DISTRIBUTION AND CONCENTRATION OF TRACE AND MAJOR ELEMENTS
IN BIOLOGICAL SPECIMENS USING PROTON INDUCED X-RAY EMISSION
ANALYSIS AND PHOTON TRANSMISSION TOMOGRAPHY

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Abstract

Trace elements within biological tissues are heterogeneously distributed. This complicates the task of deriving concentrations that represent an organ or specimen as a whole. The derivation of representative concentrations is important in the investigation of tissue health status or exposure to the individual from occupational or man-made pollution sources. Therefore, a knowledge of the elemental dispersions in biological tissues is required.

Proton induced X-ray emission (PIXE) analysis is employed in the study of the elemental heterogeneity of porcine liver, kidney, heart and lung. Specimens are analysed in two different modes. One method involves the extraction of sub-samples that are dried, homogenised and manufactured into thick target pellets. This approach however limits the spatial resolution on which elemental distributions may be derived and hence thick specimen sections that can be irradiated directly may be preferred. This type of target though suffers in that surfaces are irregular and proton irradiation and X-ray take-off angles are ill defined. The effect of these surface imperfections upon X-ray yield in PIXE are investigated by the development of simple stylised models. The physical parameters of these models are varied and the elements most affected and dominant factors in modifying X-ray yield are identified.

The trace element content for like tissues between targets in the form of pellets and freeze-dried sections are compared and mostly excellent correlation is found. The analysis of specimens in either of these modes stresses the high elemental inhomogeneity of biological samples. A quantitative determination of this elemental heterogeneity is made by the derivation of sampling factors, the minimum mass of material required to reduce elemental variations to a given level of precision. Those sampling factors derived by utilising the data from pelletised targets agree well with the limited values from the literature, whereas a large difference is found for those calculated from thick specimen target sections. This disagreement is thought to be due to the failure of sampling factor theory at the small sampling mass employed in the analysis of the latter targets.

Photon transmission tomography was investigated for the ability of the technique to provide a measure of biological specimen heterogeneity, differentiate between different composite tissues and identify regions of interest. This may prove useful for the selection of sub-samples for subsequent trace element analysis. Biological specimens were scanned
in fresh and dry states to ascertain the most favourable sample preparation technique to best achieve the above aims, the dry sample states were preferred. Freeze-dried specimens are imaged under differing scanning parameters and their data compared to theoretical values derived from PIXE and Rutherford backscattering (RBS) analysis. Good agreement is found. Regions of interest may be identified in tomographs, this being due to density variations rather than elemental variations, tissues of similar but different composition not being differentiable due to image noise which is a product of finite counts in reconstructed images. However, with the improvement of photon counting statistics in images, these tissues may be more discernible from one another in tomographs thus making photon transmission tomography a viable technique for the selection of representative sub-samples for subsequent elemental analysis.
This thesis is dedicated to the memory of my grandmother,
Olive Louise Beach
Acknowledgments

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Chapter 1
Introduction

Research into the influence of elements upon the health or disease status of biological systems has not been entirely reliant on the technological developments in the latter part of this century. As far back as the 17th century, the essentiality of Fe to sustain human life was discovered. However, most of our knowledge of trace elements in human and animal health was acquired over the last 30 years and only now is the complicated picture of their interactions and functions becoming apparent [IYE91].

The elements found within biological specimens may be categorised depending upon their level of concentration, the demarcations of major, minor and trace being >1%, 0.001%-1% and <0.001% [JOH88] of the total body weight, respectively. The major and minor elements consist of H, C, N, O, Na, Mg, Si, P, S, Cl, K and Ca [UND71] and it is interesting to note that these constitute 99% of all living matter [DAV72]. The remaining trace elements fall into three categories namely those essential, non-essential and toxic to living matter. Essential trace elements are classified as those whose deficiency constantly results in impairment of a biological function from optimal to sub-optimal [UND71]. This may be shown diagrammatically (Figure 1.1). Insufficient concentrations of an essential trace element lead to deficiency effects, these however disappear as concentration is increased and a plateau of normal physiological function is encountered which is bounded by a given trace element concentration range [SCH76]. As the concentration of the element is increased further, excretion of excess requirements becomes difficult and physiological function is impaired leading to a state of toxicity and ultimately, lethality. Hence, all essential trace elements may be regarded toxic when present at sufficiently high levels. However, the toxic trace element category covers those elements that are detrimental to living matter at low levels of concentration and the metals Pb, Cd and Hg are usually only bounded within this group. Most of the essential trace elements lie between Z=20 and Z=34 (inclusive) and include V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, and Se. A number of essential trace elements lie outside this atomic number range and include F, I, Sn and Mo. Gallium and germanium are also bounded by the above essential trace element Z limits (i.e. Z=31 and Z=32 respectively), they however have not
been found to possess any biological function and thus are classified as non-essential elements as are Al, Sb and Ag to name a few [UND71]. This perhaps is the most ambiguous classification for trace elements as the possibility exists for them to have yet undiscovered biological functions rather than none at all. Of the trace elements that are categorised as non-essential, these reflect more the interaction between animal and the external environment. Liebscher and Smith [LIE68] put forward a method by which an element may be described by identifying the concentration distribution that they take. As essential elements are physiologically regulated by their resident tissues, concentration distributions tend to normal or at least symmetrical in shape, whereas in the case of non-essential elements no physiological regulation occurs and element levels are more prone to dictation by external factors. This leads to a low but significant frequency of high concentrations and thus log-normal concentration distributions are encountered.
There are a number of reasons why trace element contents of biological tissues are a source of interest. These relate to assessing the relationship between man and the surrounding environment, the medical diagnosis of disease and occupational hygiene [CHE84, IYE86]. However, many of these assessments rely upon accurate knowledge of baseline data which describe average elemental concentrations for a given tissue. The provision of these baseline or reference data is complicated insomuch that an average individual is difficult to define and factors such as age, sex, geography, diet, smoking habits and occupational exposure play an important role in determining their trace element status [IYE82, VER84, AAL87, IYE88]. Therefore baseline data may only be accurately provided for a well defined group of individuals and ‘unless the complete history of any sample is known with certainty, the analyst is well advised not to spend his time analysing it’ [VER84]. However, there exists a number of publications that serve as guidelines for approximate concentrations of elements found in various tissues, that by Iyengar et al [IYE78] probably being the most well known, other examples are those by Tipton and Cook [TIP63a, TIP63b], Hamilton et al [HAM72], the International Commission on Radiological Protection [ICR75] and Iyengar [IYE85].

Given a knowledge of the range of concentrations expected in a well defined population it may be possible to use elemental levels to indicate the presence of various diseases or exposure to man-made pollutants. The elements belonging to the toxic grouping are usually of main interest in studies regarding the effects of industrial and pollution exposure upon man and the environment. Bone is a commonly employed biological sample in the investigation of Pb contamination as this is the main site of the accumulation of this element (bone contains >90% of the total Pb body burden) and provides a measure of long term exposure [SAM89]. Most studies identify a positive correlation between Pb bone concentration and age [SAM89, OCO80] which may be linked to environmental (heavy industry, automobile emissions) and occupational factors. A positive relationship is also identified between this element and smoking [NUS65]. Smoking also contributes toward elevating Cd levels in the kidney although industrial and occupational exposure contribute and again a positive correlation between this element and age is noted [PIS72, ELI76]. Perhaps one of the most sensitive organs for indicating pollution exposure are the lungs as these continually sample the atmosphere. This tissue has been employed in many studies upon the industrial and occupational impact of trace
elements upon human health [MOL67,BAR82].

The trace element contents of various organs and tissues may be utilised to indicate the presence of a variety of disorders. Elevated levels of copper in the liver may demonstrate the existence of Wilson’s disease or cirrhosis [NOO81,WAT84,HEC87] whereas Pb, neoplastic growth in some other part of the body [KOE79]. Uda et al [UDA87] investigated if element levels, especially those of Ca, Ti, Cr, Fe and Zn, could be used as indicators of cancer in kidney, testis and urinary bladder, and a link was discovered. In a similar study, the same group identify lower concentrations of iodine in injured thyroid glands when comparing them to normal organs [MAE87a]. However, no decision upon the benign or malignant nature of the growth may be made by sole analysis of I, and comparison of levels of other elements was recommended to provide an indication of this. Analysis of aluminium in brain tissue may indicate Alzheimer’s disease [KRI89] although there is large debate about this relationship at present [LAN92].

It is apparent that trace element levels of various biological organs and tissues may be used to indicate pollution exposure and the presence of disease. In order to derive conclusions upon either of these subjects, accurate derivation of concentrations must be assured. This not only involves determination and reduction of analytical errors and the identification of biological factors (e.g. diet, geography) that may influence baseline data but realisation that differing trace element levels may be derived which are dependent upon where sub-samples are extracted from a specific tissue or organ [IYE82]. For example, the kidney is essentially composed of two discrete tissues (the cortex and medulla) each with their own individual physiological function, and for this reason differing trace element contents are encountered between them [LIV71,TAN87]. In fact, various elemental contents are not constant throughout the cortex, e.g. Zn and Cd, and concentration gradients exist [LIV71] which make the extraction of representative sub-samples from this composite tissue difficult indeed. Bone presents problems in that it consists of two differing types of mineralised hard tissue, namely compacta (cortical) and trabecula (cancellous) bone [BEH81,KID82]. Compacta is hard and forms the shafts of long bones and the covering of flat bones, whilst trabecula is found within the metaphyseal region and the cortical covering of flat bones. As the composition for certain elements e.g. F and Pb, is found to differ between these two types of mineralised tissue, care is required when comparing concentrations between bones as they may contain
differing proportions of compacta and trabecula and also problems may arise in that samples include marrow, fat or blood which could affect results considerably [BEH81,GAW82,SAM89]. The brain has been determined to be heterogeneous in composition, one reason being varying proportions of grey and white matter in particular areas (e.g. dry weight concentration of Zn in grey matter is 70µg/g whilst in white matter 30µg/g) [MAE87b] whereas the lung, albeit lobulised, is essentially physiologically homogeneous throughout, but elemental differences occur between areas due to aerosol deposition [BAR82,VAN82]. Elemental inhomogeneities have also been found to exist in liver [KOE79] and prostate [LIN90].

It has been demonstrated that trace element inhomogeneities occur in a large number of differing tissues and organs, if not all of them. However, the issues of if a single sub-sample may be used to represent the organ as a whole, and if so, how large this should be are addressed only with low frequency. Therefore, further work is needed in this area so that elemental levels derived from a single or small number of sub-samples may be reliably employed in the diagnosis of disease or measurement of exposure to man-made pollutants. A single or small number of sub-samples may only be available due to standard biopsy practices or as a result of limitation in numbers which are stored in biological and environmental specimen banks. Specimen banks have been established so that baseline environmental data and trends may be identified and to provide the opportunity for retrospective analysis of samples thus allowing the application of present knowledge and advances in analysis procedures [WIS89].

It is the aim of this work to address the above issues regarding the heterogeneous nature of biological specimens so that representative trace element contents may be derived which reflect the nature and history of the tissue rather than the position within the specimen where samples were extracted. This is achieved by the analysis of selected biological organs by proton induced X-ray emission (PIXE) analysis and proton transmission tomography, each technique possessing its own capabilities and being applied to differing analysis situations.

Proton induced X-ray emission analysis is a technique where a beam of hydrogen ions is used to determine the concentration of elements (~Z≥14) in a target, by the detection of characteristic X-rays (K and L) resulting from the ionisation of inner electron atomic shells [JOH76]. PIXE is particularly useful in the field of biomedical
research as this technique has good detection limits (typically ppm), the minimum of which are in the atomic number range of the essential trace elements (K X-rays) and heavy toxic trace elements (L X-rays) and also in many cases, the degree of sample preparation is small thus limiting the risks of contamination and production of spurious results. If required, a proton micro-beam may be employed thus yielding a technique with the ability to determine elemental concentrations on a low spatial scale, typical resolutions being of the order of 1\(\mu\text{m}\) [GON87] although sub-micron dimensions are achievable [WAT82]. This mode of PIXE analysis will enable the study of elements at the cellular level which is important in furthering the understanding of their roles in health and disease [WAT84].

Samples of porcine liver, kidney, heart and lung were analysed by PIXE in two different modes. One of these modes involved the selection of sub-samples from defined positions within the organs which were homogenised and made into cylindrical pellets. As a minimum mass of material is required for pellet production, the achievable spatial resolution of the technique is compromised and hence the use of specimen slices that could be analysed directly was investigated. However, the employment of these sample sections is complicated in that their surfaces should be flat and inclined at a known angle to the proton beam [CAM84] which is not as easily achievable as in the case of pelletised targets. The effects of these surface imperfections upon PIXE are examined by the development of stylised models and results are presented. Both types of sample are mounted upon aluminium target plates and analysed by a 2 MeV energy proton beam at the University of Surrey Van de Graaff facility. Proton induced X-ray emission (PIXE) and Rutherford backscattering (RBS) spectra are collected simultaneously, the latter being for the quantification of the matrix composition (C, N, O, Na, P/S, Cl, K) which is used in the calculation of trace element concentrations. Elemental levels are presented for various portions of the above mentioned organs and significant differences highlighted or employed to demonstrate variation trends across sections. These concentrations are used to calculate sampling factors which are the minimum mass of material required to reduce derived element variations to a given level of precision, and to decide upon suitable sample extraction sites. Information pertaining to these criteria will aid in the selection of sub-samples such that their trace element contents are representative of a tissue or organ as a whole. Hence these levels may be reliably employed in the study of their roles.
in dictating disease or indicating industrial or occupational exposure.

Photon transmission tomography is a technique that enables imaging of the linear attenuation coefficient through selected slices of an object [BRO76]. The linear attenuation coefficient at a specific photon energy is a function of material composition and physical density [JAC81] and hence by scanning biological samples it may be possible to identify regions of interest e.g. fat deposits, and gain a measure of specimen inhomogeneity. This would prove useful in the selection of representative sub-samples for subsequent trace element analysis by a technique such as PIXE.

Samples of porcine liver, kidney and lung were scanned using a discrete energy radioactive source ($^{241}$Am) employed in a single pencil beam geometry configuration with a HpGe semi-conductor detector. Images were reconstructed by the filtered back-projection algorithm, and tomographs and their associated data compared between samples and scanning parameters. Tissues were imaged in fresh and freeze-dried states to ascertain the optimum specimen preparation procedure for region of interest identification and to provide a measure of elemental heterogeneity. Scanning parameters e.g. photon energy and pixel size, were varied and their effects upon tomographs noted. Data from the tomographical scans were employed to identify regions of interest, and measures of the variation of the linear attenuation coefficient given which may be attributed to image noise, and specimen density and elemental fluctuations. An estimation is provided for each. Data derived from the tomographical scans and from the elemental analysis of samples by PIXE and RBS are also compared in order to establish their degree of correlation.
Chapter 2
Proton Induced X-ray Emission (PIXE) Analysis

2.1 Historical background

X-ray emission analysis relies upon the detection of characteristic photons, the intensity of which indicates the level the element from which they originate is present within the sample under investigation. A number of techniques may be employed to excite these characteristic X-rays. These include electron beams, continuous bremsstrahlung, near monoenergetic photons from X-ray tubes, monoenergetic photons from radionuclides and charged particles as first demonstrated by Chadwick [CHA12] using an alpha particle emitting source.

Prior to the 1960s, most X-ray detection work was carried out using wavelength-dispersive analysis based upon crystal diffraction, but this allowed only single element determination at a time. However, toward the end of that decade the emergence of lithium drifted silicon Si(Li) semiconductor detectors enabled high resolution X-ray spectroscopy, and hence determination of many elements simultaneously. During this period attention was also given toward decreasing limits of detection in X-ray emission analysis and the use of ion beams and in particular protons in the MeV range, were suggested as characteristic X-ray exciters [BIR64]. This would greatly reduce the bremsstrahlung background which is produced when charged particles are decelerated within a sample, and is large when using an electron beam. The magnitude of this background is a major factor influencing detection limits (see section 2.2.4).

