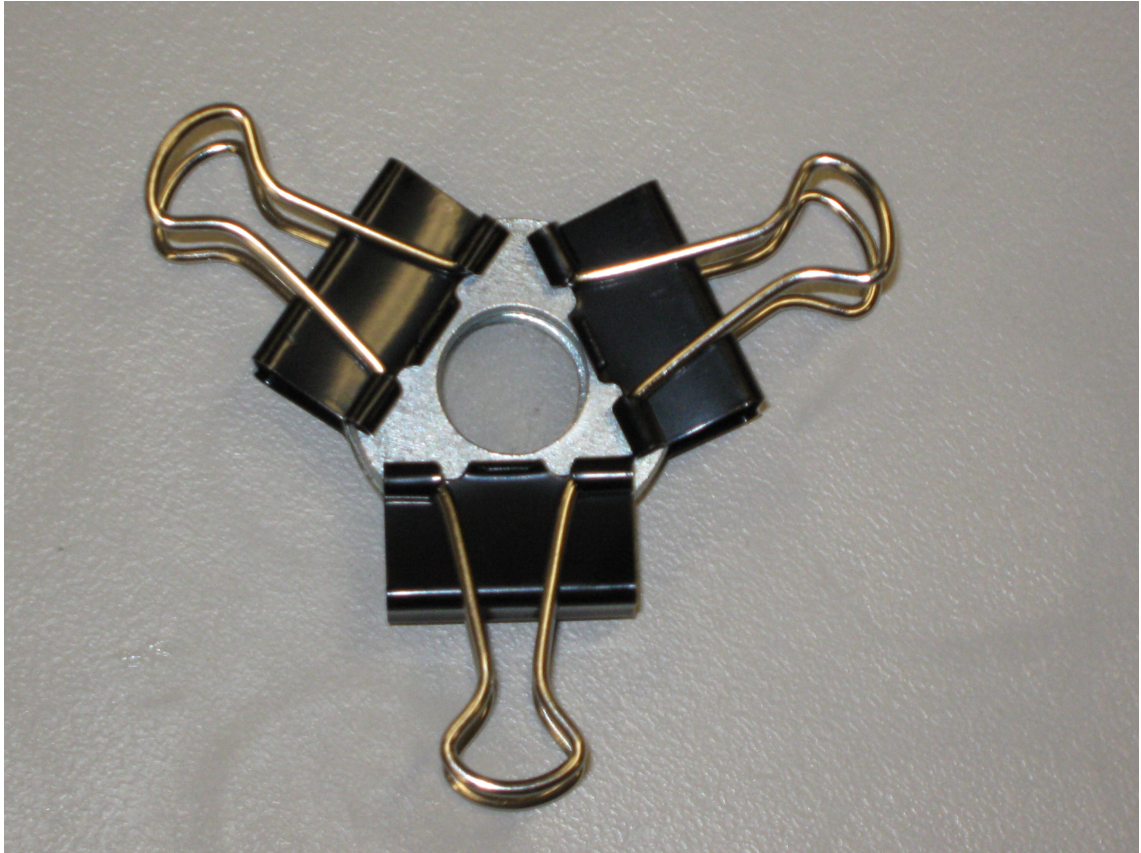


Figure 2.6: Sample Holder

Several window materials were tested including simple glass slides, different thicknesses of polypropylene, black polyethylene and mylar. Some of the sample spectra of these materials can be seen below.

Note that spectra in figures 2.7, 2.8, 2.9 have transmissions greater than 1.0, this is due to the gain settings used; as some materials absorbed a significant amount of light in some parts of the spectrum the gain settings of the spectrometer was set to double the incoming electronic signal, this is demonstrated by the transmission level of some of the spectra exceeding 1.0. This was done here to ensure a spectra was produced that had a transmission level throughout the spectrum significantly higher than the background and noise levels. The spectra in figure 2.10 had no additional gain applied.

As can be seen most of these have a high IR absorption in the wavenumber range of interest and so were unsuitable. Other materials like  $\text{CaF}_2$  and KBr that are often used in FTIR devices were considered and discarded without testing as they were deemed unsuitable,  $\text{CaF}_2$  because it cuts off transmission at around  $1200\text{ cm}^{-1}$  which is undesirable as there are several prominent peaks associated with DNA in the  $800 - 1500\text{ cm}^{-1}$  region of the spectrum and KBr because it is hygroscopic and the samples were liquid and contained a large amount of water (solid samples or other solution types could be used however as discussed earlier this results in a significant change to the spectrum and an inevitable loss of data). So while the final choice of  $80\mu\text{m}$  thick sheets of polypropylene may not be ideal as its spectra does contain several large peaks in the  $800 - 1500\text{ cm}^{-1}$  region and cuts off entirely from  $2800 - 3000\text{ cm}^{-1}$  it was deemed a necessary compromise as a lot of useful data can still be obtained from the rest of the spectrum without any need to process the sample in any way before measurement.

Once the cell samples were irradiated and ready for FTIR measurements a fixed amount of  $0.30\text{ mL}$  of the culture was transferred into the sample holder using a pipette to ensure accurate measurements. This was done under a bacterial culture hood. Then the sample holder was placed in the

sample compartment of the Bomem and with a suitable gain setting chosen measurements were taken.

It has been mentioned previously that FTIR methods are appropriate to observe physical changes induced in biological samples. To do this physical structures in relevant molecules must be readily observable in an FTIR spectrum. The spectrum in figure 2.11 is from Jung [1] and was produced using FTIR methods. It can be seen that there are clearly identifiable peaks throughout the 800 - 4000  $\text{cm}^{-1}$  region. In the spectrum of a whole cell these peaks and others can be used to identify DNA and proteins, while changes to the position or amplitude of these peaks helps in identifying physical alterations that have taken place.

For details of using the Bomem FTIR Spectrometer refer to Appendix A.

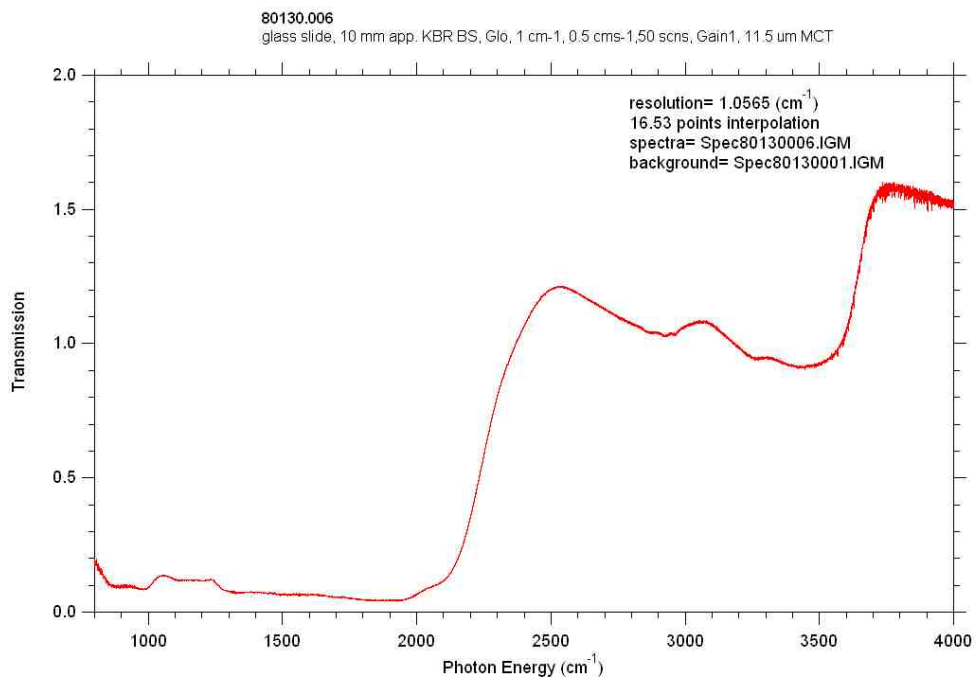


Figure 2.7: Glass Slide

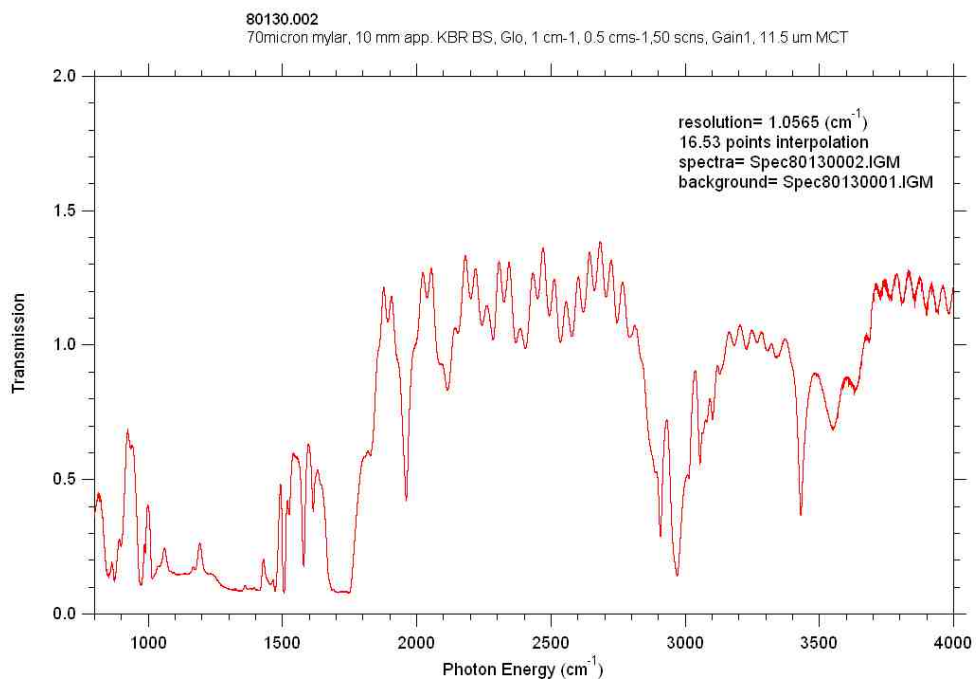


Figure 2.8: 70  $\mu$ m thick Mylar

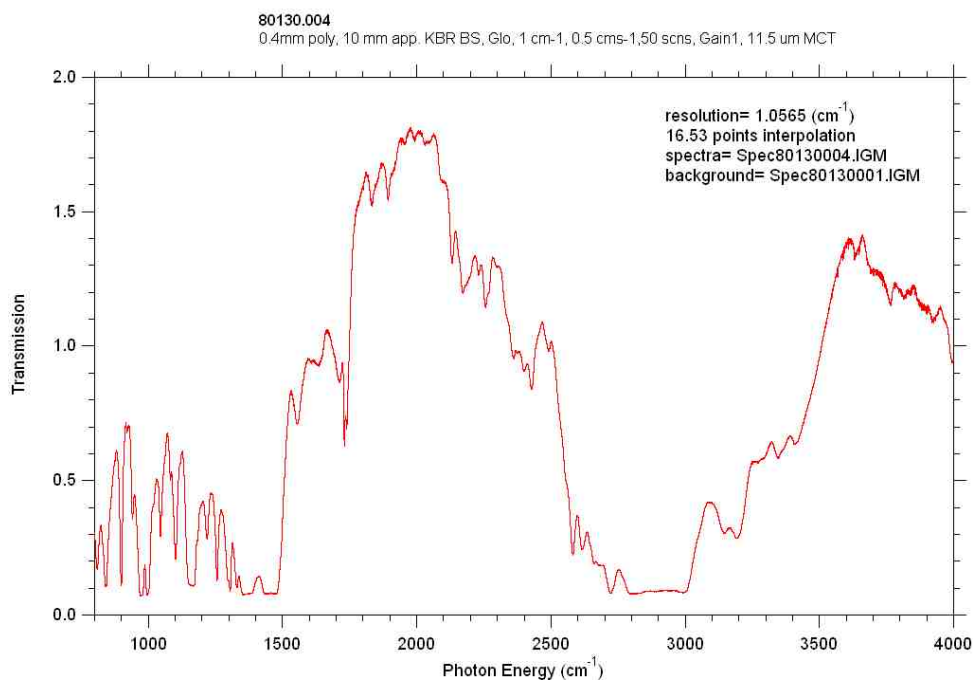


Figure 2.9: 0.4 mm thick Polypropylene

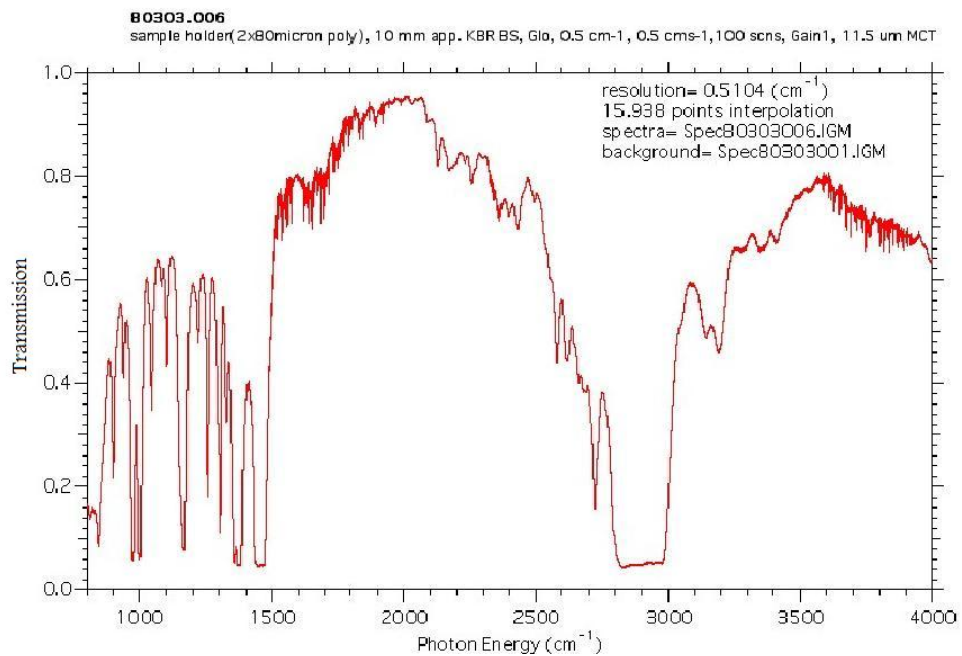


Figure 2.10: Spectrum of empty sample holder with two sheets of  $80\mu\text{m}$  thick polypropylene