Johansson et al [JOH70] were the first to employ an ion beam for X-ray emission analysis when they studied solutions of elements deposited upon carbon foils using a 1.5 MeV proton beam from a Van de Graaff generator. Results demonstrated that very small absolute amounts of individual elements could be detected by this technique. Van de Graaff accelerators, which were becoming increasingly available at this time due to the nuclear physics community forsaking them for higher energy machines, have the advantage of delivering a large number of ions over a short period of time. This is one factor limiting the employment of alpha particle emitting radionuclide sources for X-ray excitation.
Since the paper by Johansson et al [JOH70] in 1970, particle induced X-ray emission or proton induced X-ray emission analysis as it is more widely known due to a proton beam being employed in most cases has grown rapidly and has found many applications in biology, medicine, environmental analysis, geology and archeometry providing detection limits of typically parts per million (ppm). Recent advances of this technique have seen the development of the proton microprobe which allows mapping of elements on a slightly poorer spatial resolution than electron microscopy, but superior with respect to detection limits. The development and fundamentals of proton induced X-ray emission analysis may be found in any of the numerous reviews found in the literature [JOH70, CAH80, MIT81, KHA81, JOH88].

2.2 Basis of the technique

A typical PIXE spectrum (Figure 2.1) consists of a number of characteristic X-ray peaks superimposed upon a continuous background which originates mainly from the

![Example X-ray spectrum from the PIXE analysis of porcine kidney using a 2 MeV proton beam.](image)
sample matrix or backing material. The characteristic peaks correspond to either K\(\alpha\) and K\(\beta\) transitions for low to medium atomic number elements or L X-rays for heavier elements. The continuous background can be seen to possess a maximum at lower energies. The decrease in the continuum at the lowest energies is due to the absorption of X-rays in the detector and its filters and the effect of sample self absorption. The spectrum may also be complicated by the presence of silicon K escape peaks and sum peaks.

2.2.1 Characteristic X-rays

The nature of characteristic X-rays may be understood by the use of the Bohr model of the atom. In this, orbital electrons are classified in shells and designated by means of quantum numbers. These electrons may be grouped together into the K, L, M, etc (Figure 2.2) shells of decreasing binding energy, and with the exception of the K shell, each consists of a number of subshells of slightly different energy.

Figure 2.2 may be redrawn in the form of an energy diagram (Figure 2.3) and used to illustrate the possible electron transitions for an ionised atom. In this diagram the more strongly bound electrons are near the bottom. When ionisation occurs an electron from an outer shell moves to fill the vacancy in the inner shell, and in theory, an X-ray is emitted (see section 2.2.2). The important transitions are tabulated below.

Table 2.1 The important electron level transitions to PIXE analysis.

<table>
<thead>
<tr>
<th>X-ray line</th>
<th>K series</th>
<th>L series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial atomic level</td>
<td>Final atomic level</td>
<td>Initial atomic level</td>
</tr>
<tr>
<td>(\alpha_1)</td>
<td>K</td>
<td>L(_3)</td>
</tr>
<tr>
<td>(\alpha_2)</td>
<td>K</td>
<td>L(_2)</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>K</td>
<td>M(_3)</td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>K</td>
<td>(N_3 + N_2)</td>
</tr>
<tr>
<td>(\beta_3)</td>
<td>K</td>
<td>M(_2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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</tbody>
</table>
Figure 2.2 The Bohr atomic energy levels illustrating orbital electron grouping [DYS90].

Figure 2.3 Atomic level diagram showing the main K and L X-ray transitions [JOH88].
The important features to note about the \( K \) series are the \( \alpha_1, \alpha_2 \) doublet and the \( K_{p_1} \) and \( K_{p_2} \) lines which are also resolvable doublets in heavier elements. The \( K_{p_1} \) lines lie to the right of the \( K_{\nu} \) lines in the energy spectrum (at a higher energy) by 2.5% for Na to about 15% for U [DYS90]. In the \( L \) series the strongest line originates from the \( L_m \) energy level as this contains the most electrons (and hence are the most probable of being ejected) and end in the \( M_{p_1} \) and \( M_{n_1} \) levels. These are transitions \( L_{\nu_2} \) and \( L_{\nu_1} \) respectively and their photon energies lie between 1/10 and 1/6 of the \( K \) spectra.

### 2.2.2 Characteristic X-rays from proton bombardment

#### 2.2.2.1 Ionisation cross-sections

When a proton of the order of MeV energy impinges upon a target there is a high probability that an inner shell electron will be excited and removed from the incident atom. This probability is of the order of \( 10^{24} \) cm\(^2\) (barns). The main interaction between this proton and target atom is related to the Coulombic fields of the particles. A number of models have been developed to describe this process, the three most commonly used being the plane wave Born approximation (PWBA), the semi-classical approximation (SCA), and the binary encounter approximation (BEA) [MIT81].

Johansson and Johansson [JOH76] employed the BEA model and took advantage of it offering a scaling law for the ionisation cross-sections including only electron binding energy and proton energy. They expressed the fundamental relationship of the BEA in the parameterised form:

\[
\ln(\sigma_i\mu_i^2) = \sum_{n=0}^{5} b_{i,n}[\ln(\frac{E_p}{\lambda U_i})]^n
\]  
(Eq 2.1)

where determination of the parameters \( b_{i,n} \) was by the fitting of a 5\(^{th}\) order polynomial to the experimental proton ionisation data available at that time. The values of the coefficients of \( b_{i,n} \) may be found in Table 2.2. In equation 2.1, \( i \) represents the vacant shell (\( K \) or \( L \)), \( \sigma_i \) the cross-sections (in units of \( 10^{-14} \) cm\(^2\)), \( E_p \) the proton energy (eV), \( U_i \) the \( K \) or \( L \) shell ionisation energy (averaged over the three sub-shells in the latter case) and \( \lambda \) the ratio of proton to electron mass (\( \lambda = 1836.1514 \)).
Table 2.2 Fitted coefficients for calculation of $\sigma_k$ and $\sigma_L$ via equation 2.1.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>2.0471</td>
<td>3.6082</td>
</tr>
<tr>
<td>$b_1$</td>
<td>$-6.5906 \times 10^{-3}$</td>
<td>$3.7123 \times 10^{-1}$</td>
</tr>
<tr>
<td>$b_2$</td>
<td>$-4.7448 \times 10^{-1}$</td>
<td>$-3.6971 \times 10^{-1}$</td>
</tr>
<tr>
<td>$b_3$</td>
<td>$9.919 \times 10^{-2}$</td>
<td>$-7.8593 \times 10^{-5}$</td>
</tr>
<tr>
<td>$b_4$</td>
<td>$4.6063 \times 10^{-2}$</td>
<td>$2.5063 \times 10^{-3}$</td>
</tr>
<tr>
<td>$b_5$</td>
<td>$6.0853 \times 10^{-3}$</td>
<td>$1.2613 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Although this expression is the most simple and holds well for elements of low atomic number, deviations occur as we move to heavier elements e.g. Ag. However, advances in PIXE require that cross-sections be calculated more accurately. Development of the PWBA model resulted in the ECPSSR treatment. This treatment involved the correction for energy loss ($E$) during the collision, deflection and velocity change of the projectile due to the Coulombic field ($C$), perturbation of the atomic stationary states (PSS) by the projectile and relativistic effects ($R$) [JOH88]. An extensive publication of the ionisation cross-sections for the K, L$_{\pi}$, L$_{\mu}$ and L$_{\mu}$ subshells by ECPSSR may be found by Cohen and Harrigan [COH85]. These are found to be adequate for use in K shell ionisation in PIXE. A graphical representation of these cross-sections for selected elements is found below (Figure 2.4). Note the increase in ionisation cross-section for target element $Z$ as the proton energy $E_p$ increases, reaching a maximum with the velocity of the projectile matching that of the ejected $i$th shell electron. The proton energy is given by $E_p = (M/m)U_i(Z)$ where $m$ and $M$ are the mass of the electron and proton respectively. Further increase in the proton energy results in a slow fall off of the cross-section, while another notable feature is the sharp fall off of $\sigma$ with $Z$.

2.2.2.2 X-ray production cross-sections

The concept of ionisation cross-sections for proton bombardment has been discussed. However, the cross-section of greater importance to PIXE analysis is that predicting the probability of X-ray production rather than initial shell ionisation. This is given for K-shell transitions by:
Figure 2.4 Dependence of ionisation cross-sections with proton energy and target atom Z number. Values predicted by the ECPSSR model [COH85].

\[ \sigma^X_K = \sigma^I_K \omega_K k \]  

(Eq 2.2)

where \( \sigma^X_K \) and \( \sigma^I_K \) are the X-ray production and ionisation cross-sections respectively, \( \omega_K \) is the fluorescence yield and \( k \) the transition probability. A similar relationship applies to the L shells although this is more complex due to the multiplicity of the subshells.

2.2.2.3 Fluorescence yield

When an atom becomes ionised, it may return to a state of lower energy by emitting an X-ray photon when an electron in a higher shell fills the vacancy in the lower shell. Alternatively a radiationless transition may take place. These radiationless transitions are in the form of Auger and Coster-Kronig effects. In the Auger process, the energy associated with the X-ray is used to create a vacancy in a higher shell with the ejection
of an electron. Hence, although the initial X-ray photon will not be emitted, as its energy was used to create an Auger electron, a vacancy in a higher shell will exist which may produce the emission of a characteristic X-ray of higher order.

The Coster-Kronig effect involves a redistribution of electrons in atomic subshells of the same principal quantum number (and hence the K shell is excluded from this process). For example, in the L shell, electrons may undergo radiationless transitions from \( L_{\text{m}} \) to \( L_{\text{n}} \) and \( L_{\text{r}} \) and from \( L_{\text{n}} \) to \( L_{\text{p}} \). The energy that becomes available from this may cause the ejection of electrons from outer shells (e.g. M) whose binding energy is smaller than that made available by the Coster-Kronig transition.

These competitive effects to the emission of characteristic X-rays are described by the fluorescence yield \( \omega \). This is defined as the ratio of emitted X-rays due to the transitions of a particular shell to the number of primary vacancies created in that shell. The relationship of \( \omega \) (K and L shells) with atomic number may be seen in Figure 2.5. Note that the fluorescence yield for both shells increases with Z number. A semi-empirical formula has been derived for \( \omega \) and is given by:

\[
(\frac{\omega_i}{1-\omega_i})^{1/4} = \sum_{n=0}^{3} B_{i,n} Z^n
\]  
(Eq 2.3)

where \( i \) is the K or L shell, \( Z \) the atomic number and \( B_n \) fitted coefficients which may be found in Table 2.3 [BAM72]. Attention must be drawn to the fact that \( \omega_i \) is the mean value for that shell.

Experimental values of fluorescent yield for the K shell are known precisely, and there is a good fit of data to equation 2.3 (uncertainty for \( \omega_k \) ranges from 3.5 % for

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>( K )</th>
<th>( L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B_0 )</td>
<td>((3.70\pm0.52) \times 10^{-2})</td>
<td>(0.17765)</td>
</tr>
<tr>
<td>( B_1 )</td>
<td>((3.112\pm0.044) \times 10^{-2})</td>
<td>(2.98937 \times 10^{-3})</td>
</tr>
<tr>
<td>( B_2 )</td>
<td>((5.44\pm0.11) \times 10^{-5})</td>
<td>(8.91297 \times 10^{-5})</td>
</tr>
<tr>
<td>( B_3 )</td>
<td>(-(1.25\pm0.07) \times 10^{-8})</td>
<td>(-2.67184 \times 10^{-7})</td>
</tr>
</tbody>
</table>

Table 2.3 Coefficients for the calculation of fluorescent yields for K and L shells.
Z=10 to 20, to 0.3-0.5 % for Z greater than 40). However such precise data is not available for the L shell and hence conclusions about the accuracy of, for example, Krause's [KRA79] extensive semi-empirical values are difficult.

2.2.2.4 Relative X-ray transition probability, \( k \)

Radiative transition at a particular energy level may give rise to the emission of more than one X-ray line as transitions may originate from different energy states. This is the case for the L X-ray where photons may come from a multiplicity of states. The fraction of a specific X-ray line emitted with respect to the total number of X-rays from that shell is known as the relative X-ray transition probability, \( k \). Reference values may be found in Salem et al [SAL74].
2.2.3 Background radiation from proton bombardment

The trace elements of interest in PIXE analysis are always present within or upon a matrix, be it a carbon backing on which a material has been deposited or an organic matrix in the case of the analysis of biological materials, and mostly for this reason, characteristic peaks are superimposed on a background radiation continuum. This background may originate from a number of effects which includes projectile bremsstrahlung, secondary electron bremsstrahlung, Compton scattered gamma radiation from nuclear reactions, charge build up from poor electrically conducting samples and environmental sources [KHA81a]. The magnitude of this background sets a limit to the sensitivity which can be obtained as characteristic peaks have to be differentiated from the continuum, and thus it is of great interest to reduce it to the lowest possible levels.

2.2.3.1 Projectile bremsstrahlung

When a projectile passes through a material the deceleration it experiences from the Coulombic encounters with bound electrons results in the emission of bremsstrahlung photons, the energies of which are distributed from zero to the maximum projectile energy. The intensity of these photons is proportional to \( (F/M)^2 \) where \( F \) is the electrostatic force on a projectile of mass \( M \). For an electron and proton, where the force will be the same, it is evident that the background for electron bombardment will be far greater than that for a proton (by a crude estimate of 1836\(^2\) times). The differential cross-section for the production of projectile bremsstrahlung is given by:

\[
\frac{d\sigma_b}{dE_x} = c \cdot \frac{A_p Z_p^2 Z_m^2}{E_p E_x} \frac{Z_p - Z_m}{A_p - A_m}
\]

(Eq 2.4)

where \( E_x \) is the energy of the background radiation, \( A_p, Z_p \) and \( E_p \) are the projectile atomic mass, atomic number and initial energy and \( Z_m \) and \( A_m \) that for the matrix, and \( c \) is a slowly varying factor which is dependent upon \( Z_p, Z_m \) and \( E_p \). For a low atomic number matrix, as for example a biological specimen, the ratio \( Z_m/A_m \) is approximately 0.5 and thus it is advantageous to select a projectile where \( Z_p/A_p \) takes a similar value e.g. \(^{2}He\). Then the background due to the projectile bremsstrahlung will approach zero. However, this has minor experimental importance as other background sources predominate over this effect in most practical situations [JOH88].

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2.2.3.2 Secondary electron bremsstrahlung

This mode of background radiation originates from the ejection of secondary electrons from target atoms during the collision process and predominates over projectile bremsstrahlung. It may be shown that the maximum permissible energy transfer from projectile to electron is predicted by:

\[ E_{e}^{\text{max}} = 4 \left( \frac{M_e}{M_p} \right) E_p \]  
(Eq 2.5)

where \( M_e \) and \( M_p \) are the mass of the electron and projectile respectively. Therefore, background due to this effect will be biased toward low energies and decrease rapidly beyond \( E_{e}^{\text{max}} \). Secondary electron bremsstrahlung is further complicated by the fact that it is emitted anisotropically [MIT81].

The maximum background is found to be at 90° to the incident beam and decreases as this angle increases. Therefore, this background may be reduced by suitable placement of the Si(Li) detector within the target chamber and also reducing the sample matrix to as thin as possible so that secondary electrons may leave the target with decreased probability of creating a bremsstrahlung background.

2.2.3.3 Gamma ray background

A γ ray background occurs where there are reactions, \((p,\gamma)\) or \((p,\alpha\gamma)\), between nuclei of the constituent elements within the sample under investigation and the incident proton beam. The most common reactions are seen for the elements \(^{19}\text{F}\), \(^{23}\text{Na}\) and \(^{27}\text{Al}\) which are often found in materials and have large resonance cross-sections for protons with energy less than 3 MeV. Emitted γ rays may either be Compton scattered and seen as a high energy tail in the X-ray spectrum, or be of sufficiently low energy to appear as discrete peaks and thus confused with characteristic X-rays. Gamma ray emissions for elements with \(Z>30\) at particle energies 3-5 MeV have been found to be a dominant background source [FOL74a], and thus it is recommended that proton energies be kept to a minimum, but not decreased sufficiently to compromise X-ray yields. Also, for heavier particles of the same velocity as protons, and hence greater energy, the resonance cross-sections for γ ray emission are also larger. Thus it is of no great surprise that protons between 1-2 MeV energy are recommended as the best choice for reducing this
2.2.3.4 Background due to charge build-up

When samples are electrically non-conducting, there may be problems related to charge build-up. The resultant discharge of these samples culminates in the emission of bremsstrahlung background extending up to tens of keV of high intensity. The consequence of this discharge is a poorer signal to background ratio which leads to a higher minimum detection limit. Also system dead time may be increased. This may be combated by a lower beam current but this results in longer analysis times. An example of a PIXE spectrum observed when analysing a charging specimen is seen in Figure 2.6. This may be compared to a more usual spectrum, of similar composition and roughly the same collected charge (Figure 2.1), where the background is at a much lower level and characteristic peaks are easily discernable. The explanation for the high background due to sample discharging, as seen in Figure 2.6, is the presence of fat at this sampling point, an effect also noted by Arshed [ARS91].

![Figure 2.6 Example X-ray spectrum from the 2MeV PIXE analysis of a porcine kidney specimen exhibiting sample charging.](image)
A number of possibilities exist to reduce the background due to this effect. These include depositing an ultra thin conducting material upon sample surfaces (usually by evaporation) [PAP78, FOL74b] and mixing conducting powder with the sample before production into pellets [JOP62]. Carbon and aluminium have been employed for these purposes, and should be chosen to closely match sample matrices. Obviously, there is a finite probability that contamination may be introduced whilst executing these techniques (although this should be small if care is taken) and thus it is recommended that quality assurance checks are carried out in conjunction. An alternative approach may be adopted where electrons are sprayed from an electron gun which neutralises positive charge build-up from a proton beam and good results are obtained [AHL75a]. Ahlberg et al [AHL75a] also point out that sample charging may be eliminated by increasing the chamber pressure with little effect upon quantitative measurements.

2.2.3.5 Environmental background

As long as normal laboratory procedures are adhered to, the background contribution from this can be assumed to be negligible. This was confirmed for the experimental set-up in this work.

2.2.4 Sensitivity

One of the main advantages of the PIXE technique is its high sensitivity. This may be defined in a number of ways and is dependent upon many factors. The most commonly employed definition of sensitivity is the minimum detectable concentration. As elements of interest are always contained within a matrix which generates a background continuum, then this will dictate the sensitivity of PIXE analysis as characteristic peaks have to be differentiated from it (see section 2.2.4.1). As discussed previously, reducing the thickness of the sample (see section 2.2.3.2) or ashing materials [JOH76] may reduce this background thus improving sensitivity. Hence it is difficult to present accurate values for this measurement as it is dependent upon experimental conditions, but best values in the range 0.1-1.0 ppm are usually quoted.

Khaliquzzaman et al [KHA83] define sensitivity as X-ray yield per unit concentration per unit charge (counts/ppm.μC) and plot this as a function of element atomic number. A similar graph may be seen in Figure 2.7. This contains data for
Figure 2.7 Sensitivity variation for Kα X-rays as a function of atomic number Z.

2MeV proton analysis of animal muscle and Bowen’s kale standards.

Bowen’s kale and animal muscle (H-4) standards [MUR85] from bombardment by a 2 MeV proton beam. Note that the magnitude of sensitivity will be highly dependent upon experimental conditions e.g. source-detector spacing, absorption of X-rays in detector filters, detector efficiency. However, the nature of the curves may mostly be explained by discussion of fluorescence yield and ionisation cross-section (see section 3.3.3.3 for the contribution from detector efficiency). That is, for lower Z elements the smaller sensitivity is due to the fall off of fluorescence yield, whilst toward heavier elements this is due to the decrement of the ionisation cross-sections. Note that a maximum sensitivity is observed, the position of which is dependent on proton energy (the higher E_p the higher Z number this occurs). Therefore, within certain limits, the proton energy may be chosen to yield maximum sensitivity for an element of interest. Note that similar curves may be derived for L X-rays which peak at higher Z numbers. For a 2 MeV proton beam, the highest sensitivities are usually seen for Z=20-40 (K X-rays) and Z=75-92 (L X-rays), i.e. the elements generally of most interest in biological and environmental studies.
2.2.4.1 Minimum detection limits

For an X-ray spectrum, characteristic peaks are required to rise above the continuous background to be discerned. The presence of a peak, however does not guarantee that the element from which it originates is present within the sample. Currie[CUR68] developed criterion to allow us, with some degree of confidence, to decide this. This concept, known as the Minimum Detection Limit (MDL) is given by:

\[ MDL = N_s \geq f \sqrt{N_B} \]  

(Eq 2.6)

where \( N_s \) is the number of counts in the photopeak, \( N_B \) the number of counts in the background under the photopeak, and \( f \) the degree of confidence in the determination of the MDL. The values for \( f \) of 1, 2, 3 and 3.29 constitute confidence levels of 68%, 95.7%, 99.7% and 99.9% respectively. A plot of MDLs for the spectra shown in Figures 2.1 and 2.6 as a function of \( Z \) is seen in Figure 2.8.

![Figure 2.8 Minimum detection limits for porcine kidney. Values for a normal spectrum and one exhibiting sample charging are shown.](image)
The MDLs have been normalised to charge so that values for each spectrum may be compared and a value of \( f = 3.29 \) taken. It is apparent that the increased background from sample charging in Figure 2.6 degenerates the ability of the technique to detect small quantities of elements within sample matrices.

2.3 Mathematical formulation

There are three distinct categories that a sample for PIXE analysis may lie in. These are split upon the basis of the degree of proton stopping and X-ray attenuation that they exhibit, and thus named thin specimens, intermediate specimens and thick specimens. As all of the analysis in this work is performed upon thick specimens then this is the only category that will be discussed in depth. However, a brief summary is included for both thin and intermediate specimens.

2.3.1 Thin specimens

In the case of thin specimens the incident proton beam is assumed to experience negligible energy loss whilst transversing the sample and X-rays produced are not subject to any major degree of attenuation whilst leaving the specimen. This greatly simplifies the relationship between X-ray yield and ionisation cross-section as \( \sigma \) takes one value only for the incident proton energy (this will become apparent later). Additionally, no correction for X-ray sample self absorption is needed. Further advantages of employing thin samples are the reduction in bremsstrahlung background (as long as materials that the samples are deposited upon or mounted upon, as they frequently need to be, are sufficiently thin and composed of low Z materials that contain no elements with high nuclear reaction cross-sections e.g. Na [JOH83]) and negligible spectral interferences from X-rays generated by secondary fluorescence [WIL77]. Disadvantages of thin specimen PIXE analysis include potential contamination from sample preparation procedures, spurious X-ray signals originating from backing materials [RUS81], inability to withstand large beam current densities and low X-ray signal intensities as a result of insufficient quantities of elements. However, with thought, many of these effects may be minimised, and thin PIXE analysis has been shown to perform successfully in the analysis of, for example, microtome sections of biological materials mounted upon supportive backings [HEC87], acid digested samples deposited upon backing foils [TAN87], and environmental
pollution residues that have been deposited upon filters by suction of the atmosphere through them [HAN87] which is particularly convenient as the sample needs very little further treatment before PIXE analysis.

2.3.2 Intermediate specimens

In practice, very few 'thin' samples satisfy the criteria discussed in the previous section, and exhibit some degree of proton stopping and X-ray attenuation. Now, the relationship between X-ray yield and ionisation cross-section is not so simple as $\sigma$ is required to be integrated over the proton energy range. Also, the mass stopping power (see section 2.3.3.2), which is dependent upon energy, and X-ray attenuation have also to be incorporated into this relationship. It should be noted here, that protons are not completely stopped within the sample and thus a knowledge of the specimen thickness and composition is required. Determination of specimen thickness can be performed in a number of ways which include direct weighing, photon transmission measurements, and evaluation of the energy loss of protons. Carlsson et al [CAR81] discuss the analysis of intermediate specimens with respect to filters obtained from aerosol studies using a cascade impactor and claim that an accuracy of better than 5% is obtainable whilst employing their corrections for ionisation cross-sections, proton slowing down and X-ray attenuation.

2.3.3 Thick specimens

Thick specimens are those that completely halt the proton beam within them. The yield of X-rays from an element of concentration $C_z$ for these samples is given by:

$$Y(Z) = \left( \frac{N_A \omega_2 \beta_2 e_Z}{A_Z} \right) N_p C_z \int_{E_0}^{0} \frac{\sigma_Z(T(E)) T_Z(E)}{S(E)} dE \quad (Eq \ 2.7)$$

where $N_A$ is Avogadro's number, $\omega$ the fluorescent yield, $\beta$ the branching ratio ($\kappa$), $e_Z$ the intrinsic detector efficiency, $A_z$ the mass number, $N_p$ the number of protons, $\sigma_Z$ the ionisation cross-section, $T_Z$ the X-ray attenuation and $S$ the proton stopping power. The X-ray attenuation $T_Z$ is predicted by:
\[ T_2(E) = \exp\left(-\frac{\mu}{\rho} \frac{\cos \alpha}{\sin \theta_{TO}} \int_{E_0}^{E} \frac{dE}{S(E)}\right) \quad \text{(Eq 2.8)} \]

where \((\mu/\rho)\) is the mass attenuation coefficient of the matrix, \(\alpha\) the angle of the incident proton beam to the sample surface normal, and \(\theta_{TO}\) the X-ray take-off angle to the sample surface. The generalised geometry is shown in Figure 2.9. In equation 2.7, the integral has limits from the incident proton energy \(E_0\) to \(E=0\). In the case of intermediate targets the upper limit will take a value \(E>0\), dictated by the sample thickness, whereas this integral will not be present for thin targets and a slightly different equation predicted (without any treatment for proton slowing and X-ray attenuation). Obviously, for the calculation of X-ray yields for this type of sample, a knowledge of its matrix composition is required although there are certain iterative techniques available in some circumstances to circumvent this.

Without doubt, the main advantage of thick target PIXE (TTPIXE) analysis is the lack of sample preparation required. Samples are usually in the form of pellets made...
from homogenised powdered material or require no or little sample preparation as for a wide variety of objects ranging from bone sections to those of archaeological interest such as pottery. Obviously, the minor sample preparation required reduces the time invested in this activity, but more importantly lowers the risk of introducing contamination.

Disadvantages of the employment of this type of sample include the numerical integration of equation 2.7 as this cannot be solved analytically, and the reliance upon a data base for \( \sigma(E) \) and \( S(E) \). Other potential difficulties can arise from samples not satisfying surface or geometric requirements (see sections 5.4 and 5.5), specimen charging (section 2.2.3.4) and heating (section 4.6.1), charge integration, instability of the sample under vacuum conditions and secondary fluorescence [CAM84, CLA87a]. Most of these factors will be discussed further in this or other chapters.

2.3.3.1 Secondary fluorescence

Equation 2.7 neglects the presence of any secondary fluorescence effects which occur from bremsstrahlung due to protons and secondary electrons, secondary and Auger electrons, and primary induced X-rays. The contribution to secondary fluorescence has been proven to be negligible in the case of bremsstrahlung and electrons, thus leaving the only significant source for secondary excitation being primary induced X-rays [AHL77]. These may enhance X-ray signals for a specific element within a sample. This enhancement has been shown to be significant for elements whose K-shell energy lies just below that of an intense X-ray line and mostly effects the analysis of metallic specimens. Although secondary fluorescence is assumed to be negligible in the case of thick biological samples, often without proof, it has been shown to be significant for K, Cl, S and P in teeth from the enhancement by Ca [AHL76]. Reuter et al [REU75] provide an approximate expression for estimating the degree of secondary fluorescence enhancement that an element exhibits upon other lower Z elements within that sample. This expression yields a value for \( R_A^B \), the ratio of the number of K\( _a \) X-rays of element A arising from fluorescence induced by K\( _a \) X-rays of B to the number of proton-induced K\( _a \) X-rays of A and is given by:

\[
R_A^B = 0.5 C_B \frac{\mu_{K_a,R_A}}{\mu_{K_a,s}} \frac{r_A^{-1} \sigma_B(E_0) \sigma_A}{r_A \sigma_A(E_0) \sigma_B} \omega_{K_B} \tag{Eq 2.9}
\]
where \( C_g \) denotes the concentration of B whose fluorescence yield is \( \omega_{g,B} \), \( r_A \) the K edge jump ratio for photon attenuation in A, \( \sigma_g(E_0) \) and \( \sigma_A(E_0) \) the ionisation cross-sections at the incident proton energy, \( \mu_{k_{A,B,A}} \) and \( \mu_{k_{A,B,S}} \) the mass attenuation coefficients for B's K\( _A \) X-rays in element A and the specimen respectively and \( a_A \) and \( a_B \) the atomic masses.

As very little data is available for secondary fluorescence effects in biological samples, equation 2.9 was employed to calculate enhancements for selected elements in a biological matrix, which was assumed to be composed solely of carbon. Mass attenuation coefficients and K-edge jump ratios were taken from Storm and Israel [STO70], fluorescence yields and K-energy data from Cohen and Harrigan [COH85] and Salem et al [SAL74] and ionisation cross-sections from tabulated ECPSSR fitted data for 2 MeV protons [JOH88]. Values of enhancement ratio multiplication factors (\( F \)) for Cu, Fe, Mn, Ca, K, Cl, S and P are presented in Table 2.4, as are values for K\( _A \) and K\( _B \) X-rays for enhancing elements (top and bottom respectively). Enhancement ratio multiplication factors are defined such that \( R^*_{A,B} = F \cdot C_g \). Thus, using the values of \( F \) in Table 2.4 one may calculate enhancement ratios for selected elements within a carbon matrix given the concentration of the enhancing element. Example enhancement ratios are also presented for bovine liver standard (NBS-SRM-1577a) [MUR85]. This standard was chosen as it contains relatively high concentrations of elements. Notice that \( R^*_{A,B} \) decreases as the difference between the K-edge of the enhancement element and the X-ray energy increases (as we move to lower Z elements), an effect also noted by Ahlberg [AHL77]. The highest value of \( R^*_{A,B} \) attained is approximately equal to 0.4% for the enhancement of Cl by K, the most abundant element (9960 ppm).

Thus, it is safe to assume that the effect of secondary fluorescence is negligible in thick biological specimens as X-ray intensities are generally too low. However, care must be exercised for other types of sample.
Table 2.4 Selected enhancement ratio multiplication factors and example ratios for elements within biological matrices (carbon).

<table>
<thead>
<tr>
<th>Element</th>
<th>Zn</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Ca</th>
<th>K</th>
<th>Cl</th>
<th>S</th>
<th>P</th>
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<tr>
<td></td>
<td>1.955</td>
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<td>5.54 \times 10^{-3}</td>
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</table>

Example enhancement ratios for bovine liver standard (NBS-SRM-1577a) [MUR85].
### 2.3.3.2 Stopping powers

When a proton beam traverses a material, it experiences an energy loss which is related to inelastic interactions with bound atomic electrons. As the mass of a proton is 1836 times more massive than that of an electron, then little kinetic energy is lost per collision and hence the path of the incident proton is left virtually unchanged. This greatly simplifies matters when calculating proton ranges. The parameter which describes the loss of energy of the proton is the mass stopping power $S(E)$ which is given by:

\[
S(E) = \frac{1}{\rho} \frac{dE}{dx}
\]

Equation 2.10  

i.e. the energy lost per unit mass thickness of the matrix. It is obvious that $S(E)$ is dependent upon the density $\rho$, and the elemental composition of the sample. The term $dE/dx$ is known as the linear stopping power and is the energy lost per unit distance.