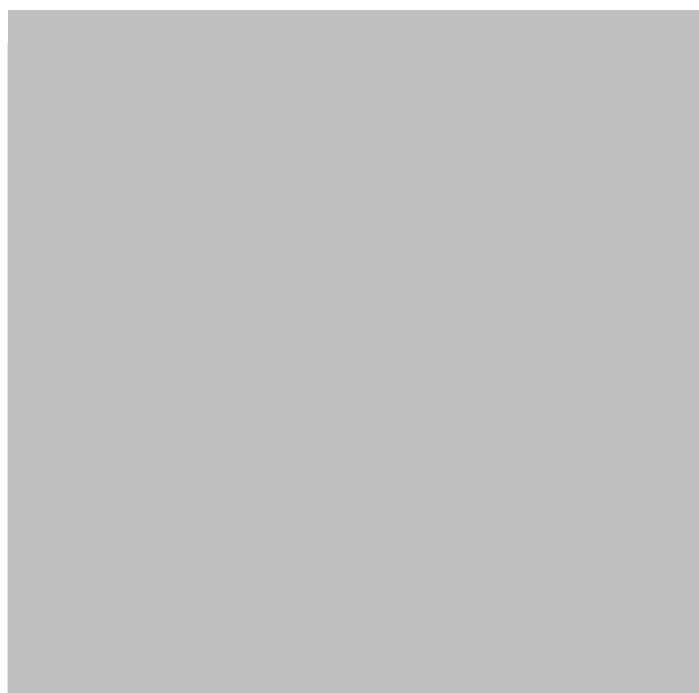


Figure 2.11: Figure from Jung [1] (referenced as Figure 2. in Jung) showing the IR spectrum of both protein and water. Changes to clearly identifiable peaks observed here are used to identify physical alterations induced.

# Chapter 3

## Results and Analysis

This chapter is divided into several parts with a preliminary data section detailing all the verification information and experimental sources of error.

### 3.1 Preliminary Data

The first data taken on the Bomem was used to verify that the sample holder was working - air tight, sample thickness was suitable, no significant noise introduced.

#### 3.1.1 Noise

A culture of cells was obtained from the Biology department with the help of Dr Mark Wilson. The culture used was U937 which is a human leukemic cell line.

As can be seen in figure 3.1 there are interference fringes with an approximate period of  $100\text{ cm}^{-1}$  throughout the spectra. This feature is due to interference of the beam in the sample; something in the sample or sample holder is causing interference or multiple reflections in the beam which is leading to the observed interference spectrum in the sample spectrum. This

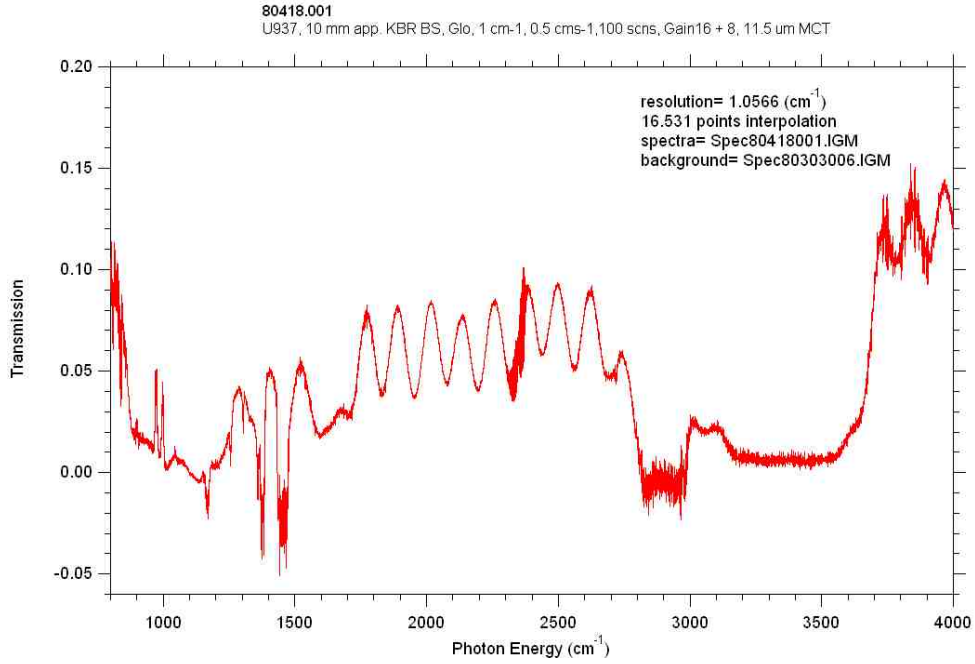


Figure 3.1: U937

phenomena is commonly observed in spectra where thin or flimsy windows are used [8] and is a result of parallel sided objects (windows) in the beam.

This feature in the spectrum is essentially noise and as it is of a large enough amplitude, of the same order of magnitude as peaks from the sample, it should be removed.

The pattern observed is essentially only a single frequency, which means that it will appear as an anomalous peak, at a position corresponding to its frequency, on either side of the main peak in the interferogram. This can be seen in figure 3.2.

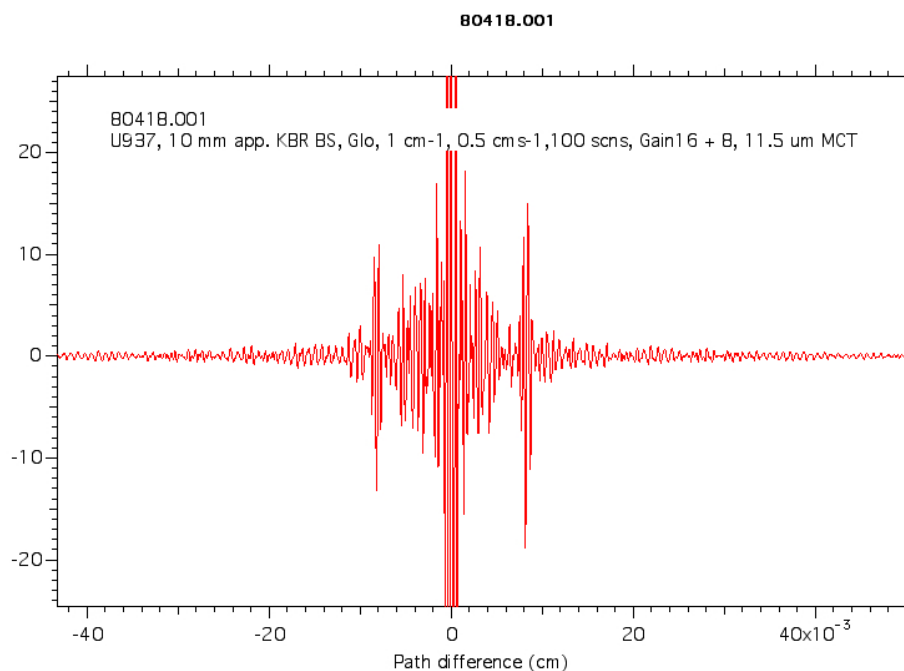


Figure 3.2: U937 Interferogram

If these anomalous peaks are removed, points set equal to zero on the interferogram, and the fourier transform taken then the interference fringes will be removed without significantly altering the rest of the data, though any features in the sample spectrum at this frequency will be lost, as seen in figures 3.3 and 3.4. Fortunately this was a fairly narrow band in the interferogram and would result in little data lost.

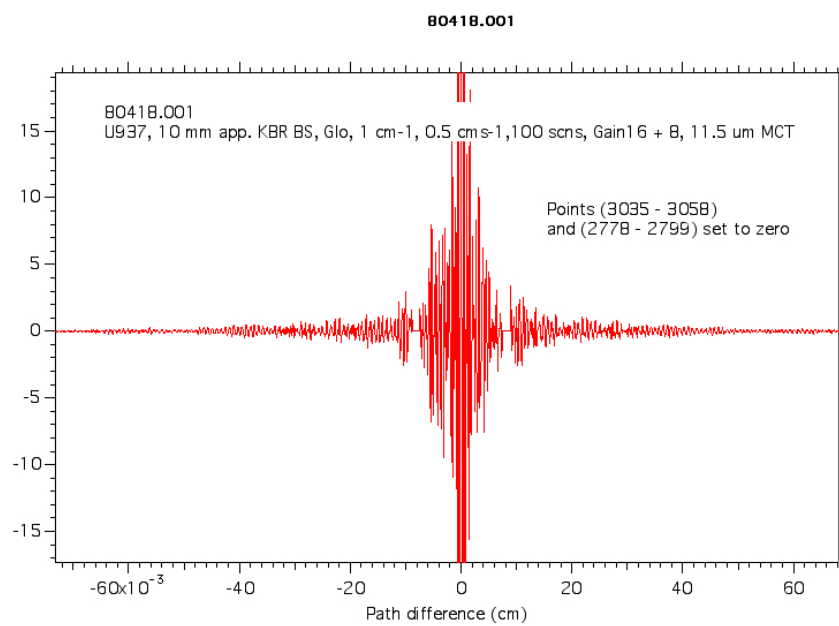


Figure 3.3: U937 Edited Interferogram

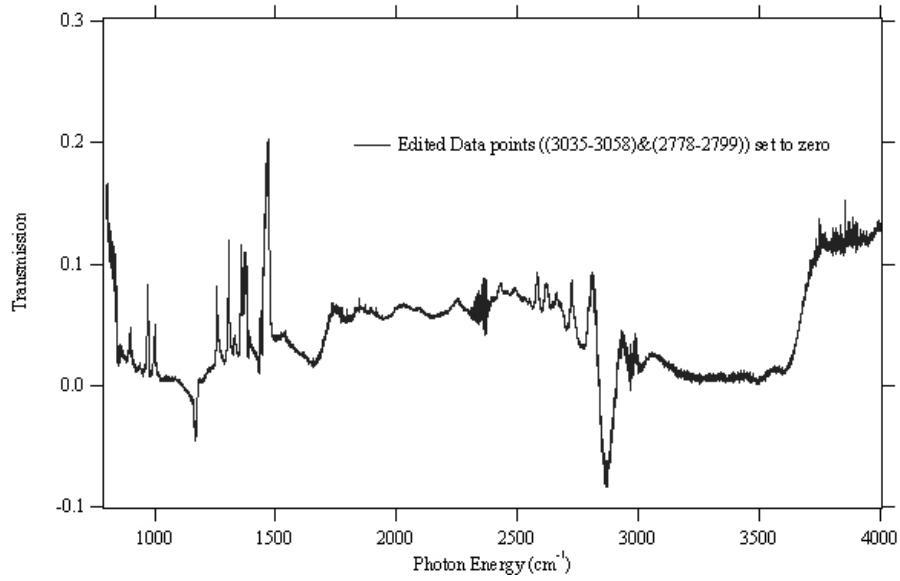


Figure 3.4: U937 Edited Spectrum

However care must be taken, when altering an interferogram, not to actually permanently remove points as this results in alterations to all of the frequency information following those points. Instead the data points must be set to zero but left in place. This does introduce a low level of noise to the final spectrum, however it can be seen in figure 3.5 that the amplitude of the introduced noise signal is small enough that it is acceptable. The noise can be seen as a sine wave of relatively small amplitude in the outer areas of the spectrum.

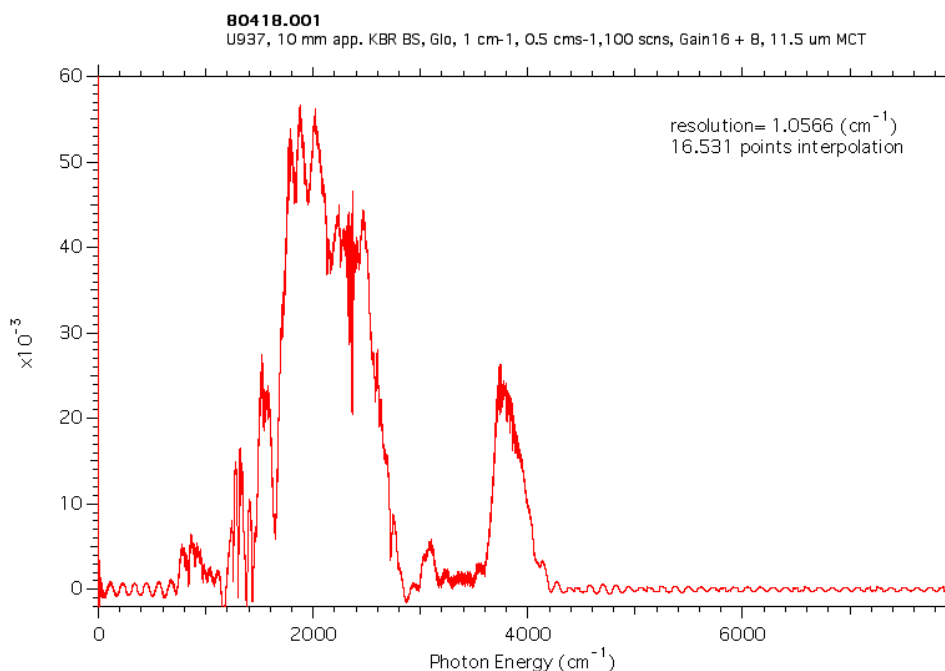


Figure 3.5: U937 Edited Spectrum Noise

Also there may be some significant noise in the spectrum if too few scans are taken - there will not be enough data to average over. For noisy spectra more scans are taken to average over so that a spectrum with transmission levels significantly greater than background noise may be obtained as the signal strength will increase directly in proportion with the number of scans and the noise, being random, will increase as the square root of the number of scans giving a signal to noise ratio:

$$\begin{aligned}
 S/N &= \frac{n}{\sqrt{n}} \\
 &= \frac{n}{\sqrt{n}} \times \frac{\sqrt{n}}{\sqrt{n}} \\
 &= \sqrt{n}
 \end{aligned}$$

Where  $n$  is the number of scans.

As has been mentioned before the window material used had several regions of very low transmission. These regions were therefore not useful when

analysing data as any small variation in the detected beam intensity in these regions, for whatever reason, would cause an inappropriately large change to the apparent transmission when the ratio of the spectrum was taken to remove the background. This can be seen in figure 3.6 below which shows the ratio of two empty sample holder spectra. This means that if the sample holder spectra remained constant a flat horizontal line would be seen at a transmission level of 1.0. Any deviation from this line will highlight variation in transmission through the sample holder.

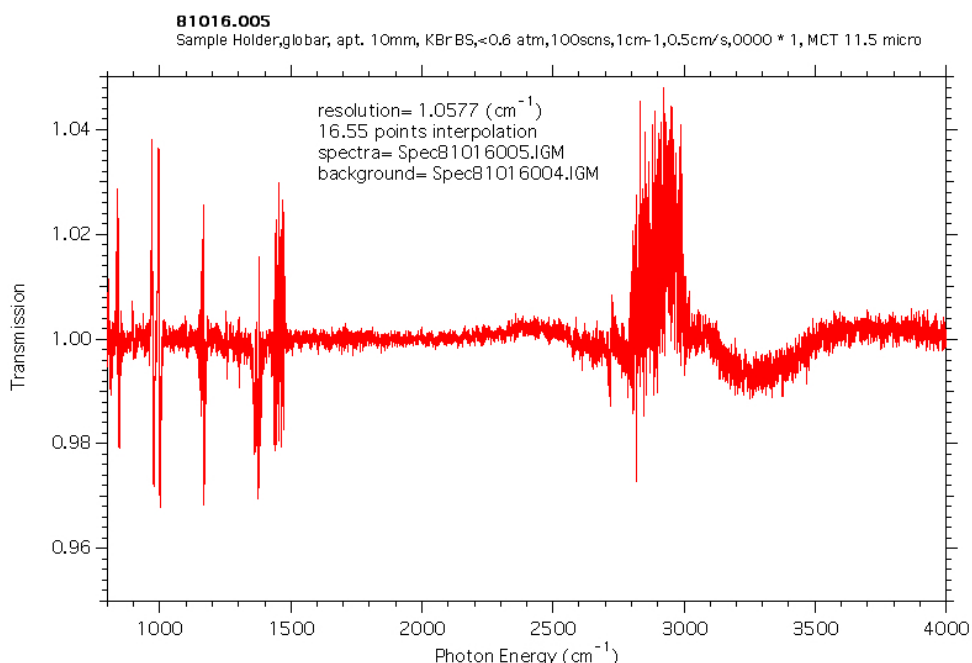


Figure 3.6: Variation in low transmission window regions

The 7 peaks/regions showing large variation in transmission (6 in the 800 - 1500  $\text{cm}^{-1}$  region and the last as the entire region from 2800 - 3000  $\text{cm}^{-1}$ ) are of no use when analysing further results. Though the peaks observed seem quite large they can in fact be the result of only a small change in transmission in those areas. As mentioned previously, in those areas as the transmission is very low any small variation in transmission that would be

insignificant in another part of the spectrum may in these areas cause a very large peak in the spectrum.

### 3.1.2 Peak Identification

Some of the features of the spectrum of cells are quite broad, spanning several tens of wavenumbers. However if a peak is narrow, around  $1 - 5 \text{ cm}^{-1}$  say, then identifying whether or not the peak has shifted in position under different conditions can be difficult unless you have accurate information on peak position. This information can be obtained by taking the second differential of the spectrum, the maxima in the differential will correspond to minima in the sample spectrum (points of absorption).

The second differential can be taken using the program IGOR (refer to Appendix C), an example is shown below.

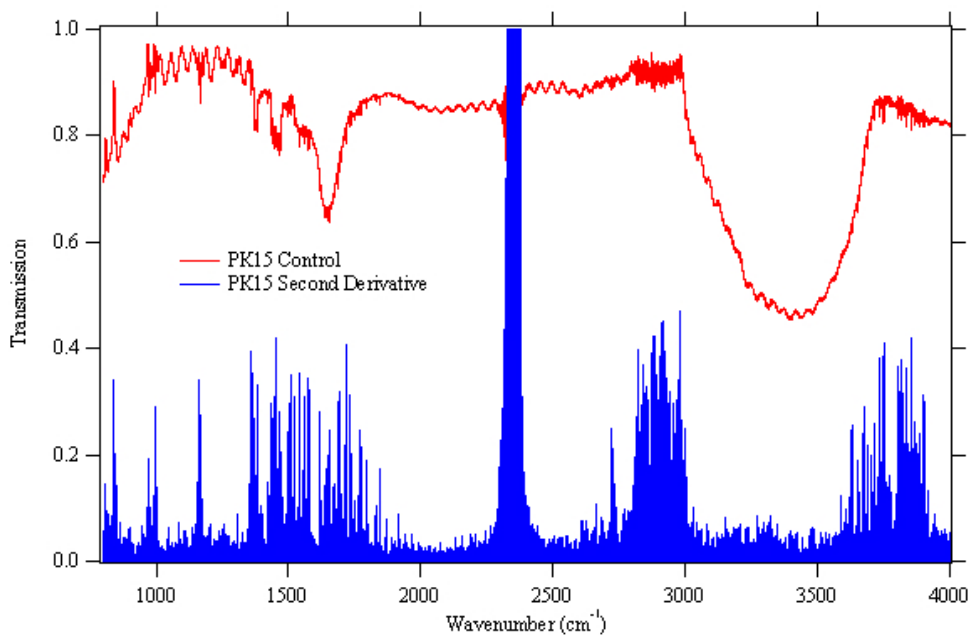


Figure 3.7: PK15 and Second Derivative

As can be seen in figure 3.7 when there are high frequency changes in the

sample spectrum the second derivative has large peaks, this can help with peak identification.

Now to identify each feature of the spectrum several sources were used [20,21,22,23,24]. The primary molecules of interest in biological samples are, generally; DNA, Proteins, Lipids and Carbohydrates. Of course water was also observed as it has a very high absorption in the mid-IR spectrum.

The main peaks for water were easily found at around  $1640\text{ cm}^{-1}$  and seen as a large region of absorption from around  $3000\text{ cm}^{-1}$  to  $3700\text{ cm}^{-1}$ . The peak at  $1640\text{ cm}^{-1}$  is also partly due to C=O stretching in the protein amide I band. Also DNA bases can have significant peaks in the region from  $1610\text{ cm}^{-1}$  to  $1670\text{ cm}^{-1}$ . This means that this wide, large peak is due to several important types of molecules. To determine how much of the absorption in that region is due to each type of molecule we must compare that region with other peaks as mentioned in the theory section earlier.

### **3.1.3 800 to $1500\text{ cm}^{-1}$**

This region of the spectrum is known as the 'fingerprint' region. It contains many of the peaks related to DNA, though they are generally fairly small in amplitude compared to peaks from other molecules they can still be observed.

As seen previously the sample holder spectrum does have several large peaks in this region with absorption greater than 95% in some of them rendering those areas useless.

This means that any data at these points must be ignored as the transmission is so low that it typically results in a large amount of noise in the spectrum at these wavenumbers.

### **3.1.4 Large peak around 1600 to 1700 $\text{cm}^{-1}$**

As mentioned, water will be the greatest cause of this peak.  $\text{H}_2\text{O}$  has a very strong absorption band in this region. The Protein Amide I band will be the next largest contributor with a strong absorption at  $1640\text{ cm}^{-1}$  and smaller than both of these will be some relatively small contribution from DNA. Though this wide band does also contain several smaller peaks.

The contribution from DNA will be due to the bases, mostly Guanine and Cytosine, and may be as small as 1 - 5 % of the amplitude of the absorption due to water. This means that looking at the variation in amplitude of this large band will probably tell us very little about changes to DNA as such a small part of the band is related to DNA and it is difficult to determine changes in amplitude with such accuracy. However looking at variation in peak position may give us some information about whether or not the chemical bonds themselves were altered.

### **3.1.5 Small Peaks Around 1700 to 1900 $\text{cm}^{-1}$**

There are several small peaks in this region that primarily belong to DNA and lipids. Though these peaks are quite small compared to others in the spectrum they are large enough to be used reliably in peak analysis however the small amplitude makes determining the exact contribution of each individual type of molecule difficult, however as they typically have small absorptions of comparable magnitude in this short region it is assumed, at least for control samples, that the DNA and lipid contributions are approximately equal.

### **3.1.6 2300 to 2400 $\text{cm}^{-1}$**

This region of the spectrum has many peaks of small to medium amplitude close together, this is due to  $\text{CO}_2$  in the sample and is probably of little interest.

### **3.1.7 Large Absorption from 3000 to 3700 $\text{cm}^{-1}$**

This is largely due to water, there will be some small contribution from other molecules including DNA, protein and lipids however they will be much smaller than the contribution of water. Thus this area of the spectrum will mostly be referred to as a water absorption region.

### **3.1.8 DNA Peaks**

Peaks from DNA that were observable were related to either bases (Adenine, Thymine, Guanine, Cytosine) or the 'backbone' (the backbone consisting of deoxyribose and phosphate molecules). These were most prominent in the 800 - 1500  $\text{cm}^{-1}$  region as already mentioned and will be analysed in more detail later if required.

### **3.1.9 Verification**

To be able to compare spectra from two different biological samples that have been treated differently (for example irradiated under different conditions) it must be determined whether or not there is any significant variation to the spectrum of multiple samples taken from the same culture.

Below in figure 3.8 are several spectra taken from different samples of the same cell culture.

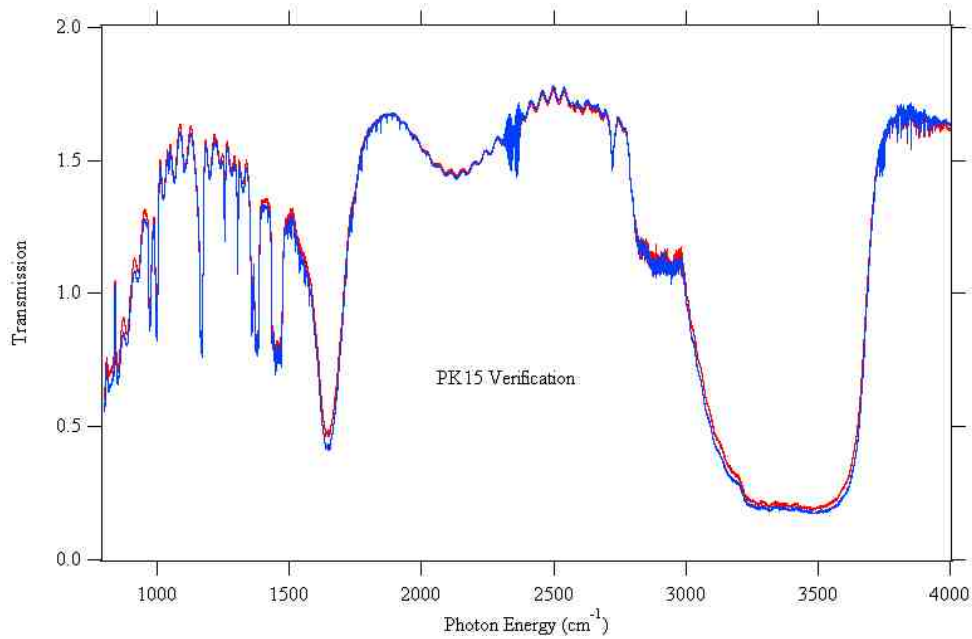


Figure 3.8: Verification - two samples of PK15 cells from the same culture

It can be seen from figures 3.8 and 3.9 that while there may be some changes to some peak intensities when samples are taken from different cultures the peak positions are the same, and when the spectra are taken from the same culture there is typically little variation in peak intensity.