A number of tabulations of stopping powers exist which includes those by Northcliffe and Schilling [NOR70], Anderson and Ziegler [AND77], and Ziegler et al [ZIE85], the latter two being the most useful and based upon the fit of parameters of semi-empirical modifications of the basic Bethe-Bloch and Lindhard theory. Although the data base of Ziegler et al [ZIE85] is the most recent, it is generally thought untested and hence values from Anderson and Ziegler's [AND77] semi-empirical fit are usually employed. In this, they split the energy range into three distinct regions and stopping cross-sections for each are predicted by:

**Region 1: 1-10keV**

\[
e(E) = A_1 E^{1/2}
\]

Equation 2.11a

**Region 2: 10-999keV**

\[
e(E) = \frac{S_{HIGH} \cdot S_{LOW}}{S_{HIGH} + S_{LOW}}
\]

Equation 2.11b
where

\[ S_{LOW} = A_2 E^{0.45} \]  

(Eq 2.11c)

and

\[ S_{HIGH} = \left( \frac{A_3}{E} \right) \ln \left[ 1 + \frac{A_4}{E} \right] \]  

(Eq 2.11d)

Region 3: \(10^3 - 10^5\text{keV}\)

\[ \epsilon(E) = \frac{A_3}{\beta^2} \ln \left( \frac{A_4 \beta^2}{1 - \beta^2} \right) - \frac{4}{\sum A_i (\ln E)^i} \]  

(Eq 2.11e)

where the stopping cross-sections \(\epsilon(E)\) has units \(\text{eV/(10}^{15}\text{ atoms/cm}^2)\) and \(E\) is the ratio of hydrogen energy to its mass \((E_p/M_{H})\) and has units of \(\text{keV/amu}\), \(\beta\) is the ratio of the projectile velocity to that of light \((v_p/c)\) and \(A_i\) the fitting coefficients which take different values for each element and may also be found in this reference.

The relationship between stopping cross-section and mass stopping power is given by:

\[ S(E) = \epsilon(E) \frac{602.22}{A_Z} \]  

(Eq 2.12)

where \(A_Z\) denotes the mass number of element Z.

2.4 Elemental concentration determination

In the determination of elemental concentrations in thick targets for PIXE analysis, two approaches may be taken. One method involves the employment of standards, be it a known concentration of a non-interfering element within the sample under investigation (internal standard) or a number of elements of known concentration in a matrix similar to that being analysed (external standard). This approach is known as
comparative analysis. The alternative method, known as absolute analysis, is dependent upon the accurate knowledge of experimental parameters and relies heavily upon the precision of parameter data bases.

2.4.1 Comparative analysis

2.4.1.1 Internal standards

In this, samples to be analysed are spiked with a known amount of non-interfering element. The most common elements employed are yttrium and ruthenium as these are not usually present within biological and environmental specimens. Concentrations, \( C_e \), for a given element are then evaluated by:

\[
C_e = \frac{C_{st} Y_e M_{st}}{Y_{st} M_e}
\]  

(Eq 2.13)

where \( M_e \) and \( M_{st} \) are the calculated X-ray yields for the element and standard respectively (calculated from equation 2.7 assuming unit concentration), \( Y_e \) and \( Y_{st} \) are the measured yields, and \( C_{st} \) the concentration of the internal standard.

Advantages of this approach include the removal of uncertainties in current integration and geometric factors such as detector solid angle. Also the effect of beam damage upon sample matrix is lessened as long as this is not so great as to cause the loss of volatile elements from the specimen (section 4.6.1) [CLA87a]. However, there is still a large reliance upon accurate knowledge of detector efficiency and ionisation cross-section and X-ray attenuation coefficient data bases in this approach.

2.4.1.2 External standards

External standards may take the form of synthetically prepared materials or those readily available which are usually of a biological or environmental nature. A classic example of a synthetic standard is hydroxyapatite doped with high purity elemental standards as used frequently in the analysis of bone [CUA87]. Examples of reference materials used for the determination of trace elements in biological and environmental samples are numerous and include blood, kidney, hair, kale, and coal to name but a few.
However, for a material to constitute a good external standard, it must satisfy a number of criteria. These include possessing a similar matrix composition to the sample being analysed, containing a wide variety of trace elements that are certified in concentration, and being as elementally homogeneous as possible [LaF74,LIB84]. The latter requirement is essential in PIXE analysis as the mass of sample probed by the proton beam is typically very small (of the order 10-100μg).

The concentration of an element in a sample calculated using an external standard is given by

$$C_e = \frac{C_s Y_s I_s(E_p)}{Y_s I_s(E_p)}$$

(Eq 2.14)

where \(C_s\) is the concentration of the same element in the standard, \(Y_s\) and \(Y_e\) are the measured X-ray yields of the element in the sample and standard respectively, and \(I(E_p)\) an integral which is given by

$$I(E_p) = \int_{E_p}^{E_0} \frac{\sigma(E)T(E)}{S(E)} dE$$

(Eq 2.15)

where \(\sigma(E)\), \(T(E)\) and \(S(E)\) hold the same definitions as they do in equation 2.7. Note that in equation 2.14, equal charge has been assumed for sample and standard.

The advantages of the external standard method are the removal of uncertainties in detector efficiency, and to a large extent minimisation of uncertainty in both the ionisation cross-section and attenuation coefficients. Extensive discussion of this technique may be found by Biswas et al [BIS81] and Khaliquzzaman et al [KHA81b,KHA83].

### 2.4.2 Absolute analysis

In some instances, trace elements of interest are not present or are undetectable in reference materials or the reliance upon standards is required to be relaxed. In the former cases, comparative analysis is either not possible or difficult and quantitative
determinations of elements is carried out upon an absolute basis. Calculation of the concentration of element Z is then achieved by rearrangement of equation 2.7 to make $C_z$ the subject of the equation. Now, in theory, the measurement of the X-ray yield, collected charge, and knowledge of the detector efficiency should enable us to evaluate the corresponding concentration. However, as one would expect, this is a simplistic view as the absolute approach relies heavily upon a large data base, the accuracy of which ultimately decides the uncertainty in results [CLA81]. Hence, not only does the accuracy of the charge collection and detector efficiency measurements dictate this uncertainty, but also the X-ray production cross-sections, proton stopping powers and mass attenuation coefficients employed.

The most convenient method of evaluating the performance of the absolute analysis technique is by measurement upon a sample of known composition. Clayton [CLA81] prepares a multi-element standard consisting of known concentrations of trace elements within a graphite matrix. He compares theoretically derived X-ray yields to measured and concludes an average difference of 7% between values for this sample. Absolute analysis is also applied to Bowen’s kale and IAEA soil-7 standard reference materials by Arshed [ARS91]. Calculated concentrations were mostly within 30% of certified values. Short irradiation time and sample heterogeneity were the reasons given for the disagreements.

2.5 Rutherford backscattering analysis

In the quantitative analysis of trace elements in thick samples by PIXE, integration of the ionisation cross-sections over the proton energy range is necessary. The dependence of the energy of the proton with depth and also the attenuation of any created X-ray are dictated by the composition of the sample. In a biological or environmental sample, the matrix composition consists of low atomic number elements e.g. C, N, O, which cannot be determined by PIXE analysis. Proton backscattering however provides information concerning these elements and is convenient as it may be performed simultaneously with PIXE [CLA87a].

Rutherford backscattering spectrometry (RBS) employing protons provides information about mass and abundancy of atoms present within the volume of interaction by measurement of the energy of scattered protons (Figure 2.10). The initial proton energy
Figure 2.10 Schematic illustrating the scattering geometry in RBS analysis.

$E_0$ and scattered proton energy $E$ are related by:

$$E = K_{M_2} E_0$$

(Eq 2.16)

where $K_{M_2}$ is the kinematic factor and given by

$$K_{M_2} = \frac{\left( 1 - \left( \frac{M_1}{M_2} \sin \theta \right)^2 + \left( \frac{M_1}{M_2} \cos \theta \right)^2 \right)^{\frac{1}{2}}}{1 + \left( \frac{M_1}{M_2} \right)^2}$$

(Eq 2.17)

where $M_2$ and $M_1$ are the target atom mass and incident ion mass respectively, and $\theta$ the scattering angle with respect to the direction of incidence. The scattering cross-section

$$\frac{d\sigma}{d\Omega} = 1.296 \left( \frac{Z_1 Z_2}{E} \right)^2 \left[ \csc^4 (\frac{\theta}{2}) - 2 \left( \frac{M_1}{M_2} \right)^2 + \ldots \right] \text{ mb/sr}$$

(Eq 2.18)
governs the intensity of the scattered protons at a particular angle, and it is more usual to state it in its differential form (equation 2.18). One should note at this point that this differential cross-section applies only to Rutherford scattering situations, and that deviations may occur from this model for lower Z elements, especially when the incident ions are protons. These deviations may be due to nuclear resonance reactions and cross-sections in these cases are called elastic cross-sections (ECS). However, as the atomic number of the target atom increases, deviations of the scattering cross-sections from those predicted by the Rutherford model decrease (Table 2.5).

It was decided to follow Arshed [ARS91] and employ elastic scattering cross-sections for elements with Z<14 (silicon) and Rutherford scattering cross-sections for all other elements.

Table 2.5 Comparison of Rutherford scattering cross-sections (RSC) and elastic cross-sections (ECS) for C, N, O and Si.

<table>
<thead>
<tr>
<th>Element</th>
<th>Proton Energy MeV</th>
<th>Scattering Angle θ (degrees)</th>
<th>Cross-section (mb/sr)</th>
<th>Ratio ECS/RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBC</td>
<td>ECS</td>
</tr>
<tr>
<td>¹²C</td>
<td>2.0</td>
<td>170</td>
<td>11.68</td>
<td>60</td>
</tr>
<tr>
<td>¹⁴N</td>
<td>2.0</td>
<td>170</td>
<td>15.96</td>
<td>80</td>
</tr>
<tr>
<td>¹⁶O</td>
<td>2.0</td>
<td>165</td>
<td>21.30</td>
<td>82</td>
</tr>
<tr>
<td>²⁸Si</td>
<td>1.98</td>
<td>170</td>
<td>65.62</td>
<td>83</td>
</tr>
</tbody>
</table>

All values for ECS are taken from [RAU85] apart from that for oxygen which is taken from [LUO85].

A typical backscattering spectrum from a sample composed of a number of matrix elements takes on a step configuration, each of which corresponds to a particular element (Figure 2.11). It may be shown that for an element A in this sample, the height of its corresponding step $H_A$ may be expressed as a ratio to another element (B) step $H_B$ by [MAY77]:

$$H_A = \frac{H_A}{H_B}$$
Figure 2.11 Backscattering spectrum of animal muscle standard taken at 165° to the incident beam of 2MeV protons.

\[
\frac{H_A}{H_B} = \frac{\sigma_A}{\sigma_B} \frac{m}{n} \frac{e_0^B}{e_0^A}
\]  
(Eq 2.19)

where \(\sigma\) is the scattering cross-section, \(e_0\) the stopping cross-section factor and \(m/n\) the ratio of the number of atoms of element A to that of B. To a good approximation \(e_0^B/e_0^A\) may be taken as unity [CHU78] and thus equation 2.19 may be re-written in the form of equation 2.20.

\[
\frac{m}{n} = \frac{H_A\sigma_B}{H_B\sigma_A}
\]  
(Eq 2.20)

Using one step, usually that of carbon in biological and environmental samples, equation 2.20 may be used to calculate the relative number of atoms of other elements. This may then be converted into relative masses for each element within the sample.
Chapter 3
Experimental Considerations for PIXE Analysis

3.1 Introduction

The proton induced X-ray emission analysis of samples in this work was carried out at the SERC funded ion beam facility at the University of Surrey. This facility contains a 50-500 KV general purpose implanter, a 35-350 KV medium current implanter, a 500-2000 KV Van de Graaff HVE implanter and a 2 MV Van de Graaff accelerator, the latter being used for all ion beam analysis. The 2 MV Van de Graaff generator is housed behind concrete shielding blocks, and accelerated ion beams pass into the analysing magnet via the exit port (Figure 3.1). This analysing magnet directs the ion beam into one of the five available beam lines, line 5 being employed for all PIXE analysis, and then it is manipulated so that it is incident upon a specific sample. X-ray and proton backscattering data are fed directly from detectors to processing nuclear instrument modules, and dumped onto a mainframe computer for subsequent qualitative and quantitative analysis. This and other aspects of experimental set-up and data acquisition and analysis will be discussed fully in the next sections.

3.2 Van de Graaff accelerator

The idea of the Van de Graaff generator was first proposed by R.J. Van de Graaff in 1931 and functions upon the principle of producing a positive ion current by the charging of a high voltage terminal. This terminal charging is by the employment of an insulating belt, positive charge being deposited upon it at corona points outside the terminal, and taken off at a second corona point within the terminal. This serves to progressively increase the charge accumulated upon the surface of the electrode and thus increase the voltage. This occurs up to a certain point and is limited by the terminal capacitance. An ion source is also situated within the high voltage terminal which coincides with the beginning of the accelerator tube. This is the point where the ion beam begins its acceleration. Van de Graaff generators may be operated in either a vertical or horizontal position, the latter being employed in our case. The main advantage of this type of generator is the high stability of ion beam energy exhibited, a fluctuation value of
Figure 3.1 Schematic illustrating the 2 MV Van de Graaff facility with exploded view of beam line 5.
0.01% at best may be cited [PER68].

3.2.1 Beam line assembly

After leaving the Van de Graaff accelerator, the ion beam is steered into a specific beam line (hereafter meaning line 5) by the analysing magnet. This is achieved in a manner which is dependent upon ion charge to mass ratio and thus any foreign ions in the beam will be excluded from that line. The steered beam then passes along the beam line, through the open gate valve, which is present to isolate the Van de Graaff generator from the line, and onto the control slits. These are an important feature of the beam line as they provide a signal which is used to adjust the energy of the ion beam. The X and Y direction beam steering plates are located after the control slits and are used for course positioning of the beam within the target chamber. These are controlled by two stable 2 kV power units. The object aperture allows the beam spot diameter to be reduced which is necessary when focusing the beam in the latter stages of setting up. The beam spot diameter is of the order of 5 mm before this. There are four settings that this niobium aperture may take which are 25 μm, 50 μm, 250 μm and 1.0 mm. The quartz view port immediately following the object aperture is used for the setting of this diameter. The scanning deflection plates (X and Y direction) control small movements of the ion beam in the target chamber and may be connected to flapping units. These flapping units allow the voltage upon X and Y plates to be varied at specified frequencies allowing scanning of the beam over areas of the sample. The advantage of this is twofold. Namely, as an effectively larger beam spot size is being used, problems with sample inhomogeneity are lessened and beam current may be increased as current density is smaller. The latter issue is important in the analysis of biological and environmental samples which can only withstand finite currents before damage occurs. This has been shown to contribute to the falsification of results and will be discussed in depth later.

The quadrupole lens system, which is housed upon a base plate thus allowing vertical and horizontal adjustment, has four individual units and is intended to be used as a 'Russian Quadruplet'. Employed in this way, the whole system behaves as a single converging thick lens which is orthomorphic i.e. produces an image the same shape as the object [COO77]. The object aperture setting is one factor which dictates the size of the focused beam spot. For this work, the 1.0 mm aperture setting was chosen which allowed
focusing of the proton beam down to the order of 200 μm. The reason for this was the relatively high beam current available with this object aperture setting. Proton microprobe studies require high beam spatial resolution and thus in this case an aperture of 25 μm or 50 μm should be chosen allowing the beam to be focused down to at best 10 μm diameter. Another factor limiting beam spot focusing are real and parasitic aberrations of the quadrupole lens system. This is discussed in depth by Grime et al [GRI82,GRI84].

Finally, the beam line is separated from the target chamber by a mechanical gate valve. This allows isolation of the beam line whilst the target chamber is at atmospheric pressure as in the case of sample plate changing. The beam line has a base pressure of 4 x 10^{-7} torr. The pressure in the target chamber is reduced by means of a rotary pump down to 10^{-1} torr and then reduced further by a liquid nitrogen cooled diffusion pump to about 10^{-6} torr. Only when the target chamber reaches this pressure may the mechanical gate valve be opened.

A detailed account of the beam alignment and focusing procedure may be found in Appendix 1. Further discussion of this subject is also presented by Thornton [THO87] and Arshed [ARS91].