This is to be expected as while all cultures were grown under as similar conditions as possible any small variation in the growth process may lead to slight variations in the samples content. For example *E. Coli* typically have a doubling rate of between 0.6 and 2.5 doublings per hour when incubating [25], so any variation to the incubation time will lead to variation in the density of cells in the culture, which would show as a difference in absorption in the IR spectra. Also the conditions under which the cultures are kept while being transported between labs or locations may vary slightly, particularly in the amount of time the cultures are kept away from the lab due to transport times or measurement times - irradiations took anywhere from 30 minutes to

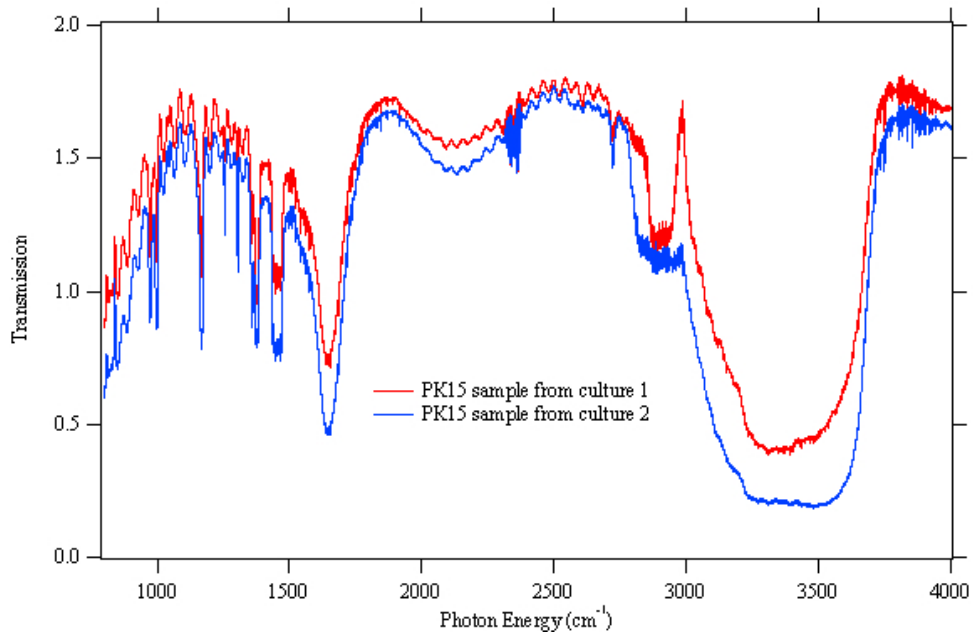


Figure 3.9: Verification - two samples of PK15 cells from different cultures

2 hours depending on the number of samples irradiated and the dose rates used. Also sometimes taking spectra can be time consuming as a single spectrum can take from 5 to 30 minutes depending on the amount of noise in the spectrum.

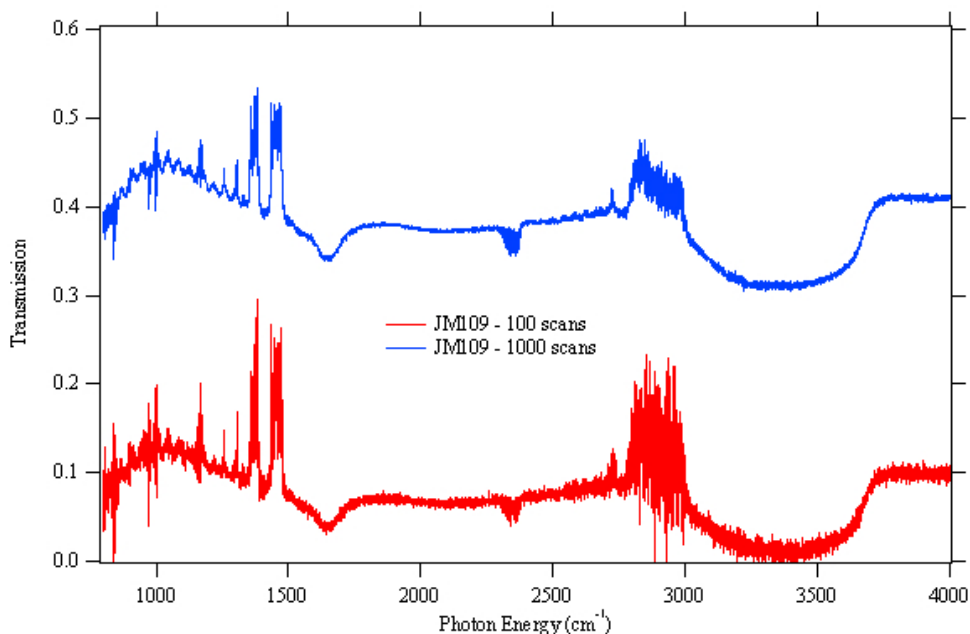


Figure 3.10: Variation in the number of scans taken impact on S/N ratio. Note the 1000 scan spectrum has been offset by 0.3 units from the bottom axis

All these small differences in the treatment of various samples may lead to differences in the IR spectrum of these samples. This means that care must be taken when comparing spectra from different samples, and that samples taken from the same culture must be kept together to ensure one does not endure different conditions to the others. However as long as this is done there should be no problem comparing samples taken from the same culture.

Essentially all of this means that while spectra may be compared from samples of different cultures, or samples from the same culture the results obtained will likely not be identical. Though trends should be easily observable whether they are alterations to absorption or peak position. It should be noted that all direct comparisons of results in this thesis are from samples from the same culture.

## 3.2 Low Dose Rate Irradiations

Samples of JM109 were irradiated using the Orthovoltage X-Ray machine at Wollongong Hospital. The survival rates are shown below. The magnet setup used had a field strength of  $0.667 \pm 0.002$  T for the gap width used. The variation in magnetic field strength over the sample volume is not known as the detector could not easily and reliably measure accurately over such small distances, the width of the sample in the beam was approximately 1.0 cm. However as the gap was reasonably small there should have been minimal variation in the magnetic field strength. Cell samples were irradiated to 10 Gy or 20 Gy.

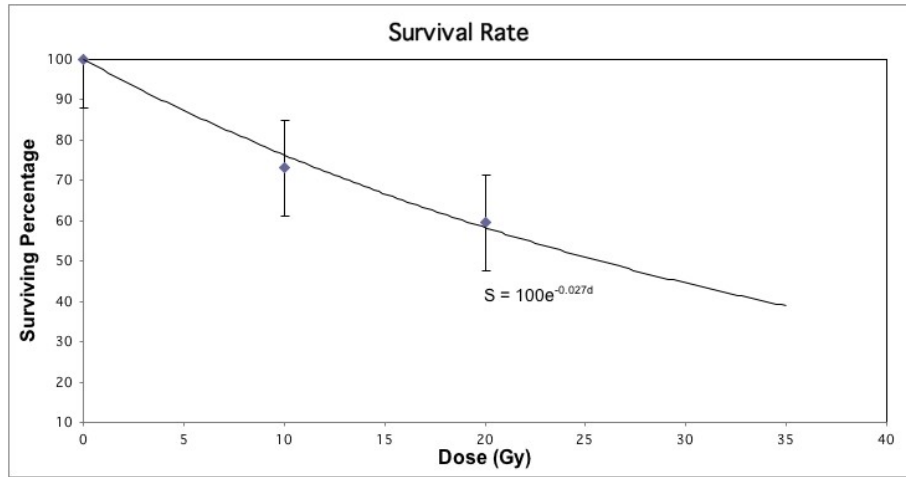


Figure 3.11: JM109 Survival Rates - Initial Orthovoltage Irradiation

Note the different survival rates of JM109 control sample compared to the first irradiations are due to differing setups in each case. Initially the samples were placed in a bucket full of water to allow for scatter, however in the second case this was not done as the relatively large magnet setup was too big to fit in a bucket or available container. This meant that the amount of scattered radiation reaching the sample would have been very different in

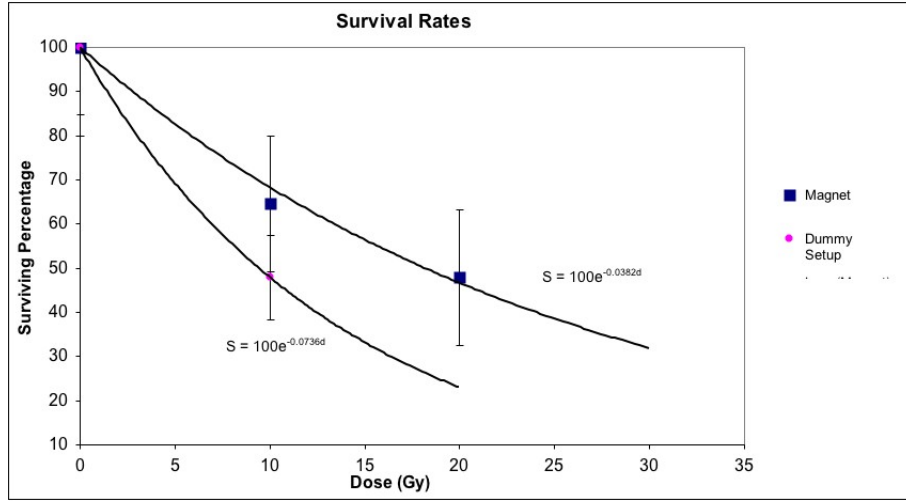


Figure 3.12: JM109 Survival Rates - Orthovoltage Irradiation with magnet setup

the two cases and the doses given in the second set of data when the magnet and dummy setup was used will be inaccurate.

Though there are not enough data points on the graphs in figures 3.11 and 3.12 to produce survival curves with acceptable accuracy over a useful range, the curves still serve to emphasize the difference in survival rates of different irradiation conditions.

It can be seen from the scatter and absorption cross sections for water and neodymium (material the magnet was made of) that the magnet would have attenuated the radiation at a rate approximately 100 times higher than water and there would have been around 1000 times as much scatter [26]. As a result of this the dose to the sample volume from scattered radiation would have been higher when the magnet or dummy setup were placed around the sample. Also the container used to hold the cell culture sample was not full and the air gap would have caused a small offset to the delivered dose, this was compensated for by increasing the delivered dose by 2.8% so to deliver 10 Gy the computer was set to  $10 \times 1.028 = 10.28$  Gy. This geometric factor

can be determined from the size of the air gap which was determined when the container was filled.

The data from the initial irradiation, in the water, matched reasonably well with the expected survival rates found by Sommers and Rajkowski [27].

The higher survival rate when the magnetic field was applied was not expected. This may be related to the low dose rate used. As *E. Coli* is much more radioresistant than human cells higher doses had to be used, however at such a low dose rate of only 68 cGy/min this took quite some time - around 15 mins for a dose of 10 Gy and 30 mins for a dose of 20 Gy. As *E. Coli* has quite a fast half life for SOS repair of DNA - only around 30 mins [28] - this means that there may have been a significant amount of repair going on even as the irradiation was taking place. The relatively fast repair rate is probably due to *E. Coli* generally having a faster metabolic rate than mammalian cells, rather than more efficient repair mechanisms or more repair enzymes. In evolutionary terms *E. Coli*, and bacteria in general, may have increased survival benefits from DNA mutation, unlike mammalian cells where mutation is very undesirable and will often lead to cell death. As SOS repair is error prone, at least compared to other types of DNA repair, this means that it may lead to a significant amount of mutation. However this will be less likely to lead to cell death in *E. Coli* compared to mammalian cells. This preference for mutation to occur will also mean that *E. Coli* is likely to have less error checking involved in its normal and SOS repair mechanisms compared to mammalian cells, which again would lead to a higher rate of mutation, though as already discussed mutation in bacteria does not necessarily lead to cell death as in mammalian cells. It should also be noted here that there is a 'kick-off' time of around 20 mins from when irradiation begins to when the first repair starts.

This means that if repair mechanisms were activated during irradiation and repair was taking place then both the repair activation mechanisms and the repair mechanisms themselves may have been altered by the magnetic field.

However as the kick-off time is longer than the period of irradiation for the low dose (10 Gy) irradiation this would seem to indicate that the field was not affecting the actual repair mechanisms, or at least not directly. Though it could still cause some change to the activation mechanisms for SOS repair and hence the kick-off time.

Though there seemed to be some effect it is not easy to determine from this data alone what it was. However there was also FTIR information from the Bomem to consider, as can be seen below.

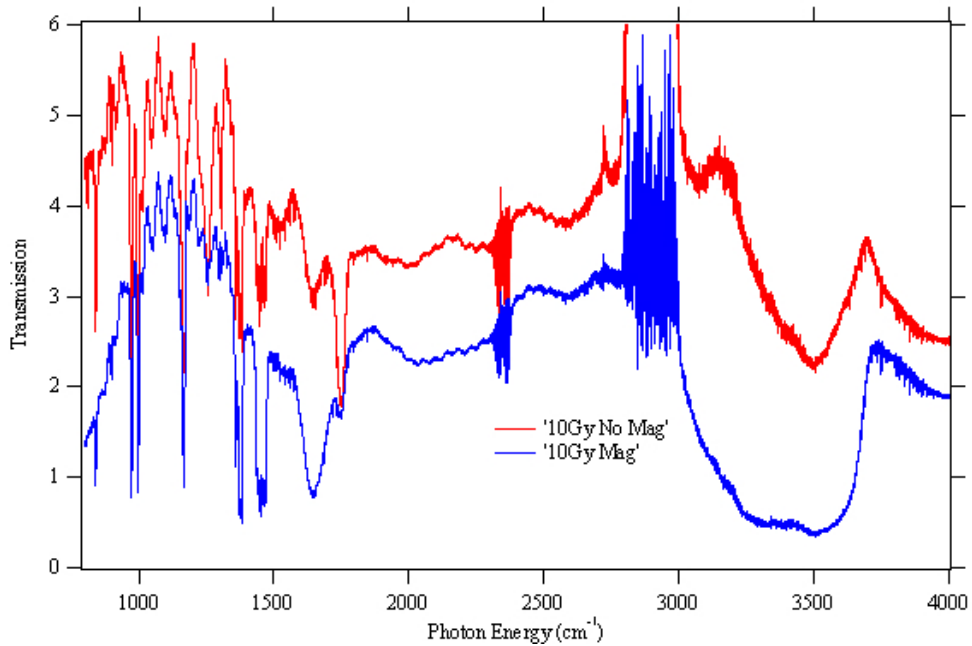


Figure 3.13: JM109 Magnet Effects on Irradiation

As can be seen from figure 3.13 the overall absorption of the two spectra are significantly different, this is a direct result of the different survival rates, with the '10 Gy Magnet' sample generally having a lower transmission - thus higher absorption - as there are more living cells to absorb the IR radiation.

However it can also be seen that some peaks have significantly different absorptions, perhaps most notably the peak around  $1750\text{ cm}^{-1}$ , which may mean that some molecules or cell structures were affected more than others by the introduction of the magnetic field.

The peaks and areas of the spectrum that were altered more than normal were:  $3000 - 3700\text{ cm}^{-1}$  (water absorption region),  $1500 - 1800\text{ cm}^{-1}$  (primarily water and protein absorption),  $800 - 1500$  (many of the observable DNA peaks are in this region).

### 3.3 High Dose Rate Irradiations

Samples of JM109 were irradiated using the Linac at Wollongong Hospital. The magnet setup used had a field strength of  $0.352 \pm 0.002\text{ T}$ . Cell samples were irradiated to 6.61, 13.22 or 19.83 Gy.

The setup used can be seen below in figure 3.14.

Physically the differences in irradiation setup were that a higher beam energy and a higher dose rate were used. Also the magnetic field strength was different as a different magnet setup was used - the one previously used was no longer available. This difference in magnetic field strength makes comparing the effects of a higher dose rate difficult as any changes may be as a result of any combination of these factors and determining which ones were or were not involved may not be possible.

Though the irradiation setup was different the samples themselves were as similar as possible, the same containers used containing the same amount

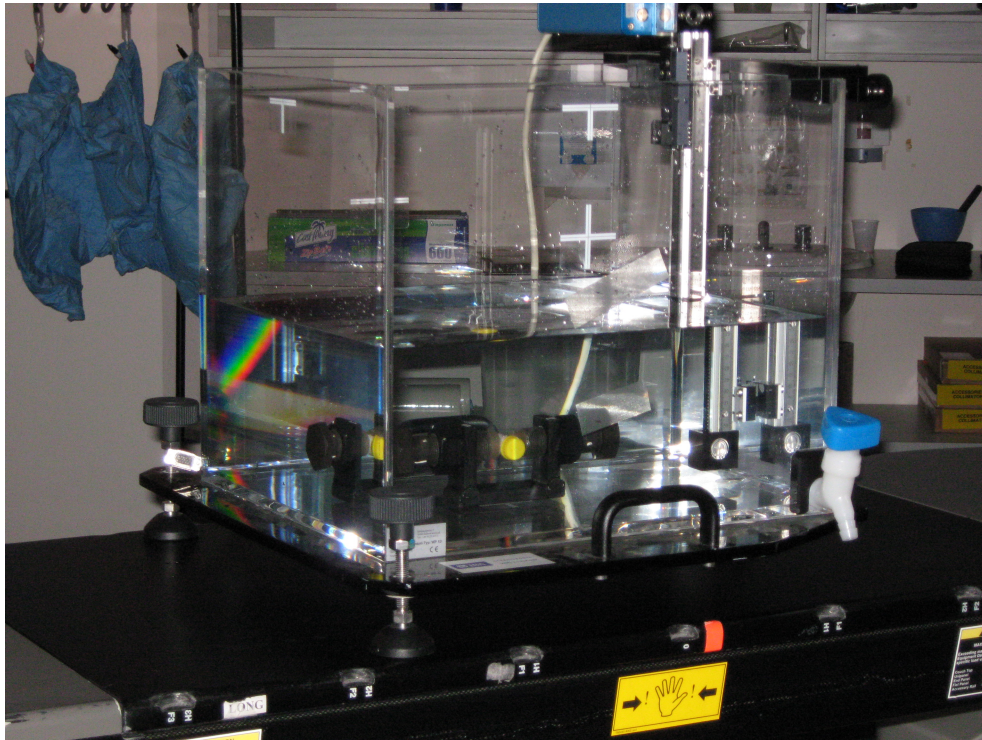


Figure 3.14: Linac Irradiation Setup

of culture grown to the same density.

Now, the RBE for gamma radiation remains fairly constant as the beam energy is increased so it is probably safe to assume that any changes to the survival rates were not related to the beam energy. The combination of high dose rate and magnetic field however may have a significant effect on the survival curve as it meant that each irradiation was completed in a short period of time compared to the repair half life, and much shorter than the kick-off time for repair. This means that the field would have had little time to influence the repair activation mechanisms and would not have had any direct influence on the repair mechanisms themselves.

It was seen that the survival rates were lower for those cells irradiated in a magnetic field. Though this data was not reliable as repetitions of the

survival data provided widely varying results. It is unclear why this was, though the relatively low doses used for some of these irradiations, 6.61 Gy, may have caused such a small number of cells to be killed that the methods used to determine survival were not sensitive enough to detect the change from the control sample. However the higher doses of 13.22 Gy and 19.83 Gy, based on previous irradiations and measurements, should have produced a clear trend in survival. As there was not enough time to repeat these measurements most of the data from this experiment was not compared to data from low dose rate irradiations or magnetic field experiments as the large variation in results was unexplainable without further work being done.

Also, as verification of the delivered dose, a strip of GaF film was placed in the dummy setup and irradiated. This can be compared to control samples to determine if the desired dose was actually delivered and also to see if the dummy setup caused any change in dose across the strip due to altered scatter/absorption cross-sections - a constant dose is desirable across the sample volume, if there is a significant dose gradient it would mean a large error in the delivered dose and so large error bars in the survival curves.

The results from the GaF films can be seen below in figures 3.15 and 3.16, including cross sections of the films grayscale readings in figures 3.17 and 3.18. The dose given to the GaF film in the dummy setup was 100 cGy.

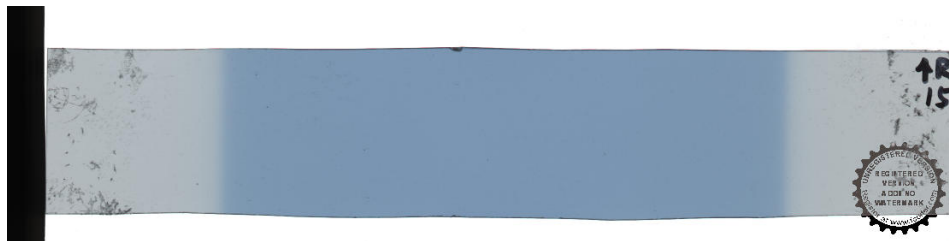


Figure 3.15: GaF sample reading

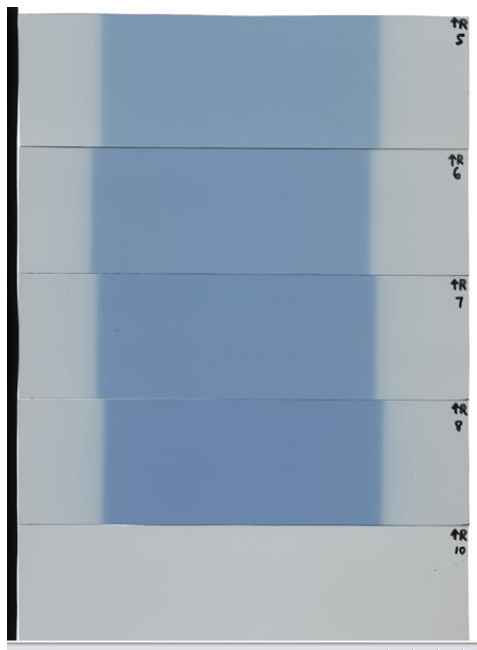


Figure 3.16: GaF control sample

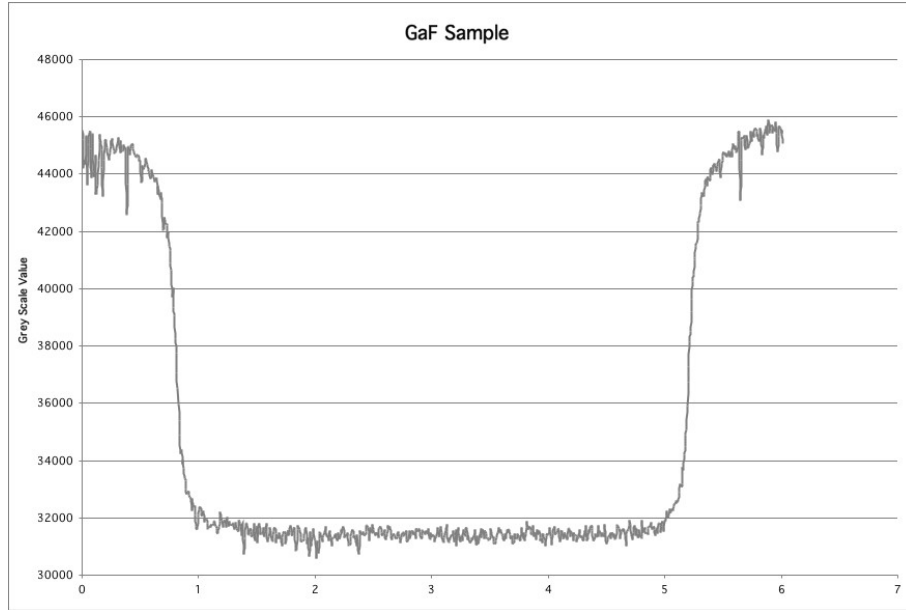


Figure 3.17: GaF sample line section

As can be seen there is no significant difference in the dose across the sample volume with the average grey scale value being  $31450 \pm 200$ , error given as plus or minus one standard deviation, which results in less than a 1% deviation from the mean. However comparing it to the control samples it can be seen that the dose in that volume is higher than the intended delivered dose, this means that the dose used in the above survival curve will be incorrect. The correct delivered dose to the GaF film in the dummy setup, as given by the control samples, was  $D = 134$  cGy. This is a 34% increase in the desired dose, due to the altered scatter and absorption cross sections from the dummy. Though this offset must be corrected for the overall trend of the graph, with the samples in a magnetic field having a lower survival, will remain essentially unchanged. When comparing this data to data taken with different irradiation setups this will become a significant issue.

It should be noted here that though this data may be used as a general

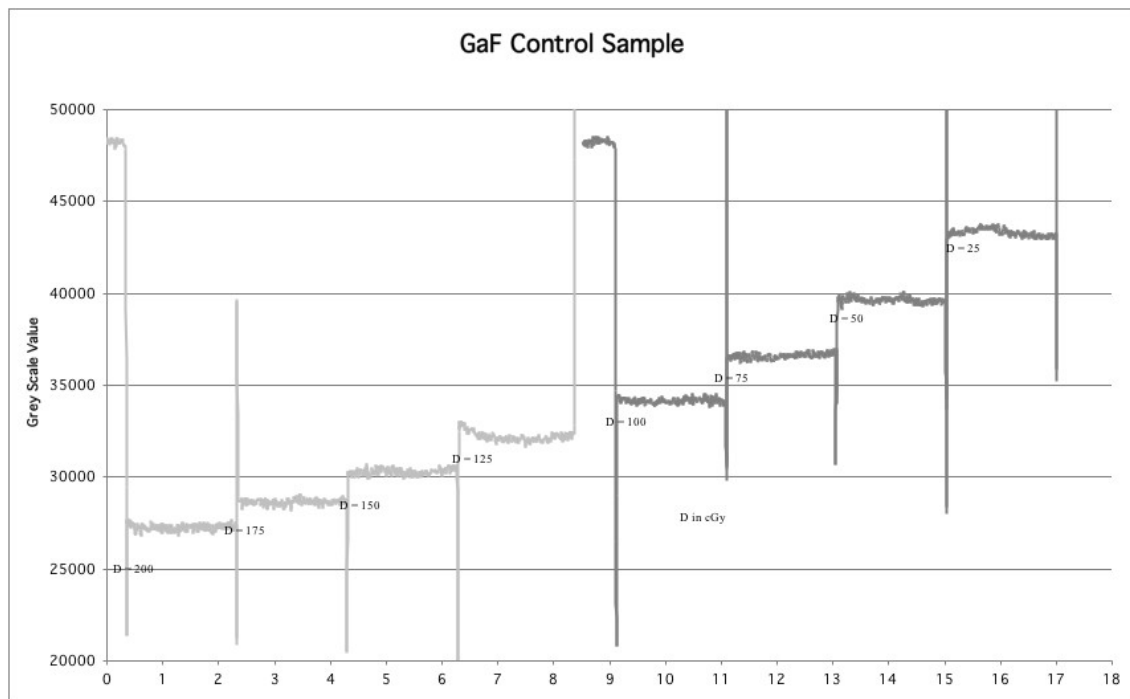


Figure 3.18: GaF control graph

guide to determine the actual delivered dose in the Low Dose Rate irradiations above, when the magnet and dummy setup were used, it will only be an approximate answer as a different magnet and dummy setup were used in those irradiations and a different beam energy as well.