### 3.3 Organisation of the target chamber

The mechanical gate valve separating the target chamber from the beam line is composed of aluminium. Aluminium is a commonly employed material and has advantages over, for example, steel in that characteristic X-rays are of a suitably low energy and may be excluded from detection by use of various filters [JOH76]. These characteristic X-rays, which originate from scattered protons interacting with the target chamber may be significant in intensity if the construction is of steel, and X-ray lines may be enhanced for Mn, Cr, Ni and Fe i.e. elements that constitute large proportions of this alloy [WEA86]. However, the utilisation of aluminium in chamber construction is disadvantaged by the fact that intense γ-radiation backgrounds may be observed from nuclear reactions ((p,γ), (p,αγ), (p,γ) and (p,γ)). These range from yields of 5.15 x 10^2 γ-rays/μC.Sr at 2.836 MeV to 4.0 x 10^4 γ-rays/μC.Sr at 1.013 MeV for a 2 MeV proton beam. The elements in steel have yields of roughly 10^2 to 10^3 γ-rays/μC.Sr [KEN80]. One method to suppress the background due to this effect is to cover the target chamber with carbon foil, a method adopted by Raith et al [RAI77] with good results. However, the
Figure 3.2 Three-dimensional illustration of the target chamber of beam line 5.
background due to this effect is not seen to be significant in X-ray spectra collected from our experimental configuration, as little high energy tailing is observed (see section 2.2.3.3). Access into the target chamber is by the removal of a plate at the rear, which is retained in place by the vacuum. Once inside the chamber, one may see the goniometer with connections for current integration and electron suppression, the optical viewing system, Si(Li) detector, surface barrier detector and view port window for illumination of the chamber. The position of these is illustrated in Figure 3.2 and nearly all will be individually discussed in the following sections.

3.3.1 Goniometer

The goniometer is a rectangular aluminium frame that the sample plate is mounted upon and may be adjusted in or out along the beam direction. Various movement of the sample is possible which includes translation in a plane which is perpendicular to the incident beam, and tilt in two axes. One of these axes is represented by $\alpha$ in Figure 2.9 whilst the other is orthogonal to this and is at right angles to the incident beam direction when $\alpha=0^\circ$. Tilt in either of these directions is employed in channelling and depth profile experiments and not employed in this work and always set to zero. Lateral translation of the goniometer is controlled by a stepper motor control, the discrete distance being set by the user. This is used to position individual samples in front of the beam. Samples are frequently in pelletised form and mounted upon an aluminium plate (aluminium plate for reasons stated earlier). Approximately 15 pellets of 5 mm diameter and 1-2 mm thickness may be attached to each plate which forms a desirable configuration as time invested in sample changing with associated evacuation of the target chamber is considerably reduced. Sample mounting upon these plates will be discussed in depth later. The sample plate is attached to a plastic electrically insulating mounting and held in place by a screw and current integration clip. There is also a circular metal turntable upon this plastic strip upon which a glass slide is attached and employed in the beam focusing procedure.
3.3.1.1 Current integration

Accurate determination of the number of protons incident upon samples in proton induced X-ray emission analysis is essential. In the case of thin or intermediate specimens this task is straightforward as proton charge is measured via a beam stop or Faraday cup positioned behind the sample [WAT82, VAN84]. This is frequently composed of graphite to minimise any contribution to background from γ-rays due to nuclear reactions. However, as the thickness of the specimen increases, charge integration becomes more difficult and may be complicated by sample charging and secondary electron effects. Approaches to combat sample charging have been discussed in section 2.2.3.4 and hence will not be pursued further. Secondary electrons, which are extensively discussed by Matteson and Nicolet [MAT79] may reach the target chamber as a result of their production along the beam line and may cause erroneous charge readings. This problem may be solved by the placement of positively biased electron stripping electrodes in front of the chamber. There is also a possibility that secondary electrons may leave the surface of the sample. This may be tackled in a number of manners, two solutions being the placement of a negatively biased ring in front of the target, the other applying a positive bias to the target plate. The latter configuration is adopted in our work and will be discussed in the following section. Another source of error in the charge integration process is associated with proton scattering in the target material. However the loss of protons from this effect is small and estimated to be less than 0.01% [CHU78].

Samples are mounted upon target plates by double sided adhesive tape. This tape is electrically insulating and hence a conducting path must be created by the application of carbon dag from the side of samples which extends to the plate surface. Carbon dag is a high purity carbon powder in a suspension of high purity water. The current integration signal is extracted from the charge integration clip which affixes the plate to the insulating plastic backing. The efficiency and variability of the current integration process is presented within the experimental chapters.

3.3.1.2 Electron suppression

Originally in line 5 design, there was a negatively biased ring electrode used to suppress secondary electron ejection from the sample surface. In theory, as this bias is made more negative, the K X-ray yield normalised to charge should increase and then
plateau, a relationship demonstrated by Malmqvist et al [MAL82] for Ag. However, this trend was not seen for our negative electrode [ARS91] which was attributed to non-concentricity of the beam with the ring. Thus this configuration for electron suppression was removed and replaced by application of a positive bias to the sample plate.

A number of authors have employed this method for secondary electron suppression, typical positive biases used being +90 V [MUS83] and +180 V [KNU80], although the figure of +90 V by Musket [MUS83] is not an optimised value. Investigation of the optimum bias on line 5 was investigated by Arshed [ARS91] with the measurement of Cu Kα X-ray yield with respect to applied voltage. This yield was noted to increase up to roughly +100 V and then plateau. A bias of +200 V was recommended for secondary electron suppression which was adopted throughout this work.

3.3.2 Optical viewing system

The optical viewing system is employed in a number of activities the nature of which dictates the mode in which it is operated. The viewing system consists of an eyepiece from which a view of the inside of the target chamber is seen. This view is formed by a number of lenses which may be placed in various positions yielding differing magnifications. The image is passed to these lenses by a 90° prism that views the target chamber. An objective lens may be placed in front of this prism which produces the maximum magnification of the lens configuration. This objective lens is mounted upon a moveable arm which is controlled outside of the chamber. Thus it may be removed from the vicinity of the sample when not in use.

When the optical viewing system is operated at maximum magnification an image of the sample surface is seen. This is used for two purposes, these being for the focusing of the ion beam by the quadrupole magnets (see Appendix 1) and for the goniometer placement. As samples placed upon target plates are inevitably of differing thicknesses, then the goniometer is required to be moved in or out along the ion beam axis so that the irradiation configuration is kept constant. By obtaining a focused image of the sample under investigation, this requirement is satisfied. With the optical system in low magnification, an image of the whole target plate is seen. This is used for coarse placement of the ion beam.
3.3.3 The Si(Li) detector

The most commonly employed photon detector in proton induced X-ray emission analysis is the lithium drifted silicon detector. This has many advantages over the hyperpure Ge detector which also provides the ability to perform high energy resolution spectroscopy although this is slightly inferior to that of the Si(Li) detector over the X-ray energy range. As the atomic number of silicon is lower than that of germanium, the hyperpure Ge detector provides superior efficiency especially for medium to high γ-rays. However, the efficiency of the detectors are comparable in the X-ray range although this is more complicated for Ge due to the K absorption edge at 11.10 keV whilst for Si this falls at 1.84 keV and hence has only minor importance in the detection of elements in PIXE as this technique usually concentrates upon \( Z \geq 17 \). The fact that the atomic number is low for silicon actually aids in the detection of X-rays as high energy γ-rays are highly transparent to this material [KNO89]. Also, X-ray escape peaks are less prominent in Si(Li) detectors.

![Si(Li) detector geometry for PIXE showing a) optimisation of efficiency](image)

Figure 3.3 Si(Li) detector geometry for PIXE showing a) optimisation of efficiency b) reduction of secondary electron bremsstrahlung background.

Two distinct detector placement configurations are usually employed in PIXE analysis (Figure 3.3). One such configuration employs the sample at an angle to the incident beam (\( \alpha > 0^\circ, \theta_{10} > 0^\circ \) Figure 2.9) whilst the other relies upon the sample surface
being perpendicular to the incident beam ($\alpha=0^\circ$, $\theta_{\text{rot}}=0^\circ$ Figure 2.9). The advantage of the latter geometrical set up is that background originating from secondary electrons is reduced. This was investigated by Kaji et al [KAJ77] who demonstrated that at $\alpha=0^\circ$, $\theta_{\text{rot}}=45^\circ$, the secondary electron bremsstrahlung is greatly reduced when comparing it to that at $\alpha=45^\circ$, $\theta_{\text{rot}}=45^\circ$ (90° to the incident beam direction). In fact, the maximum bremsstrahlung background has been found to peak at 90° to the beam. The disadvantage of the geometrical configuration with $\alpha=0^\circ$ is that sample-detector distances are increased which may in some circumstances offset the reduction in background seen in this set up [CAM81]. As secondary electron bremsstrahlung photons lie mainly below 5 keV, analysis when interest is centred upon the 10-15 keV region (e.g. Pb L X-rays) must concentrate upon efficiency maximisation. However, the angles employed in this work are $\alpha=0^\circ$ and $\theta_{\text{rot}}=45^\circ$ and hence maximisation centres upon background reduction in the lower energy region of X-ray spectra and thus greater sensitivities are acquired for lower Z elements i.e. those generally of interest in these studies.

The Si(Li) detector crystal is contained within a vacuum cryostat that extends from the dewar containing liquid nitrogen to the vicinity of the sample in the chamber. At this end is a Be window allowing low attenuation of X-rays before they are incident upon the crystal. Signals from this detector pass first to a pulsed optical feedback preamplifier before going onto further amplification and processing electronics. Specifications of the Si(Li) detector used may be found in Table 3.1.

Note that three values are presented for the distance between the front face of the crystal and Be window. The first is that specified by the manufacturer whilst the second and third are those measured by Arshed [ARS91] and Gooding [GOO89] respectively. These measurements were performed by varying the distance between a source and the detector Be window and the count rate of a particular photopeak at each of these distances noted. A linear least square fit of distance against the inverse square root of count rate derives a value for the distance that the crystal face is behind the Be window. An average of the two measured values in Table 3.1 i.e. 10.9 mm, is taken where needed (e.g. detector efficiency measurements), and that given by the manufacturer is assumed to be incorrect.
Table 3.1 Specifications of the Si(Li) detector employed in PIXE analysis.

<table>
<thead>
<tr>
<th>Description</th>
<th>Manufacturers specification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si(Li) crystal: diameter</td>
<td>6 mm</td>
</tr>
<tr>
<td>Front face area</td>
<td>28.3 mm²</td>
</tr>
<tr>
<td>Sensitive depth</td>
<td>2.75 mm</td>
</tr>
<tr>
<td>Dead layer</td>
<td>$3.0 \times 10^2 \mu$m</td>
</tr>
<tr>
<td>Au contact thickness</td>
<td>10 nm</td>
</tr>
<tr>
<td>Voltage bias</td>
<td>-500 V</td>
</tr>
<tr>
<td>Crystal front face to Be window</td>
<td>3 mm</td>
</tr>
<tr>
<td>distance</td>
<td>$11.1 \pm 0.2$ mm\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>$10.7 \pm 0.2$ mm\textsuperscript{b}</td>
</tr>
<tr>
<td>Be window: thickness</td>
<td>8$\mu$m</td>
</tr>
<tr>
<td>diameter</td>
<td>7±0.5 mm</td>
</tr>
<tr>
<td>Energy resolution at 5.9 keV</td>
<td>140 eV</td>
</tr>
</tbody>
</table>

* supplied by Link Analytical LTD, High Wycombe
\textsuperscript{a} measured by Arshed [ARS91]
\textsuperscript{b} measured by Gooding [GOO89]

3.3.3.1 Si(Li) detector calibration and linearity

Linearity of the Si(Li) detector is essential if we are to perform accurate X-ray spectroscopy. One factor limiting this linearity is the applied voltage across the crystal. A value of approximately 250 V/mm is required to avoid a degradation of linearity due to the significant loss of charge due to trapping and recombination [KNO89]. A method of assessing the linearity of the Si(Li) detector is by calibration employing X-rays of differing energy. Obviously this calibration is also required for photopeak energy identification and is given by:

$$E_i = m.i + c$$  \hspace{1cm} (Eq. 3.1)

where $E_i$ is the energy (keV) of channel $i$, $m$ the energy per unit channel (keV/channel) and $c$ the zero offset (keV). A number of methods may be employed to yield this calibration. Arshed [ARS91] doped Whatman filter papers with high purity solutions of elements and analysed these by PIXE. The method however adopted in this work was to capitalise upon the analysis of Bowen's kale standard which was frequently employed in
the comparative approach. This has an X-ray spectrum with easily identifiable photopeaks and is particularly useful as calibration and linearity checks may be performed, if wished, each time PIXE analysis is carried out. Calibration coefficients calculated by this method may be found in Table 3.2.

Note that two calibrations are given, one for a multiplexer being used in the signal processing electronics, whilst the other excluding the device. The multiplexer was introduced toward the end of this work for the purpose of data collection from RBS and PIXE analysis upon a single computer. Previous to this, two computers were employed. This will be discussed further in the following sections. Calibration points were taken from an energy range 3.31 keV (K Kα) to 14.96 keV (Rb Kα). The number of points are shown. Linear regression was applied to this data and calibration coefficients obtained. Correlation coefficients for the regression show excellent linearity of the Si(Li) detector over the specified energy range.

Table 3.2 Calibration coefficients for the Si(Li) X-ray detector used in PIXE analysis.

<table>
<thead>
<tr>
<th>Calibration parameters</th>
<th>No multiplexer (n=7)</th>
<th>Multiplexer (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gradient m (keV/channel)</td>
<td>0.04777±0.00013</td>
<td>0.08882±0.00022</td>
</tr>
<tr>
<td>intercept c (keV)</td>
<td>-1.28173±0.02600</td>
<td>-1.73482±0.02731</td>
</tr>
<tr>
<td>correlation coefficient</td>
<td>0.999980</td>
<td>0.999979</td>
</tr>
</tbody>
</table>

3.3.3.2 Si(Li) detector resolution

The energy resolution of a detector dictates its ability to resolve two photopeaks that lie closely together. It is usually quantified in terms of the width of a photopeak at half its maximum height (FWHM). The total FWHM, W_T, is dependent upon three factors and is linked to these by:
where $W_E$ is the FWHM attributed to noise from the electronic components of the signal processing chain, $W_D$ the FWHM due to the statistical spread of charge carriers and $W_X$ the FWHM from detector leakage currents and charge collection problems [KNO89]. The FWHM, $W_D$, due to the statistical spread of charge carriers is predicted by:

$$W_D^2 = 2.35^2 e F E$$  \hspace{1cm} (Eq 3.3)

where $e$ is the energy needed to create an electron-hole pair in Si (3.76 eV @ 77 K), $F$ the Fano factor (theoretical value 0.05, measured range 0.085-0.137 @ 77 K [ZUL70]) and $E$ the energy of the incident photon. The Fano factor is described as the ratio of the observed variance of charge carriers to the total number produced.

Figure 3.4 Plot of resolution (FWHM) as a function of energy for the Si(Li) detector employed in PIXE analysis.
The total FWHM was quantified over much of the energy range utilised in PIXE analysis i.e. 5.9 keV - 14.14 keV. The values obtained were from the photopeaks present within a Bowen's kale X-ray spectrum. Special care was taken to avoid peaks that overlapped with others, e.g. Rb Kα and Br Kα. A plot of energy against FWHM may be found in Figure 3.4. The value of FWHM @ 5.9 keV (Mn Kα peak) was found to be 150 eV which compares favourably with that specified by the manufacturer (Table 3.1). Resolution up to 60 keV has been found by Arshed [ARS91].

A plot of $W_j^2$ against $E$ was obtained from the data presented in Figure 3.4 in order to estimate a value for the Fano factor for Si. The contribution to resolution from leakage current, $W_j^2$, is assumed small and this and the spread due to noise, $W_n^2$, is constant across the energy range considered [KNO89]. The gradient of this linear plot is given by $2.35\sqrt{\text{eF}}$ and using the electron-hole pair energy stated above, F is calculated to be 0.179. This figure is higher than the upper Fano factor range limit given by Zulliger and Aitken [ZUL70].