The FTIR data obtained from these samples was just as varied as that from the survival curves. There seemed to be large difference in the density of cell culture samples. As all the samples were taken from the same culture this would normally indicate a difference in survival rates, however the variation was found even when testing samples that were irradiated to the same dose. This variation in results, as also mentioned in the survival curve data above, may indicate a difference in the density of cells in each of the sample containers. Though this should not have happened as all the samples were taken from the same culture which was mixed slightly to ensure that

the density was as constant as possible. Previously this had provided reliable and reproducible results, it is not known exactly what went wrong with this particular experiment though it could possibly have even been some fault of measurement techniques used in each individual case, though the repeated variation in results makes this unlikely.

However though the biological data from this experiment was not very useful the data from the GaF film was very useful in showing that the dose deposited in the sample volume was altered significantly by the introduction of the magnet or dummy setup.

### **3.4 Physical Effects of Magnetic Fields Applied to Biological Samples**

Some data was also taken investigating the effects of a magnetic field without irradiating samples.

#### **3.4.1 Cell Samples in A Magnetic Field**

FTIR spectra were taken of cell culture samples in the presence of a magnetic field. This was done by placing the permanent magnets used for the high dose rate irradiations on the Linac into the sample compartment of the Bomem. The field strength of the magnet setup here was  $0.072 \pm 0.002$  T. Unfortunately the magnet setup would not fit into the sample compartment in the same configuration as when used in the linac irradiations, the magnets had to be separated to fit the sample holder resulting in a weaker magnetic field strength.

Though the introduction of a magnetic field across the sample volume should have no effect on the IR beam background scans were taken to ensure that there was no variation (note that 'background' is taken to be when there

is nothing in the beam at all). The following graphs show the ratio of the background spectrum with a magnet present in the sample container taken with the normal background with no magnet present, figure 3.19, and the ratio of the sample holder spectrum with a magnet present in the sample container taken with the sample holder with no magnet present, figure 3.20. This means that if there is no change induced by the magnet setup being introduced then the graphs should both show a straight flat line at a transmission of 1 (showing that the two cases, magnet and no magnet, are the same).

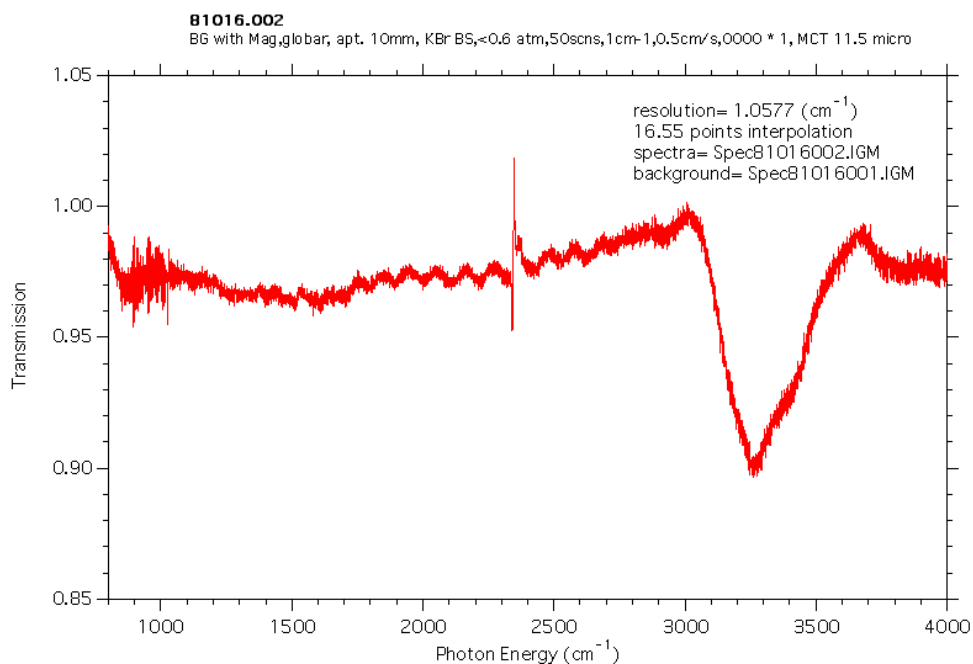


Figure 3.19: Ratio of BG with magnet to BG without magnet

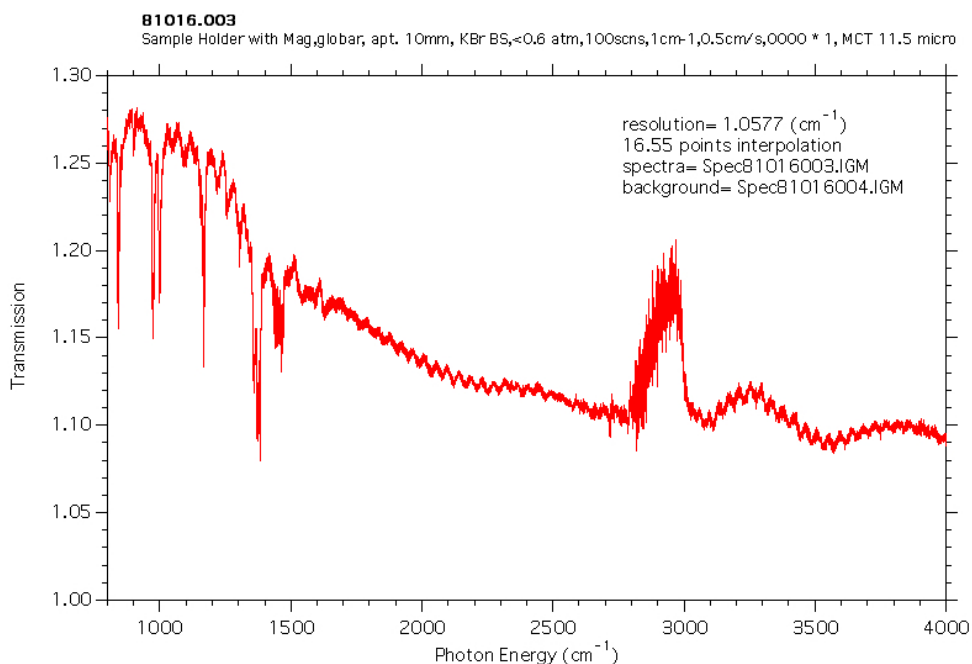


Figure 3.20: Ratio of sample holder with magnet to sample holder without magnet

As can be seen there was some change to the background in general and the sample holder spectrum also. Though the changes to the background were generally quite small (less than 4 %) except for the region from 3000 - 3700  $\text{cm}^{-1}$ , the water absorption region, where there was a significant decrease in transmission of upto 10 %. This could be due to some water vapour being in the sample compartment which was then altered by the magnetic field, though the pressure in the compartment was quite low ( $< 0.6$  atm) there could still have been enough water vapour to observe a change of this magnitude.

The change to the spectrum of the sample holder, however, was more significant. A sloping baseline can be seen with upto a 28 % increase in transmission. This is quite a large difference that cannot be ignored. Though it was easily compensated for by ensuring that all following spectra of cell

samples that were taken in the presence of a magnetic field were taken with the ratio of the sample holder in a magnetic field as the background.

To investigate the effects of a magnetic field on biological samples PK15 cells were used, these are porcine kidney epithelial cells. The difference in the spectra was quite pronounced, as can be seen below in figures 3.21 and 3.22.

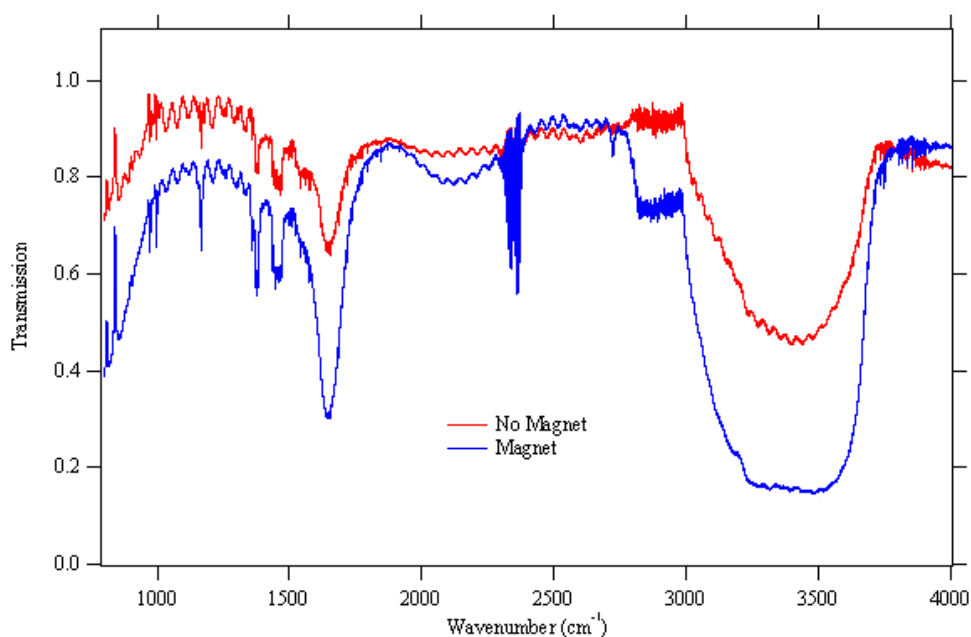


Figure 3.21: PK15 with and without magnet in sample holder

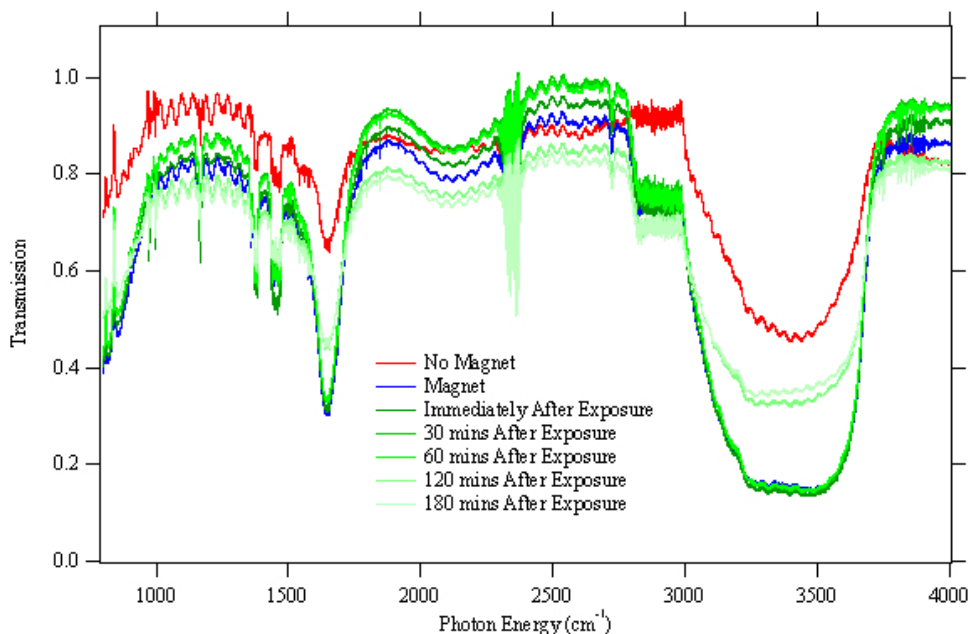


Figure 3.22: PK15 after magnet removed from sample holder

As can be seen in figures 3.21 and 3.22 there are significant changes to the spectrum when the magnetic field is placed over the sample and the changes do not disappear immediately once the magnet setup is removed.

Some of the areas with the most significant changes are in the region of water absorption, as seen in the wide peak at around  $1640\text{ cm}^{-1}$  and from  $3000\text{ to }3700\text{ cm}^{-1}$  where there is a much higher absorption when the magnetic field is present. This implies there are more of that particular type of bond - most commonly the H bonds in water, which may imply that the magnetic field causes water molecules in the sample to bond with each other more often [29]. There are some results from others [19,29,30] that indicate that a magnetic field will effectively cause water molecules to bind together more often, at least when in the liquid phase, which would support data from this study. This was primarily based on a study of the radial distribution function of water molecules in a static magnetic field which showed that

the average distance between oxygen and hydrogen molecules, when a static magnetic field was applied, was decreased. This was taken to indicate both a strengthening of the H bonds in water and an increased probability of H bonds being created between water molecules. Though quantitatively the significance of this effect on biological systems is still being studied. However there are also some published results indicating that there would be no change in the amount of bonds or strength of bonds in liquid water in the presence of a magnetic field, this would seem to go against the results of this study. This area is still under investigation with no definite answers found yet.

Any change to water in a cell may not normally have a significant biological impact, unless the cell is under irradiation. As mentioned previously when irradiated, water molecules can be broken into free radical pairs which can lead to damage to DNA possibly resulting in mutation or cell death. If the H bonds in water are significantly strengthened then this may result in the creation of fewer free radicals when the cell is irradiated as it would take more energy to break each of the bonds. It could also potentially cause a change in the lifetime of free radicals, if the water molecules were broken up into free radical pairs and separated by a smaller distance then it would be easier for them to recombine into a water molecule.

JM109 was also looked at with and without a magnetic field present (the same experimental setup was used) with similar, though not identical, results as can be seen in figure 3.23.