### 3.3.3 Si(Li) detector efficiency

Knowledge of the Si(Li) detector efficiency as a function of energy is essential when performing PIXE analysis upon an absolute or in some cases comparative basis. This efficiency, $e_T$, is a function of the intrinsic efficiency $e_i$ of the crystal sensitive volume which is corrected for the photon energy dependent geometric factor $f_g$, the transmission of photons through the beryllium window $f_{be}$, gold contact $f_{Au}$ and frontal dead layer $f_d$, and the radial dependence efficiency $f_R$ (equation 3.4) [HAN73].

$$e_T = e_i f_g f_{be} f_{Au} f_d f_R$$  \hspace{1cm} \text{(Eq 3.4)}

The geometric factor corrects for the variation of penetration of X-rays in the detector volume. That is the effective solid angle $\Omega_e$ subtended from source to detector will lie between $\Omega_1$ and $\Omega_2$ depending upon the energy of the incoming photon. Absorption of X-rays in the gold layer and dead layer of the crystal is significant if the incident photons are below 5 keV energy. Attenuation in the Be window is relevant for all energies. Empirical formulae for the linear attenuation coefficients over specific energy ranges for the above absorption corrections is given by Cohen [COH80]. As the response of the Si(Li) detector will not be constant across the crystal surface and is of a 'bell
shape' in form with maximum response in the centre of the crystal [COH80, ALB89], then a factor $f_R$ is required to correct for this.

There will also be attenuation of X-rays from detector filters used in conjunction with PIXE analysis. These are employed to reduce system dead time that originates from intense low energy X-rays from low atomic number elements. These elements that are high in concentration in biological and environmental materials cause degradation of sensitivity for higher Z elements as was demonstrated by Albury [ALB89] in the analysis of Bowen’s kale standard. The introduction of a 350 μm mylar filter which is used in this work, attenuates low energy photons and improves the sensitivity of the technique for medium to high atomic number elements. The treatment however, for X-ray filters employed will be quantified independently from the Si(Li) detector efficiency.

The Si(Li) detector intrinsic photopeak efficiency was determined by the employment of $^{241}$Am, $^{54}$Mn and $^{57}$Co point sources obtained from Amersham International plc, UK. The energies and relative intensities of photopeaks employed are given in Table 3.3. This data is taken from Cohen [COH80], Hansen et al [HAN73] and Browne and Firestone [BRO86]. Errors on relative intensities are shown where possible.

Sources were mounted upon an aluminium cap that fitted onto the front end of the Si(Li) detector. This cap contained a hole such that photons were not attenuated by it whilst transversing towards the detector. The total distance from source to detector crystal front face was calculated to be 73.4±1.3 mm thus yielding a geometric solid angle, $\Omega$, of $(5.242±0.18)\times10^3$ Sr. Using this and the information from Table 3.3 the intrinsic photopeak efficiency $\varepsilon_E$ is calculated by:

$$\varepsilon_E = \frac{N_A}{A b t} \cdot \frac{4\pi}{\Omega} \cdot e^{\mu x} \quad \text{(Eq 3.5)}$$

where $N_A$ is the peak area counted over time $t$, $b$ the branching ratio, $A$ the source activity and $e^{\mu x}$ an approximate correction for the attenuation of photons in the polystyrene covering that surrounds the source. The polystyrene covering is 0.5 mm thick ($x$) with linear attenuation coefficient $\mu$. The linear attenuation coefficients were calculated by the mixture rule [JAC81] assuming a composition of 7.75% H and 92.25% C with density 1050 kg/m$^3$ [WEA86] and mass attenuation coefficients were taken from Storm and Israel [STO70]. A plot of the intrinsic photopeak efficiency as a function of energy (5.41 keV-
Table 3.3 Energies and relative intensities of photons employed in the efficiency calibration of the Si(Li) detector employed in PIXE analysis.

<table>
<thead>
<tr>
<th>Source</th>
<th>Line</th>
<th>Photon energy $E_x$ (keV)</th>
<th>Photons/decay b</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{241}$Am</td>
<td>Np M</td>
<td>3.3</td>
<td>0.0635±0.0060*</td>
</tr>
<tr>
<td></td>
<td>$L_1L_3 $</td>
<td>4.82</td>
<td>0.0017±0.005</td>
</tr>
<tr>
<td>$^{54}$Mn</td>
<td>Cr $K_{\alpha}$</td>
<td>5.41</td>
<td>0.220±0.002</td>
</tr>
<tr>
<td></td>
<td>Cr $K_{\beta_1}$</td>
<td>5.947</td>
<td>0.029±0.002</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>Fe $K_{\alpha}$</td>
<td>6.400</td>
<td>0.489*</td>
</tr>
<tr>
<td></td>
<td>Fe $K_{\beta_1}$</td>
<td>7.058</td>
<td>0.058*</td>
</tr>
<tr>
<td>$^{241}$Am</td>
<td>Np $L_1$</td>
<td>11.871</td>
<td>0.0081±0.0003*</td>
</tr>
<tr>
<td></td>
<td>Np $L_{\alpha}$</td>
<td>13.927</td>
<td>0.132±0.003</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>$\gamma$</td>
<td>14.412</td>
<td>0.095*</td>
</tr>
<tr>
<td>$^{241}$Am</td>
<td>Np $L_{\eta}$</td>
<td>15.861</td>
<td>0.0033</td>
</tr>
<tr>
<td></td>
<td>Np $L_0$</td>
<td>17.8</td>
<td>0.194±0.004</td>
</tr>
<tr>
<td></td>
<td>Np $L_{\gamma}$</td>
<td>20.8</td>
<td>0.0496±0.002</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
<td>26.35</td>
<td>0.0236±0.0001</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
<td>33.2</td>
<td>0.0014±0.0001</td>
</tr>
</tbody>
</table>

All data is taken from [COH80] apart from * [BRO86] and †[HAN73].

33.2 keV) may be found in Figure 3.5. For each of the data points there is an associated error which originates from uncertainties in solid angle, source activity, branching ratio and counting statistics, the latter generally being the most significant. Also illustrated in Figure 3.5 is the response of the detector as predicted by equation 3.4. As we may see, the experimental values of efficiency for $E_x\geq10$ keV compare well with the theoretical data points. However, for $E_x<10$keV there is a large divergence. There are a number of possible reasons for this, namely source self absorption corrections that have not yet been
Figure 3.5 Experimentally measured intrinsic photopeak efficiency for the Si(Li) detector employed in PIXE analysis. A theoretical fit (from equation 3.4) is shown as a solid line plot.

applied to experimental data points, ice build-up on the crystal surface as a result of leakage in the vacuum cryostat and Si K-edge escape peaks. When a photon interacts with the detector crystal, a Si K X-ray may be produced which escapes from the active volume and hence that photon is registered in a photopeak which corresponds to 1.74 keV energy less than the initial incident photon. Although this effect increases as the incident X-ray energy decreases down to the K-absorption edge of Si, its magnitude is less than 1% for most elements of interest in PIXE and hence neglected by Cohen [COH80].

Cohen [COH82] addresses the problem of ice build-up upon the detector crystal surface and introduces a correction factor $f_{\text{ice}}$ for this to equation 3.4. Now the experimental values of efficiency are correlated to theoretical by:

$$\epsilon_E = \epsilon_T f_{\text{ice}} = \epsilon_T \exp(-0.447 X_{\text{ice}} E_X^{-2.92})$$  \quad (Eq 3.6)

where the exponential function is an empirically fitted one [COH82] and $X_{\text{ice}}$ represents
the ice thickness in microns and \( E_X \) the energy of the incident X-ray photon (keV). The weighted least-square method was employed to fit \( \varepsilon_f \) to experimental values of \( \varepsilon_f \) to yield a value for \( X_{\text{Ice}} \) at the minimum chi-square point. All data points were employed in this fit (Figure 3.6) and a value of \( X_{\text{Ice}} = 428 \mu m \) yielded. This is considerably larger than any thickness stated by Cohen [COH82] of tens of microns, and hence it may be concluded that another factor is dominant in reducing experimental intrinsic efficiencies from their theoretical values.

The radioactive sources are absorbed into a 1 mm diameter ion-exchange resin and correction for source self absorption is difficult. One method to overcome this problem is to use liquid sources deposited upon backing materials to reduce source self absorption to a minimum as demonstrated by Cohen [COH80]. This option was not available at the time of analysis. However, it is thought that photon absorption in the ion-exchange resin is the main reason for lack of correspondence of experimental and theoretical efficiencies at lower energies. This conclusion is arrived at by single element

![Image]

Figure 3.6 Theoretical Si(Li) detector efficiency and weighted least square fit to experimental points to derive the thickness of ice present on the crystal surface.
comparative analysis in PIXE (see section 4.6.2), this approach relying heavily upon accurate knowledge of the Si(Li) detector efficiency. Theoretical values employed in this work predicted elemental concentrations in samples to a good degree of accuracy.

3.3.4 Surface barrier detector

The Ortec EG & G surface barrier detector is mounted at 165° to the incident beam direction in the chamber. A large angle is required to obtain maximum difference in energy between protons backscattered from atoms of similar but different masses. The optimum angle is found to be at 180° as predicted by equation 2.17. This however is a practically unrealistic angle and hence one which is smaller but close to it employed.

The energy resolution of the surface barrier detector was measured by a triple element alpha particle emitting source under vacuum conditions (4-6×10⁻⁵ torr). This source contains the radioisotopes ²³⁹Pu, ²⁴¹Am and ²⁴⁴Cm of main α-particle energies 5.144 MeV, 5.486 MeV and 5.805 MeV respectively [BRO86]. The resolutions (FWHM) obtained for each of these energies are 26±4 keV, 22±4 keV and 25±4 keV. Potential sources of resolution degradation are from edge effects of the detector and noise generation from light and associated pulse processing electronics. The problem with edge effects may be resolved by the introduction of a collimator allowing protons to fall only in the centre of the device. This was the approach taken in this work with the use of a brass collimator with 5 mm diameter (19.6 mm²) circular hole. However, further collimation was needed to reduce dead time problems. Surface barrier detectors are sensitive to light as these photons possess enough energy to create electron-hole pairs. Hence a dark target chamber is required whilst making proton scattering measurements otherwise a large noise background will be generated. Measurements upon the efficiency of the surface barrier detector were not made, however Arshed [ARS91] notes that as the range of 2 MeV protons in silicon is 47 μm, then the detector may be assumed 100% efficient.

3.4 Data acquisition

A schematic representation of the electronic devices utilised in the acquisition of data from the surface barrier detector and Si(Li) detector is illustrated in Figure 3.7.
A description of this diagram is as follows. The surface barrier detector which is biased at a voltage of +75 V d.c. is connected to an Ortec 142 pre-amplifier. This pre-amplifier possesses four further connections to a precision pulser, detector bias power supply, pre-amplifier power supply and amplifier for the detector output signal. After shaping of the signal in the amplifier using a 0.5 μs shaping constant and amplification, the linear output signal is fed into the front face panel of the multiplexer. Meanwhile signals from the Si(Li) detector after passing through the pulsed optical feed-back pre-amplifier are processed by the Link analytical pulse processing unit experiencing shaping and amplification. The linear output signal from this unit is then fed into a second input on the front face panel of the multiplexer. There are four input connectors in all, only two being utilised here. The purpose of the multiplexer is to differentiate between each input signal so that they may be processed by the multi-channel buffer (converted from analogue to a digital signal), and thus when sent from this device they are registered in the correct portion of the multi-channel analyser program which is run on a Sun Spark 1 workstation. Previous to the introduction of the multiplexer there was a second multi-channel buffer and computer and the signal from each detector sent to either of these. The present set-up however simplifies data collection as acquisition may be controlled from one keyboard.

The multi-channel analyser program, which is named XMCB [ROB93] and was written in-house, runs under X-windows. XMCB possesses 4k channels, however half of these are ghost as they are used for communication with the multi-channel buffer. The remaining 2k channels are split into 4 segments, one for each of the inputs to the multiplexer, each of 512 channels.
Figure 3.7 Schematic representation of the electronic components utilised in the acquisition of data from surface barrier and Si(Li) detectors.
Control of the multi-channel analyser software is by the mouse which is clicked onto various positions of the display to execute different functions. Essentially for each analysis, the previous spectrum is cleared using the RESET function and STARTed. The PIXE or RBS spectrums are viewed by activating the specific segment. One should note however that only a single segment may be viewed at any one time. The spectrums may be displayed in either autoscale or manual scale modes and if required regions of interest (ROI) set. Also whilst in data accumulation mode, real time, live time and acquired charge are displayed. However, the acquired charge will only be correct if the current range is set to the same value on the integrator. The default range is 100 nA. On saving the data, the PIXE and RBS spectrums are written to file on the network under the filenames specified by the user. A title may also be accompanied with the data which is saved in rows of 10 channels. This data may then be analysed by XRBS and PIXAN which is software for the manipulation of RBS and PIXE spectrums respectively.

3.5 Analysis of RBS and PIXE spectrums

The software that enables the analysis of RBS spectrums, XRBS, was written at the University of Surrey [ROB93] and runs upon X-windows. Conversely, PIXAN is a suite of programs written in FORTRAN 77 which has been developed at the Australian Atomic Energy Commission, Lucas Heights [CLA86].

3.5.1 RBS spectra analysis

Rutherford backscattering spectrums are loaded into XRBS for subsequent analysis. Before this however, calibration of the software is required. This is done by identifying a specific edge within the spectrum. Using the calibration option the corresponding channel number is entered along with the beam irradiation parameters e.g. beam energy (2 MeV), ion source (proton), take-off angle (165°). Now for a specific element, which is chosen by clicking onto that element in a 'pop-up' periodic table window, the corresponding channel number is presented.

Data is extracted from a RIO panel. These regions of interest are set by clicking the middle mouse button at specific points upon the spectrum display. A maximum number of 20 regions of interest may be set in all, and these may be retained for each data file which greatly reduces the time spent executing this activity. The extracted data
is used to calculate mass proportions of major elements as described in section 2.5.

The XRBS software has many more functions than that indicated above e.g. statistical analysis, polynomial fitting. These however are not discussed here as they are not employed in the RBS spectra analysis in this work.

3.5.2 PIXE spectra analysis

PIXAN [CLA86,CLA87b] consists of two programs namely BATTY and THICK. BATTY is based upon a model that describes the X-ray spectrum as modified Gaussian peaks on a background and parameters are determined by non-linear least square fitting. There are two inputs to BATTY, namely the X-ray spectrum data (SPECTR) and experimental set-up data (DSET1). DSET1 contains the energy calibration coefficients, detector parameters, the X-ray filter thickness, the type of background fitting required, if sample self-absorption is needed (and if so specification of the sample matrix concentrations), if sum peak correction is required, the 'shopping list' of elements of interest, and the energy range for analysis.

In general, there are two types of background fitting available i.e. polynomial and iterative. Arshed [ARS91] compares the two backgrounds in the analysis of Bowen's kale standard and concludes that a polynomial background is advantageous over iterative as more favourable chi-square values are yielded in characteristic peak fitting. A 5th order polynomial was employed in most of this work, and the fitting procedure checked by observation of chi-square values and where possible plotting the fit against the spectrum as strongly suggested by Clayton [CLA86]. One such plot may be found in Figure 3.8 for Bowen's kale collected for a total charge of 10.54 μC. An initial 5th order polynomial background is illustrated which has been fitted between 2.0 and 20.0 keV resulting in a poor fit with a chi-square value of roughly 2×10². A marked improvement is observed when the fitting range is reduced to 3.0 to 20.0 keV with the reduction in chi-square value to roughly 5. A similar decrease in the chi-square value may be experienced by increasing the order of polynomial fit.

The matrix construction, which is obtained from RBS analysis, is used to correct for sample self absorption of X-rays. This correction becomes more significant as the energy of the photons decreases i.e. the element atomic number decreases. Also corrections are made for silicon K-escape peaks, sum peaks and peak tailing.
Figure 3.8 Plot of a PIXE spectrum of Bowen’s kale illustrating poor and good polynomial background fits from BATTY [CLA86].

The ‘shopping list’ specifies the elements that BATTY will search for. These will be Kα X-rays for atomic numbers up to roughly Z=50 (Sn) and Lα X-rays for Z numbers upwards, although it may be possible to search for Lα X-rays down to Z=40 (Zr).

Output from BATTY (see example in Appendix 2) contains parameters for the background fit i.e. chi-square value, root mean square value and polynomial coefficients followed by the total collected charge and data for Kα or Lα peaks for each of the elements. Presented for each element is the peak area $N_A$, the error associated with the peak area $S_N$ (equation 3.7), chi-square fit of a Gaussian to the Kα or Lα peak, and the minimum detectable limit (MDL). In equation 3.7, $N_B$ is the number of background counts.

$$S_N = \sqrt{(N_A + 2N_B)}$$  
(Eq 3.7)
below the peak which is taken over a region ±3σ from the centroid. The minimum detectable limit is given by equation 2.6 where a value of f=3.29 (99.9% confidence level) is employed. The MDLs are also presented for elements not found within the sample analysed.

THICK calculates X-ray yields for specified elements assuming unit concentration. That is, it essentially evaluates equation 2.7. Control of THICK is by DSET6. This file contains information pertaining to the detector geometry (e.g. sample-detector distance, take-off angle, detector diameter), energy limits for integration, filter thickness, charge, ‘shopping list’ of elements of interest and matrix construction. In the case of 2 MeV proton analysis upon thick specimens the lower integration limit, EHI, is set to 2 MeV whilst the higher integration limit, ELO, 0.1 MeV. In theory, ELO should be set to zero, however THICK allows only a lower limit of 0.1 MeV as the contribution of X-rays below this energy is deemed insignificant. In the case of intermediate samples, ELO will take a value greater than 0.1 MeV which is dictated by the thickness, composition and density of the specimen under analysis. Charge is specified in microCoulombs, and is usually set to unity for the ease of trace element concentration determination. Thus one output file from THICK may be used for samples of the same matrix composition and multiplied by the collected charge rather than DSET6 being altered for each charge resulting in larger processing times and file numbers.

Output from THICK is basically in the form of three columns of data. For each element is given the thick specimen yield (mg/kg/μC), the thin specimen yield (μg/cm²/μC) and the integral as specified in equation 2.7. Use of data from the first column with that from BATTY in the manner specified by sections 2.4.1.1 and 2.4.1.2 allows quantification of trace elements in thick samples upon a comparative basis.
Chapter 4
Analysis of Homogenised Biological Samples

4.1 Introduction

In the quantification of trace element concentrations in a particular organ or tissue, one should be aware that the nature of the sub-samples extracted to derive these levels will dictate the quality of the determination. These sub-samples may be made available to us from autopsy or biopsy procedures and hence the amount of material analysed will be limited. The percutaneous biopsy technique is a well established diagnostic procedure [HEY84] and is increasingly used for the investigation of Wilson's disease and primary biliary cirrhosis in liver by the analysis of specimens for copper [NOO81]. However, the employment of sub-samples not only means that we should be concerned with potential contamination risks, but also effects due to homogenous trace element distributions [IYE76]. The degree of specimen homogeneity governs how well trace element levels of samples extracted from different organ or tissue positions agree. Disagreements due to this effect are a consequence of biological specimens consisting of different composite tissues each possessing its own individual biological function. To circumvent this problem, one needs to set out sampling protocols such that adherence to these results in the derivation of reliable values. These values may be compared to others from similar biological tissues from different subjects with a degree of confidence that any variation in levels does not originate from effects of heterogeneity but from other factors e.g. age, dietary habits, disease/health status, smoking history.

Elemental homogeneity in biological specimens, and in particular liver, kidney and heart, is discussed in the following sections. Methods to quantitatively evaluate specimen homogeneity in terms of percentage standard deviations and sampling factors for each element are presented. Trace element analysis is performed upon the above organs by 2 MeV proton-induced X-ray emission with simultaneous collection of RBS spectra. Sub-samples were extracted from various positions within these organs homogenised and pelletised for analysis. Elemental concentrations are derived, where possible by the comparator method although some absolute analysis is performed. These methods along with a variation of the single element standard technique are compared and
their relative merits and disadvantages discussed. Quantification of specimen elemental homogeneity is also presented and highlights the need for adherence to specially developed sampling protocols.

4.2 Review of existing sampling studies

4.2.1 Liver

The liver is the largest gland in the body and lies in the right hypochondrium under cover of the lower ribs [PAS76]. It accounts for 2.5% of the total body weight in an adult and is wedge shaped which can further be divided into two lobes (Figure 4.1). This division of the larger right lobe and smaller left lobe is marked by the falciform ligament on the anterior surface, and on the visceral and posterior surfaces by fissures for the ligamentum teres and ligamentum venosum. The lobes, however, are useful topographically, but are neither vascular or biliary units. Blood from the portal vein enters the liver at the porta hepatis which then divides within the liver to finally enter an anastomosing system of sinusoids. This provides 80% of the hepatic blood supply, while the remaining 20% comes from the hepatic artery which also divides repeatedly within the liver to meet the sinusoids. Hepacytes in the vicinity of these sinusoids secrete bile which drains away through the bile ducts. This bile is then conveyed from the liver to the duodenum and the gall bladder which stores and concentrates it. Bile is an important aqueous solution that enables the digestion and absorption of ingested fats. Apart from bile production, the liver has many more metabolic functions. These include plasma protein synthesis, carbohydrate metabolism, vitamin metabolism, alcohol metabolism and inactivation of drugs, poisons and hormones.

Without doubt, the liver is the most studied organ in sampling experiments. Generally, conclusions drawn from these experiments either points to the liver being elementally heterogenous or elementally homogenous. Studies by Schicha et al [SCH70] and Lievens et al [LIE77] in essence lean toward the former belief. Schicha et al [SCH70] extracted 1 cm³ (weight not specified) samples from two livers and determined their Co, Fe, Sb, Se and Zn content using instrumental neutron activation analysis (INAA). Percentage standard deviations were mostly below 20% which rose to 50% for the non-essential element antimony. They conclude that the analysis of a single sample of liver will not yield trace element results that are representative of the whole organ. Lievens et
Figure 4.1 Anterior view of the liver illustrating its lobes, blood supply and biliary duct system.

al [LIE77], although not obtaining variations across their 5 livers as great as those by Schicha et al [SCH70], still find marked dispersions. Forty sub-samples of 1.5 g dry weight were used to determine 25 elements in all by neutron activation analysis (radiochemical and instrumental). Standard deviations for the essential trace element group were mostly below 10% whilst those that are non-essential or toxic exhibited greater deviation.

Studies conducted by Koenig et al [KOE79] and Perry et al [PER77] point to the liver being an elementally homogenous organ. The study by Perry et al [PER77] involved the trace element analysis of ten 1 cm³ (no mass given) samples, taken from
each liver, by emission spectography. Elemental homogeneity was expected due to the liver composing of essentially a single cell type and for most of the 16 elements analysed, this was true (even for the toxic elements Cd and Pb). Significant deviations between sampling sites were however observed for Fe, Sr and Ba, variations in iron possibly being due to different blood content of sub-samples. Aalbers et al [AAL87] claim that determinations in liver for the elements Cr, Fe and Rb may be enhanced by an average 15%, 22.3% and 8.6% due to the presence of residual blood. The work of Van Eijk et al [VAN74] refutes findings by Perry et al [PER77] in the case of heterogenous behaviour of iron in liver. Surgical needle (composition unknown) biopsies of 1-2 mg dry weight extracted from the left lobe, middle and right lobe of the organ showed deviations which were comparable to the standard error of the experimental procedure. Hence, they conclude negligible influence of sampling site upon iron determination.

Koenig et al [KOE79] extract 8 samples of 0.8 g each from three different areas of a single human liver (right lobe, middle and left lobe) and determined contents of 18 different trace elements using proton induced X-ray emission analysis. They found that although small differences occurred between sampling sites, as long as 'enough tissue' is extracted and homogenised to average out the liver microscopic lobular structure, choice of sample site is insignificant for most elements. Sampling location only becomes significant for toxic elements such as lead which may become concentrated in the presence of neoplastic diseases. In fact the question of liver elemental homogeneity becomes even more complicated when the organ is diseased.

Nooijen et al [NOO81] studied hepatic copper content for the indication of biliary cirrhosis. Sample weights of 5 mg were extracted by percutaneous liver biopsy and elemental variations quantified. They conclude that the use of a single biopsy reflects a reliable measure of hepatic copper content. However Kostic et al [KOS87] who were interested in the elements Zn, Fe and Sb in liver in different cirrhotic and cancerous states stress that not one individual sample is representative of the whole organ. Rather extraction of samples of the tissue of interest (e.g normal, cancerous) should be taken as these have their own trace element content.

There seems to be a split of attitudes concerning the degree of elemental homogeneity of the liver. One way around this would be to use homogenised samples from a whole organ [ZEI84, ZEI88, TIP63a]. This option however is rarely available to
us and it is more usual to be presented with a number of sub-samples to derive an average organ element concentration. In the past, researchers have tested the viability of employing a single sample to represent the liver as a whole. Some conclude that this approach is feasible, whilst others do not. The main failure to agree is linked to the sample size extracted for analysis. The smaller the sample, the larger the variation between samples and vice versa. Therefore researchers using smaller sample sizes will inevitably conclude larger elemental variances than those using larger sample sizes. As the weight of the sample increases, there will come a point where variances due to element heterogeneity become insignificant (this is predicted by the sampling factor and will be discussed later). This is the mass which will yield representative results about the whole of the organ. There has been a failure to quantify this mass apart from an attempt by Heydorn [HEY84] who takes data presented by Schicha et al [SCH70] and Lievens et al [LIE77] and calculates it to be 200 g for Br, Co, Mn, Rb and Zn. The value for Fe is a little higher whilst that for Se a little lower.

4.2.2 Kidney

Normal adult kidneys weigh 120-160 g each and measure roughly 11.5 cm in length [DAR80]. When sectioned coronally an inner region called the medulla is revealed which is interfaced to the outer region, the cortex (Figure 4.2). The cortex exhibits a granular appearance due to the random arrangement of tiny glomeruli associated with nephrons, the functioning units of the kidney. Distinction between the cortex and medulla is made easily by the examination of their boundary, the corticomedullary junction, which is marked by the presence of arcuate arteries. The cortex is usually 6-8 mm thick. The medulla extends inwards to the papillae which have radiating streaks which lead to the tip where collecting ducts (ducts of Bellini) open into the surface. The renal substance which stretches from the papillae tip to the corticomedullary junction base is termed the renal pyramid. Between these pyramids extends renal cortex known as the columns of Bertin. The renal pelvis forms a funnel shaped drainage system which extends from the papillae to the ureter.

The kidneys are responsible for the removal, by filtration, of metabolic waste and excess materials from the blood by the process of excretion and urine formation. They also regulate the composition and physical properties of the blood by monitoring the acid-
base balance, osmotic relationship and the content of organic and inorganic solutes. Other organs of the urinary system perform the simple task of transport, storage and elimination of urine [CRO76].

It is apparent that the kidney is an extremely physiological heterogenous organ, and hence it is of no great surprise that recommendations point to trace elements being reported individually for each tissue type [KOl81]. Hamilton et al [HAM72] report elemental concentrations for cortex and medulla separately. However, many authors present values for cortex only as a substantial degree of uncertainty exists in the ability to separate the medulla from this tissue. Even analysis of the cortex is difficult as differences in trace element content are seen for example between the subcapsular and corticomedullar regions. Livingstone [LIV71] investigated the distribution of Zn, Cd and Hg across human kidneys and found preferential collection of these elements in the renal
cortex. In fact he discussed a concentration gradient for Zn and Cd which decreased from a maximum in the subscapular cortex to a lower value at the juxtamedullary region and was constant across the medulla. This concentration gradient was parallel for the two elements which may be explained by their chemical similarities. It is postulated that Cd and Zn are transported to the kidney by the protein metallothionein [PIS72,TAN87]. Metallothionein is mainly synthesised in the liver and one of its main functions is detoxification of heavy elements and in particular cadmium [BRE87,BRE90]. Cadmium is a toxic element and has been shown to be at elevated levels in kidneys for subjects exposed to it from industry and smoking [ELI76]. In addition to Cd, Hg and Zn, the elements Mn, Ni, Cu and Rb [TAN87] also preferentially collect within the cortex, whereas Ca, K, Fe, Na, Mg and P exhibit no preferential collection site [TAN87, SUB82].

Little work exists upon the sample size needed to gain representative trace element results about this organ. One of the few reports by Damsgaard et al [DAM82] quantifies the sampling constant of Se and Zn in renal medulla to be 194 g and 208 g respectively. This, they say, is comparable to the total weight of one kidney. These masses highlight the degree of elemental heterogeneity of this organ.

4.2.3 Heart

The heart essentially consists of four chambers, the left and right atria and the left and right ventricles (Figure 4.3). The heart functions in the following manner. Blood returning from the circulatory system enters the right atrium via the venae cavae. Having passed through the tricuspid valve it is pumped into the right ventricle. From here it leaves via the pulmonary valve to the pulmonary artery which supplies the lungs with blood. The blood now being oxygenated passes back into the left atrium which is then pumped through the mitral valve and into the left ventricle. From here, the blood is finally pumped from the left ventricle, through the aortic valve and into the aorta which supplies the circulatory system with oxygenated blood [PAS76].

A report by the World Health Organisation [WHO74] identifies that problems may occur with the multi-trace elemental analysis of heart samples which are of the order of a gram or so. Of the few reports on this subject, that by Schicha et al [SCH72] identify significant variances (15-70%) for the elements Co, Fe, Se, Zn and Sb for adjacent 0.4-1.0 g muscle samples extracted from the left ventricle. The largest variance occurs for the
non-essential trace element antimony. They conclude that a single tissue sample cannot represent the trace elemental status of the whole organ. A similar experiment conducted by Aalbers et al [AAL87] (0.8 g muscle samples from the left ventricle), identifies an intra-regional variation for the elements As, Hg, Cd, Mo, Na, Co and Zn to be 10%. For the elements Cu and Se a slightly lower value of 5% was attained. The greater degree of Cu homogeneity compares well with the conclusions of Heydorn [HEY84] who specifies for this element in bovine heart that a sampling constant indistinguishable from zero is yielded (upper limit 10 g).
4.2.4 Lung

The lungs are the most sensitive organ to environmental changes and come into contact with 15000 litres of atmospheric air daily [BAR82]. Air is inhaled into the lungs via the trachea which splits into two large bronchi before one passes into each lung. The large bronchus that feeds the right lung splits further into three smaller bronchi whilst the left into two. These supply each of the lobes with air (Figure 4.4). The left lung has only two lobes as the heart lies on this side. The bronchi repeatedly split, becoming smaller in diameter until eventually terminal bronchioles are formed. Upto this point, the walls are relatively thick and used only for conducting the air into the lungs. Beyond the terminal bronchiole is the respiratory bronchiole which contains alveoli. The walls of this part of the respiratory tract is much thinner as these (alveoli) are the parts of the lung that exchange gases with the blood [PAS76].

Figure 4.4 Illustration of the lungs highlighting lobular segmentation.
Trace elements that are present within the lungs may be split into two categories, namely those that are deposited in them by the inhalation dust particles and those that form part of the natural composition of lung tissue [BAR82]. Particles of dimensions 1-2 μm exhibit the higher deposition rates, whilst those of size <0.1 μm may be deposited in the alveoli [VAN82]. If these particles are not cleared by various mechanisms, which are dependent upon their composition, reactive tissue may grow around them forming a permanent record of environmental exposure to that individual. However, some dusts e.g. those containing various hard metals from industrial practices, are soluble in the surrounding biological fluids and are linked to a number of lung diseases [ED90]. Bartsch et al [BAR82] study the distribution of elements throughout the lung and identify in some cases gradients from a higher value in the apex (essentially lobe 1, Figure 4.4) down to lower values in the base of the lung. The existence of this gradient for a particular element infers that its concentration is mainly due to the deposition of dust particles. Bartsch et al [BAR82] identify the elements K, Ca, Cu, Zn and Rb to show no significant difference in concentration between sampling sites, whereas Ti, Cr, Ni and Sr exhibit the above described concentration gradient. The findings of Molokhia and Smith [MOL67] reiterate the independency of concentration to sampling area for Cu and Zn, and add the elements Sb, As, Cd, Hg and Mn to the list of elements that are found to be more concentrated in the lung apex. Heydorn [HEY84] also notes that Cs is concentrated in the apex. Although Vanoeteren et al [VAN82] do not identify the presence of concentration gradients in the lung, they state that for most of the elements listed above, they exhibit an inhomogeneous distribution the degree being dependent upon the element. No sampling factors for trace elements in lung tissue were found in the literature and thus it is difficult to assess the degree of heterogeneity of this organ.