As demonstrated in figures 3.21, 3.22 and 3.23 the introduction of a magnetic field caused the greatest change to the areas of the spectrum related to water absorption, inducing a change not only to the absorption but also the shape of the spectrum in the region from  $3000 - 3700 \text{ cm}^{-1}$  in particular. As was the case with PK15 cells the peaks related to water absorption in

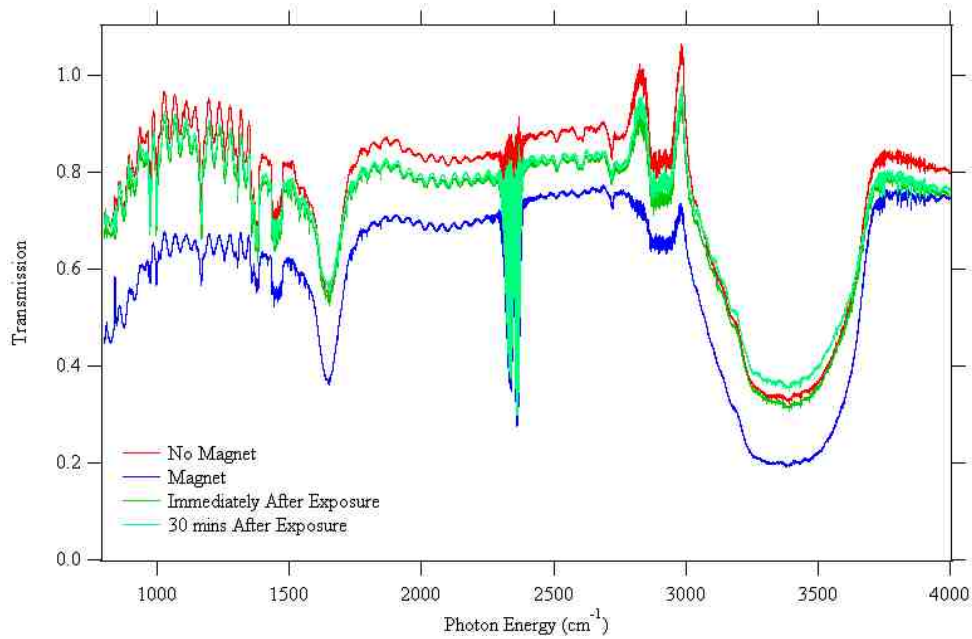


Figure 3.23: JM109 with and without magnetic field in sample holder

the JM109 spectrum returned to their original state after the magnet was removed, though this happened much faster than in the PK15 cells. Though there was a smaller increase in absorption seen here compared to the absorption change seen in PK15 where there was a large difference.

This was repeated with JM109 cells to determine the validity of these results. In total three sets of spectra from different samples showed very similar results.

### 3.4.2 Magnetic Fields Influence On Growth Rate

Here mixtures of cell samples and LB were exposed to a static magnetic field of strength  $0.212 \pm 0.002$  T for a period of 5 minutes before being incubated.

As can be seen from the spectra in figure 3.24 the pre-incubator exposure to a magnetic field definitely seemed to have an effect on the growth of the cells. The higher absorption of the sample that was exposed to the magnetic

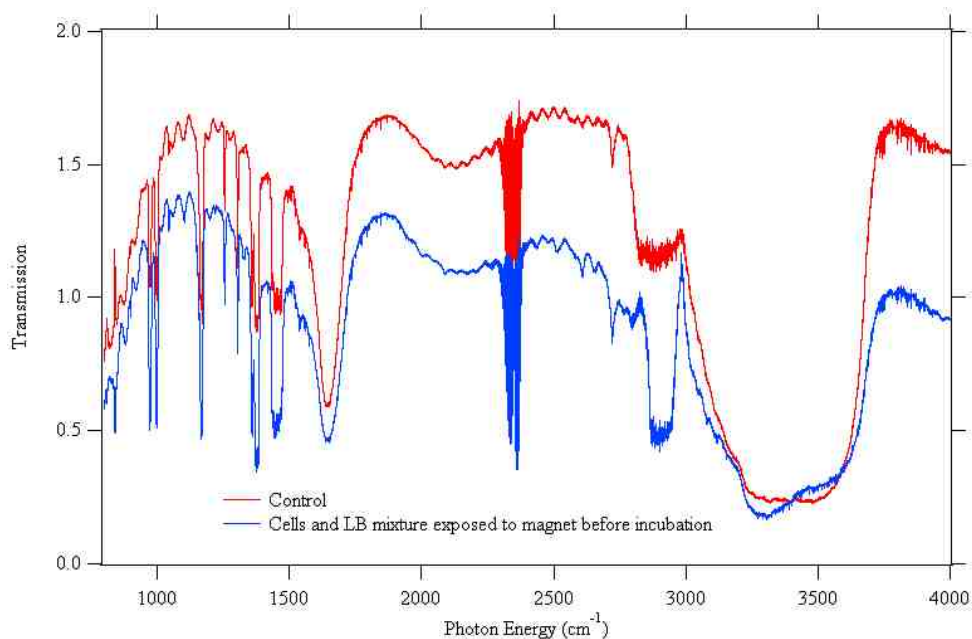


Figure 3.24: JM109 Growth Rates as Influenced by Exposure to a Magnetic Field Before Incubation

field before incubation indicates a higher cell count. As the samples were seeded with one colony each in approximately the same volume of growth medium this seems to indicate the test sample had a faster growth rate than the control sample. This may be a result of the magnetic field influencing the cells or possibly influencing the growth medium.

Of course probably the simplest explanation for this result is that the exposure to the magnetic field simply 'excited' the cells into their growth phase. When cells are stored in a cool room, as mentioned in the Introduction and Experimental Methods chapters above, they essentially go into stasis. This means that when a plate of cells is removed and used to seed a culture there is a 'kick-off' time before the cells enter the growth phase and start proliferating. If the exposure to the magnetic field induced a change into the growth stage then it would be as if that culture had been incubating for a longer period of time than the control sample, which would result in a greater

cell density and hence a greater absorption in the IR spectrum.

This was repeated several times to ensure the validity of these results. A total of three sets of spectra from different samples all showed very similar results.

It must also be mentioned here that if there was a significant change to the growth rate of the cells then the IR spectrum would probably be significantly different as the growth rate of cells has a direct effect on both the composition and size of cells [25] - altered cell composition would result in either an increase or decrease in the number of each particular type of chemical bond found in the cell which would show as a change in intensity in parts of the IR spectrum, changes to cell size would also lead to an increase of molecules (and thus bonds) in the cell. This means that if the growth rate of cells was altered by the exposure to the magnetic field then the spectra would also be different, though it is not possible from FTIR data alone to differentiate between this and the probably more likely case of simply having a higher cell density.

# Chapter 4

## Discussion

### 4.1 Biological Changes Induced By Magnetic Fields

Consistently it was seen that there was a significant change in the regions related to water absorption when a magnetic field was introduced - around the  $1700\text{ cm}^{-1}$  and from  $3000 - 3700\text{ cm}^{-1}$ . Though of course this may not be completely due to water - the protein amide I band has a large absorption in the  $1700\text{ cm}^{-1}$  region and so it is entirely possible that some of the changes seen in the spectrum in that region were due to protein alterations.

These changes may not normally have a significant biological impact, however if the sample is being irradiated then any change to the strength or number of bonds in water molecules could have a large impact on the damage done to individual cells. This is because the majority of damage from radiation is typically caused by free radicals which are created when water molecules are broken, as described earlier.

The region of  $3000 - 3700\text{ cm}^{-1}$  showed some peaks shifting to lower wavenumbers, this indicates an increase in the strength of those particular bonds. This increase to the strength of the bonds in water would mean that more energy would be needed to break them, thus higher energy photons

would be required to break the bonds. As most of the damage to cells is done by free radicals, and most free radicals are created from water (as described earlier) this could easily reduce the number of free radicals created.

The region of 800 - 1500  $\text{cm}^{-1}$  showed a general increase in absorption when the magnetic field was introduced. This was unusual as this region is related to DNA and protein in general and an increase in absorption across this region would imply that more chemical bonds were being made. This could mean anything from cross-linking in DNA where molecules start to bond with each other several times rather than just singly, or a large change to the conformation of proteins that would lead to new bonds being created between previously unbound areas. Either way such a drastic change was not expected and though other reasons for the increased absorption in this region should be considered no alternatives have yet been found.

Also interesting was that though there seemed to be significant changes to the spectrum of cells in a magnetic field, after the field was removed the first part of the spectrum to return to normal was that related to water with the rest of the spectrum largely remaining in its altered state. This seems likely as the chemical bonds in water are much stronger than most of the bonds in other biological molecules present in a cell and so would be more inclined to return to their natural state if there was no external stimulus to hold them there, whereas most other molecules having weaker bonds may simply stay in their altered state permanently (or take longer to revert), or perhaps stay in the altered state until something actively causes them to revert.

That said, after the magnetic field was removed there was eventually changes seen to almost all parts of the spectrum of PK15 cells and though these changes all initially moved the spectrum back to its original state there was significant variation in the speed of change. Also as can be seen each

part of the spectrum seemed to react differently once the magnetic field was removed with the low wavenumber region of around  $800 - 1500 \text{ cm}^{-1}$  moving slowly back to its original state but not actually reaching it, the mid region of  $1700 - 2800 \text{ cm}^{-1}$  showing a lot of variation and ending up with an even greater change after the magnet was removed than when it was present and the high wavenumber region of  $3700 - 4000 \text{ cm}^{-1}$  also acting like the mid region in that greater variation was seen after the field was removed. Though it must be stressed that this data, from PK15 in a magnetic field, was not repeated for verification (due to equipment problems) so to determine what the cause of these changes are and what effect they will have on the cells, the experiment should be repeated to check that the alterations to the spectrum that were seen were actually caused by the magnetic field and not by some other unaccounted for stimulus and further experiments investigating cell survival and life cycle should be performed to analyse what, if any, biological changes are induced.

However even though not verified the data is interesting and should still be analysed. Another interesting feature of the spectrum was the extra peak seen at around  $2700 - 2750 \text{ cm}^{-1}$  which was not seen until the magnetic field was applied and remained there even after the field was removed. This was strange as there were not any nearby peaks for this to be related to in the normal unaltered spectrum so it could not easily be explained as a shift in bond energy in a particular molecule. However there are several peaks of significant magnitude in the  $2800 - 3000 \text{ cm}^{-1}$  region that were not observable due to the opacity of the window material in this region. It is possible that one of these peaks was shifted to a lower wavenumber by the introduction of the magnetic field, this could be achieved by the field altering the conformation of a molecule directly as the molecules are

composed of a distribution of charges. The other possible explanation is that the conformation of a molecule was so altered that it created a new chemical bond between two previously un-bonded parts of the molecule. This would require a much greater change to the conformation of any molecule and so would seem less likely to occur, though not impossible.

A change that was consistently seen was the alteration of H bonds. This was seen in the change to water molecules and also the change to the low wavenumber region of the spectrum,  $800 - 1500 \text{ cm}^{-1}$ , which is related to DNA and protein bonds, many of which are H bonds. This is an interesting phenomena, though it is unclear exactly why H bonds should be affected more commonly than other types of bonds or even if this is simply a coincidence. Though if there is a greater probability of the application altering H bonds over other types of bonds then it could be very important as many of the most significant bonds in cells (at least as relating to radiotherapy) are H bonds. Besides the obvious example of water, DNA is also composed of many H bonds connecting bases together. If the strength or number of these bonds is altered it could significantly change the probability of mutation as this is governed by the breaking and incorrect re-connection of these bonds.

Another interesting change observed was that to the growth rate of cultures exposed to a magnetic field before incubation. There was a definite increase in cell density seen in the cultures that were exposed to the field, though the increase in absorption in the IR spectrum was not constant with areas due to water absorption showing a smaller change than all other parts of the spectrum. This follows from earlier observations that though the field caused changes to the bonds in water these bonds seemed to essentially go back to normal after the field was removed. The shift to lower wavenumbers of some of the peaks in the  $3000 - 3700 \text{ cm}^{-1}$  region was also seen just as

in the spectrum of cells in a magnetic field. This may indicate that not all changes to water induced by the field were reversed after its removal.

As mentioned earlier probably the simplest explanation for this observed increase in cell density is that the application of the field somehow 'excites' the cells into their growth phase. This could be by any of several mechanisms from alterations to ions or ion gates allowing for greater movement of ions or molecules across the cell membrane causing a faster absorption of nutrients from the medium into the cell, or a direct change to DNA or other molecules related to cell replication. There is too little data to determine the cause of the change or even prove that it is this 'excitation' mechanism. Though even so it seems obvious that there is *some* kind of change induced by the application of the magnetic field and this may be beneficial.

One problem with using radiotherapy to treat cancer can be that of the Oxygen Enhancement Ratio (OER). When a tumour grows large enough some parts of it are too far from blood vessels to be able to get oxygen and because of changes to the cell (in becoming cancerous) it can no longer induce the creation of new blood vessels to supply it, even if new vessels could reach these areas. This means that some layers of cells in large tumours are starved of oxygen, but as their ability to undergo apoptosis has been removed they do not die but rather simply stop growing and essentially 'deactivate'. If for any reason oxygen is again supplied to the cells, for example when radiotherapy destroys the cells closest to the blood vessels and allows the 'deactivated' cells to gain supply back, then the cells 're-activate' and start growing again.

This fraction of oxygen deprived cells is actually harder to kill using radiotherapy (when dealing with Gamma radiation. Heavy ion beams have very different radiobiological relations) than the actively growing cells. This is quantified by the OER, which is the ratio of doses in hypoxic cells to well

oxygenated cells required to produce the same biological endpoint, this shows that generally as the fraction of hypoxic cells increases so the RBE of the radiation decreases. This means that for a given dose of Gamma radiation there will be a higher surviving fraction of cells in a hypoxic tumour area than in a well oxygenated one. This makes killing tumours difficult as when the well oxygenated areas are killed off some of the hypoxic areas are given access to blood vessels, and thus oxygen, again and begin to grow meaning further treatment is required.

The reason for a reduced level of cell kill in hypoxic cells is unclear. However it seems to be related to a reduced effectiveness of free radicals, or perhaps a reduced number of free radicals created.

If however the hypoxic cells could be 're-activated' during radiotherapy they may be easier to kill, reducing the effect of OER and meaning that lower doses, or fewer fractions, may be used to treat some patients. This 're-activation' may be possible through the application of a magnetic field, as seen already there seems to be some change induced in cells when exposed to a field, though it is unclear exactly what that change is. Though at this time this is only an idea and much more research must be done to determine exactly what in the cell is being altered by the magnetic field and what biological change this will lead to.

## **4.2 Irradiation of Biological Samples in a Magnetic Field**

There was definitely a difference in the survival rate and the IR spectrum of cells irradiated in the presence of a magnetic field compared to those irradiated without a field. Though again the data obtained was not repeated for verification due to equipment problems. However the most significant

changes were seen in the areas of 1700 and 3000 - 3700  $\text{cm}^{-1}$ , which is consistent with what was seen in the other sections of this study, this supports the validity of these results without actually verifying them.

The largest change was seen in the region around 1700  $\text{cm}^{-1}$  related to primarily water and the protein amide I band as can be seen in figure 4.1. This showed a large change in the structure of the two main wide peaks with either a significant shift to lower wavenumber of one of them indicating a strengthening of the bonds this peaks represents, or a large change in intensity of the two different peaks which would indicate a reduced number of bonds (where the transmission is increased) or an increased number of bonds (where the transmission is reduced). As the area the one peak is reduced by matches almost exactly with the area the other is increased by the former explanation - a shift to lower wavenumber of one of the peaks rather than the formation of new bonds - would seem to be the most likely explanation.

This would mean that either water bonds or the bonds of the Protein Amide I band, or both, were significantly strengthened in the magnetic field. Which in turn would make them harder to break when irradiated and would explain the increase in absorption in this region.

A similar result can be seen in the 3000 - 3700  $\text{cm}^{-1}$  region where there is an increase in absorption of particularly the lower wavenumber section of this region. This would indicate an increase in the number of bonds of water molecules.

It is unclear whether or not this would have any significant impact on the production of free radicals, or the life time of free radicals.

A higher survival rate was seen here in the samples irradiated in the presence of a magnetic field, this was found using the biological methods

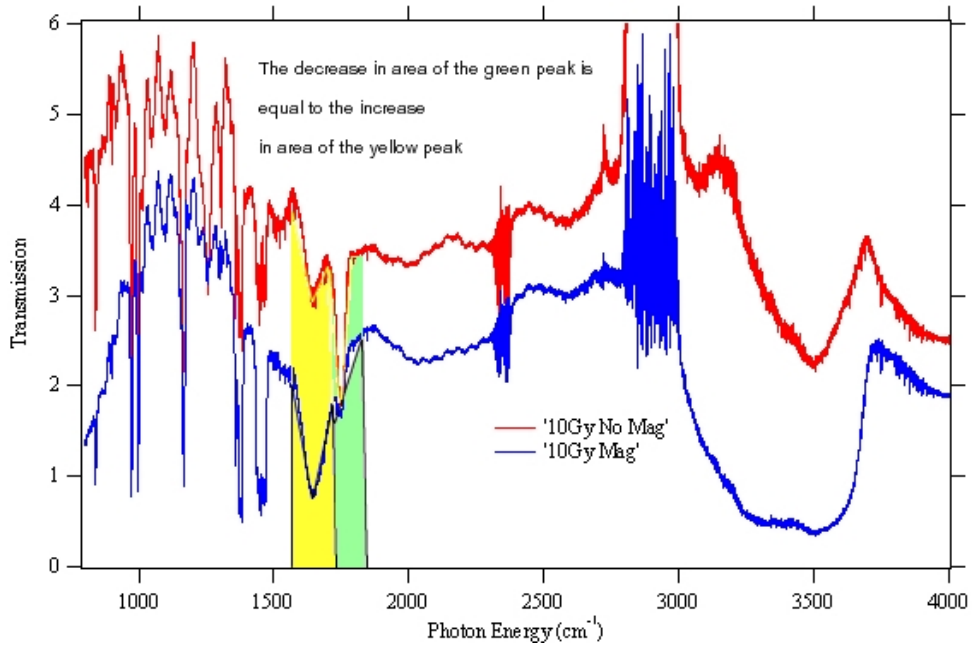


Figure 4.1: JM109 10 Gy Magnet and No Magnet  $1700\text{ cm}^{-1}$  peak zoom with areas highlighted (Note curves are not offset, data seen as recorded)

described earlier of growing known amounts of each sample at different densities and supported by the FTIR data which showed an overall increase in absorption of the sample that had been irradiated in the presence of a magnetic field, this indicates there were more living cells present in the sample (or at least cells with intact chemical bonds, without which they could not remain viable).

The survival rates were found to be 48 % for those cells irradiated to a dose of 10 Gy without a magnetic field and 65 % for those irradiated to the same dose in the presence of the  $0.667 \pm 0.002\text{ T}$  field. This is a significant increase in survival and was not expected as previous experiments done by the Centre of Medical Radiation Physics at Wollongong University have shown results indicating a decrease in survival rate when a magnetic field of similar strength is applied. However there are some quite large differences between

those experiments and this one, the largest being the cell type used. Previous experiments were done using mammalian cells, not bacteria.

As discussed above the presence of a magnetic field does cause some change to the growth of cells, quite possibly exciting cells into the exponential growth phase. The initiation of repair of DNA is similar to cell growth in that there is a lag of around 20 mins from when the initial damage occurs to when repair begins. If the presence of a magnetic field could somehow speed up the initiation of repair (as an 'excitation' mechanism) then the cell repair mechanisms may be more effective. This would be because the repair mechanisms being initiated sooner would mean that they would have a head start on repairing damage - there would be less damage initially for them to repair (as the time the cells had been irradiated for when repair begins would be less than if the magnetic field was not present) and so repair may be more effective. If so this would probably lead to a higher survival rate in cells irradiated in the presence of a magnetic field. This would not be expected in other experimental setups if the irradiation time was decreased significantly (and thus dose rate increased significantly), as there would be little or no time for the repair mechanisms to gain their head start, or probably if mammalian cells were used rather than bacteria as the much longer repair half life would mean that any advantage gained would be reduced significantly unless the irradiation time was correspondingly increased (though this would lead to very large irradiation times on the order of several hours for a typical 2 Gy fraction which would be entirely impractical).

However though the data found in this study would support these ideas it is not enough to prove them. As such more research into this area should be done to determine what the biological impacts of the observed physical changes to the cells are and whether they are significant to radiotherapy.

Though it seems apparent there are significant physical changes to some molecules, particularly water, as indicated by changes to the IR spectrum.

## Chapter 5

### Conclusions

It has been seen that the application of a magnetic field to biological samples can have a significant impact on the growth and chemical structure of cells. Of particular interest was the observed change induced in water as it may have significant impact if applied to radiotherapy. Any change to the structure of water could mean a significant change to the number of free radicals created or the lifetime of those free radicals when cells are irradiated. As free radicals are responsible for the majority of damage caused by Gamma radiation this is a very important relationship that requires greater understanding. As such the changes induced in the structure of water by the application of a magnetic field should be investigated further as well as independently tested as much of the research in this area by others so far has produced conflicting results.

There is still a lot of work that can be done in this field. Powdered DNA samples could be used to determine whether the application of a magnetic field may cause more of a change to bonds in bases or in the backbone of DNA, this was not able to be determined in this study as the peaks relating to DNA typically overlapped with relatively large peaks from other molecules like proteins or lipids. Though general trends could still be observed, like an

overall increase in absorption indicating an increase in the number of chemical bonds, detailed data about exactly which bonds in DNA were influenced was not obtained.

Also more data relating to the formation and lifetime of free radicals in a magnetic field should be obtained. This is an important relationship to investigate as there may be several mechanisms working against each other - for example an increased number of bonds potentially decreasing the lifetime whereas the direct influence of the magnetic field may increase the lifetime of free radicals - and so the overall impact of the application of a magnetic field remains unknown.

The effect of a magnetic field on H bonds is another area that may be investigated as H bonds play a very important role in the creation of free radicals and the mutation of DNA, both of which are very important when considering radiotherapy.

Some of the data found here should be verified properly, particularly the irradiation data. As mentioned this was not done due to equipment problems: several problems occurred however the primary concern was with the water cooling system for the Globar light source in the spectrometer. This broke down many times and was not always able to be quickly repaired causing the spectrometer to remain out of use for several months in total which seriously inhibited the ability to take data.

## *Appendix A - Bomem*

### Bomem Use

Water cooling system must be turned on, want about 150 - 200 kPa of pressure. Check that water is flowing.

Turn on Global

Config on PC should be in: scan mode = local, vacuum = evacuation (when scanning), set detector to appropriate sample position

Purge sample compartment. Put sample in sample compartment and screw on lid (don't screw lid with vacuum on)

Set to evacuation, turn on pump and release yellow valve slowly

Want source pressure to be  $< 0.6$  atm

Create new folder (name: date) in c:bdata and in c:fourier-guest-bomem

Change file it saves to  $>$  options  $>$  pref

Choose new folder (date)

File name: BG [background], DTGS [detector], coat. BS [beamsplitter], Glo [source], 1 cm<sup>-1</sup> [resolution], 0.5cm/s [mirror speed], 200 scns, 0.2 atm, gain 8X4 [detector X pc], 10 mm [aperture size], left [sample place] mount + mask [comment]

Click scan/start to see alignment and laser stable (should be green) and want signal (%ADC) between 40 - 60 (min 20, max 80) otherwise change gain appropriately

When source pressure  $< 0.6$  atm click Raw Spectrum to take data

To change sample close valve then switch to purge and change the sample

When done move .igm file to bomem-2007[year]-[foldername] and use Mac to analyse data in Igor

Fourier data  $>$  bomem  $>$  folder opens in IGOR

My macros > fourier transform

'Ratio' function to remove background

## *Appendix B - Time Line*

### GENERAL PLAN:

1. Literature review
  - a. Radiobiology in general
  - b. Magnetic field effects on cells and use in radiotherapy
  - c. FTIR
    - i. Use with biological samples
    - ii. Appropriate sample holder
    - iii. Window material
  - d. Cell growth
    - i. Determine appropriate cell type to use
2. Learn to use Bomem (FTIR spectrometer) and associated software
  - a. Design and make a sample holder
  - b. Find appropriate window material
3. Biology lab
  - a. Learn to grow cells
  - b. Learn how to determine surviving fraction of cells in a sample
4. Wollongong Hospital contacted to arrange irradiations of samples

The previous work (steps 1, 2 and 3) was started December 2007-January 2008 and completed around June-July 2008.

Up until this point the FTIR device and associated equipment had failed and required repairs several times resulting in it being unavailable for a total of around 4-5 weeks. At this point this was not a significant impediment as there was little work to be done requiring the spectrometer (mostly test-

ing window materials and cell types to find an appropriate combination for further experiments). At this point it was believed that the major problem that had caused equipment failure (the breakdown of the water cooling system that was essential to using the spectrometer) had been solved and furthermore even if it continued to cause problems the equipment had only been unavailable for 4-5 weeks out of 6-7 months, the author believed that even if similar problems were encountered there would still be enough time to complete the vast majority of desired experiments. Note that the FTIR spectrometer had been used for extended periods, before this thesis began, without encountering any serious equipment problems. At this time (2008 in general) the only other users of the spectrometer had minor time requirements, it was easily available for enough time for this thesis to be completed on time.

At this point the following was attempted:

#### 5. Experiments completed

- a. Cells grown in the Biology labs at Wollongong University (Bacterial Culture Lab)
- b. Irradiations done at Wollongong Hospital
- c. Samples returned to University labs for study (both FTIR lab and Biology lab)

#### 6. Analyse data on continuing basis

Now from around July-August 2008 the experiments began (growing and irradiating cell cultures, measurements taken on spectrometer and survival determined). However early on in this period (around mid August) the water

cooling system for the spectrometer failed and needed repairs. This ended after around one week with the system being shutdown and the backup system activated. Later, around September 2008, the backup water cooling system also failed and required repairs. This was fixed within one week; however it continued to breakdown and require repairs and maintenance over the next several months. During this time the longest period the equipment was unavailable was two weeks, however it was unavailable for a total of over 9 weeks.

This caused significant problems as the main point of this thesis was to investigate the damage done to biological samples irradiated in the presence of a magnetic field using FTIR spectroscopy. As it could take hours to get several useable IR spectra from a experiment it often meant that it would take several days in total to get results from an experiment as background readings had to be taken, repeated measurements of irradiated samples were taken and measurements of control samples were taken . If (as happened twice) the spectrometer became unavailable due to equipment failure during the process of acquiring spectra of irradiated samples then the samples had to be stored and measurements continued when possible. This lead to further experiments being done on the change to cells IR spectrum over time to ensure that if samples were stored in cold storage it would not significantly alter the data obtained from them. This meant that the longer the spectrometer was unavailable the more experiments had to be done to ensure that data taken was valid. This further reduced the time available to complete the original planned experiments.

## *Appendix C - IGOR*

### IGOR (by Wavemetrics)

A software tool used for graphing and analysis of data. Specifically suited to use in FTIR spectroscopy as data can easily be graphed, Fourier transforms can be easily and quickly applied to data, Peak Analysis function allows absorption peaks to be easily identified. For more information refer to <http://www.wavemetrics.com/>

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