4.3 Methods for assessing specimen trace element homogeneity

The discussion in the previous sections has highlighted the degree of elemental heterogeneity of biological specimens. This makes difficult the task of choosing a single sample that is representative of the whole organ. Use of single samples is often forced upon us as, for example, only limited masses are available from standard biopsy procedures. Even the use of multiple samples presents problems concerning the representative value of results. A method to avoid these problems would involve the
homogenisation and trace element analysis of a whole organ. As mentioned earlier, this option is rarely available to us. To gain representative trace element results, one needs to consider issues concerning the number of samples to be taken (if it is possible to take more than one), how large these should be, and from where in the material they should be extracted [KRA81]. These questions however can only be answered with some knowledge of the homogeneity of the specimen. This and a method for quantifying the mass of material needed to yield representative results from a single sample (sampling factor) are presented in the following sections.

4.3.1 Specimen homogeneity in PIXE analysis

A method of quantifying the elemental homogeneity of a specific specimen involves the analysis of replicate samples of the same mass. Now the standard deviation for each element for the replicate analysis is given by:

\[ \sigma_T = \sqrt{\sigma_a^2 + \sigma_h^2} \]  

(Eq 4.1)

where \( \sigma_a \) is the standard deviation due to the analytical procedure and \( \sigma_h \) is the standard deviation due to elemental inhomogeneity within that specimen [LIE84, KAJ84]. Hence the standard deviation due to inhomogeneity is given by equation 4.2.

\[ \sigma_h = \sqrt{\sigma_T^2 - \sigma_a^2} \]  

(Eq 4.2)

The analytical standard deviation in PIXE analysis may approximately be given as:

\[ \sigma_a = \sqrt{\sigma_c^2 + \sigma_e^2 + \sigma_s^2} \]  

(Eq 4.3)

where \( \sigma_c \), \( \sigma_e \) and \( \sigma_s \) are the standard deviations associated with the charge integration process, fluctuation in proton energy and counting statistics of each individual photopeak respectively [ARS91]. The standard deviation due to peak counting statistics is predicted by equation 3.7. Fluctuations in charge integration may be measured by comparing the carbon signal (normalised to charge) between the RBS spectra. Typical values for this work are presented with inhomogeneity data (see section 4.6.1). Arshed [ARS91] assumes
that fluctuations in proton energy are a negligible contribution (less than 1%) when the Van de Graaff accelerator is running under stable conditions. This assumption is backed up by Persico et al [PER68].

There are a number of salient points which should be noted when quantifying sample elemental homogeneity in this manner. Firstly, the variance due to the analytical process must be quantifiable and less than fluctuations due to element heterogeneity [YOU67]. If this requirement is not satisfied variance among samples is masked by the variance from the analysis method. Secondly, the mass of sample will dictate the values of homogeneity obtained. Lieser and Tu [LIE84] analysed 100 mg and 250 mg samples of biological reference material by neutron activation analysis. As expected, higher degrees of elemental heterogeneity are noted for the smaller sample aliquot.

### 4.3.2 Sampling factor

It is of great interest to be able to specify a sample weight such that its analysis yields results that are negligibly affected by specimen heterogeneity. The concept of the sampling factor allows us to calculate this. The sampling constant, as it was previously known, was originally developed for geochemical analysis [WIL64,ING73,ING74, ING76]. However Heydorn [HEY84] later applied it to the analysis of biological materials, in which he described a random determinand distribution in a uniform matrix. Mathematically the sampling constant (K₁) is predicted by:

$$K_1 = R^2m$$  \hspace{1cm} (Eq 4.4)

where m is the sampling mass of n replicate determinations, and R the relative standard deviation in per cent [HEY87]. Thus K₁ represents the minimum sample size needed to keep the variability due to lack of homogeneity at less than 1% (68% confidence level). Equation 4.4 may be re-written in the form of equation 4.5.

$$K_1 = 10^m m \sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}$$  \hspace{1cm} (Eq 4.5)

The variable $\bar{y}$ represents the average value of n determinations of y, whilst $\bar{\sigma}^2$ the average variance accredited to the analytical procedure and this is predicted by equation 4.6.
\[
\frac{1}{\sigma^2} = \frac{1}{n} \sum_{i=1}^{n} \frac{1}{\sigma_i^2}
\] 

(Eq 4.6)

Again, it should be noted that if the replicate analysis is not under statistical control the quantification of the sampling constant is impossible i.e. \(\sigma_h^2\) must be significantly smaller than \(\sigma_s^2\). Heydorn and Damsgaard [HEY87] note that for a heterogeneous distribution of trace elements within a uniform matrix, the sampling constant will be different for each element. For this reason, Spyrou and Al-Mugrabi [SPY88] use the term sampling factor in preference to sampling constant.

The concept of sampling factors has become particularly useful in the assessment of elemental heterogeneity of standard reference materials. Certified elemental concentrations are needed to be known accurately in these, and fluctuations due to heterogeneity kept to a minimum. In a study by Spyrou et al [SPY90], sampling factors were determined for 5 different standard reference materials by INAA and PIXE. Values from PIXE instead of being stated in terms of mass, were given in number of beam spot positions on the target required for the attainment of representative trace element levels. See appendix 4 for the application of sampling factors to biological tissues.

4.4 Sample extraction and preparation

Intra-organ elemental variations were investigated by the analysis of homogenised sub-samples extracted from porcine kidney, liver and heart. Porcine samples were chosen due to the ease of procurement and their physiological and anatomical similarities with human tissues. Also, problems associated with bio-hazards are avoided e.g. hepatitis.

Organs were specially ordered from a local abattoir so that delay between extraction and preparation was kept to a minimum. Instruction was given to leave any connective tissue or blood vessels attached to the organ. It is important to keep the time between extraction and preparation to a minimum as trace element compositions have been shown to change significantly with delay [IYE81, IYE82].

On collection, the organs were immediately transported to the University for preparation under clean-room conditions. Vinyl gloves were worn throughout all preparation procedures, but great care was taken to wash off any talcum powder with de-ionized water as this has been demonstrated to be a source of contamination of trace
elements [AAL87]. All preparation equipment (containers, tray for specimen preparation) apart from surgical blade, were composed of polystyrene or polyethylene to reduce risks of trace element contamination. These were washed in a solution of Decon 90® and de-ionized water which is a recognised method of cleaning equipment. A stainless steel scalpel was used to excise samples from specific sites within each organ. This has the potential of introducing contamination from the elements Co, Cr, Cu, Fe, Mn, Ni and Zn [VER73,BEH80]. Iron is one of the major components of steel and hence the largest contamination originates from this element. However, it is usually found in large concentrations in biological specimens and thus its relative enhancement is small. Care though, must be taken for Cr and Ni as concentrations may be enhanced in the liver by a factor of 2 [VER73] or 5-10 [MEI79] depending on the method of sample extraction. As levels of these elements are of the same order in other organs, then similar enhancements may be expected. Contamination effects from the stainless steel surgical blades was avoided by extracting a sample which was larger than needed. The surface of the sample was easily excluded from the dried sample by using a contamination free polyethylene spatula.

Specimen sub-samples were taken from positions (described next) under the organ capsule or surface. This was done to avoid contamination which may originate from non-clean room procedures at the point of organ extraction to even the surroundings of the organ whilst still in the animal [BEH81]. Extracted sub-samples were briefly rinsed in de-ionized water, blotted by Whatman filter paper to remove excess blood, biological fluids and water, weighed and immediately transferred to their own individual air-tight polyethylene bag. Samples were then frozen at -12°C to inhibit composition changes. Much controversy surrounds the issue of sample washing. Some researchers recommend repeatedly rinsing, whilst others condemn it altogether. It is felt that brief rinsing is required to reduce the dependence of some trace element concentrations (Cr, Fe, Rb) on the presence of blood [AAL87] and hence a rinsing protocol as suggested by Koityohann et al [KOI81] was adopted.

Table 4.1 contains information concerning the sampling from kidney, liver and heart. Note that three individual hearts and livers were analysed. Presented in this table is the number of sub-samples extracted from each organ, a description from where they were extracted, their wet weight, dry to wet weight ratio, and replicate analysis weight
(dry weight). For kidney, initially a single specimen slice was extracted and freeze dried (described next). An average dry/wet ratio of 0.167 was measured. We would expect this value to be lower for tissue composed solely of medulla as it contains more water than the cortex [WHI91]. Two circular sub-samples of approximately 50 mm diameter were extracted from this slice and scanned by photon transmission tomography. The resultant tomographs were used for selecting a total of 24 sub-samples from the two circular samples. These were chosen to represent the two component tissues and also any adipose present. A full description of the scanning and sub-sample selection procedures may be found within chapter 6 and chapter 7.

A feature to also note about Table 4.1 is the range of sample wet weights extracted from liver and heart. This originates partly from the reasons associated with contamination from the surgical blade but also as it was thought more sensible to weigh out replicate masses when specimens were in a dry form and less prone to contamination and degradation. The large variation seen for heart is due to valves being included in the range. The dry/wet weight ratio for liver and heart muscle respectively is roughly 0.28 and 0.20. These compare well to those presented for human tissues, values being 0.29 [ICR75] and 0.283 [MAE84] for liver, 0.28 [ICR75] for heart and 0.24 [ICR75] and 0.2 [MAE84] for kidney.

Once the samples had been completely frozen, they were transferred to their own clean polystyrene petri dish and placed in the vacuum chamber of the freeze drying unit. Up to roughly ten samples could be dried simultaneously by this technique. An Edwards rotary pump evacuated the sample chamber down to roughly 8×10^2 torr, water being liberated by a process of sublimation and being held by a cold trap at a temperature -50°C to -60°C. Freeze drying was monitored by periodic weighing of the samples, this process being complete when weights became static. This method was believed more accurate than observation of the pressure gauge, although slightly more time consuming. Throughout the freeze drying procedure samples could be seen to lighten in colour due to their loss of water. Typical drying times for samples were of the order 48 hrs apart from the kidney slice which took 67 hrs mainly because of its weight and thickness.

There are distinct reasons that freeze drying was chosen in preference to other drying/matrix reduction techniques such as wet ashing, low temperature ashing, high temperature ashing, alcohol dehydration and microwave drying. Alcohol dehydration
techniques may be immediately dismissed as they result in serious loss and contamination of elements in biological tissues [GAL84, OTH79]. The same is noted for high temperature ashing. Without doubt, the best of the alternative drying procedures involves low temperature ashing. For most elements no loss or contamination is seen, apart from P, Cl, Br and Se [GAL84]. Microwave drying has similar capabilities to this technique [KOH80]. Lyophilization has been demonstrated superior with respect to insignificant element loss [OTH79] and even retention for the notoriously volatile elements Cr, As, Br and Hg has been shown to be good [LaF73, GOE79]. However, the disadvantage of this technique lies with the amount of matrix material left after drying. Smaller amounts remain when using ashing techniques for biological sample preparation e.g. of the order of 20% of the freeze-dried mass, with the result of better trace element detection limits in PIXE analysis [GAL84]. This is due to trace elements being more concentrated in the ashed material and a lower bremsstrahlung background. Lyophilization was however selected for biological specimen drying as the elements lost by alternative matrix reduction methods are valuable to the homogeneity study.

After freeze drying, the biological specimens had their outer surfaces removed with a clean polyethylene spatula, and a specific mass weighed out as indicated in Table 4.1 ready for homogenization. Samples were homogenised using a porcelain pestle and mortar and made into 5 mm diameter circular pellets with a stainless steel pelletiser. Washing in de-ionised water of all homogenisation and pelletising equipment was carried out between the preparation of each sample to minimise risks of cross-contamination. Contamination from the pestle and mortar and the pelletiser themselves was checked by following a similar preparation technique for a pure compound (lithium carbonate). The PIXE analysis of this indicated that no contamination was occurring. Once the biological samples were pelletised, they were mounted upon an aluminium backing plate by double sided adhesive tape. A total of 15 samples of 5 mm diameter could be mounted upon a backing plate at one time. It has been demonstrated that no leaching between this adhesive tape and biological samples occurs [ARS91]. The samples had carbon dag applied to their sides (for the reasons stated in section 2.1.1.1), carefully avoiding application to their upper surface which is analysed, and then were stored in a desiccator ready for proton induced X-ray emission and Rutherford backscattering analysis.
Table 4.1 Information pertaining to the sub-sample selection from porcine tissues employed in homogeneity studies

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of sub-samples (n)</th>
<th>Description of sub-samples</th>
<th>Sub-sample weight (wet-weight)</th>
<th>dry/wet weight ratio mean ± SD</th>
<th>Replicate analysis weight (dry-weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>24</td>
<td>1.5 cm thick slice cut through centre along length of organ. 24 dry sub-samples of cortex, medulla and fat taken from this</td>
<td>109.7 g</td>
<td>0.167</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Liver 1</td>
<td>14</td>
<td>3× left lobe, 5× right lobe central, 6× right lobe peripheral</td>
<td>1.23 - 1.97 g</td>
<td>0.277 ± 0.012</td>
<td>0.31 g</td>
</tr>
<tr>
<td>Liver 2</td>
<td>13</td>
<td>3× left lobe, 3× right lobe central, 7× right lobe peripheral</td>
<td>3.62 - 8.75 g</td>
<td>0.282 ± 0.007</td>
<td>1.28 g</td>
</tr>
<tr>
<td>Liver 3</td>
<td>13</td>
<td>3× left lobe, 4× right lobe central, 6× right lobe peripheral</td>
<td>2.87 - 7.98 g</td>
<td>0.274 ± 0.005</td>
<td>0.89 g</td>
</tr>
<tr>
<td>Heart 1</td>
<td>10</td>
<td>1× right atrium, 3× right ventricle, 1× left atrium, 3× left ventricle, 1× aortic valve, 1× mitral valve</td>
<td>0.33² - 9.15⁻ g</td>
<td>0.204 ± 0.006³</td>
<td>0.72 g†</td>
</tr>
<tr>
<td>Heart 2</td>
<td>11</td>
<td>As for heart 1 + 1× pulmonary valve</td>
<td>0.18⁻ - 13.61⁻ g</td>
<td>0.203 ± 0.017⁷</td>
<td>0.57 g†</td>
</tr>
<tr>
<td>Heart 3</td>
<td>10</td>
<td>As for heart 1</td>
<td>0.23⁻ - 9.21⁻ g</td>
<td>0.204 ± 0.011¹</td>
<td>0.54 g†</td>
</tr>
</tbody>
</table>

² heart muscle, ³ heart valve.
4.5 Experimental parameters for analysis

The backing plate with samples was mounted in the target chamber which was evacuated down to the order of $10^{-6}$ torr. On reaching this pressure, the target chamber gate valve was opened allowing the 2 MeV proton beam to irradiate the samples. This beam had been previously collimated employing the 1.0 mm aperture setting, and was focused down to a beam spot size of roughly 200 μm. The beam scanning area was set and analysis of the samples begun. Proton induced X-ray emission and Rutherford backscattering spectra were collected simultaneously on the Sun Spark workstation, and stored on the mainframe ready for quantitative analysis. When a large beam scanning area was chosen (~ 3 mm x 3 mm) only a single spectrum was taken for each pellet, as this was thought as representative of the sample as one may achieve. Hence long irradiation periods achieving better counting statistics were adopted collecting a total charge of between 7 μC and 10 μC at a beam current 8 - 10 nA. Low beam currents were adopted to lessen specimen damage which may lead to falsification of results. If smaller scanning areas were used multiple sampling sites and even lower beam currents were employed.

4.6 Results and discussion

4.6.1 Intra and inter pellet elemental variations

The efficiency of the sub-sample homogenisation process will dictate the representativeness of the trace element results obtained. That is, if we wish to compare the results between two or more homogenised samples, we must be certain that they are representative of their extracted specimens. This issue is extremely important in PIXE analysis because we are analysing only small portions of the extracted sample (typically 10-100 μg) and hence large differences in trace element results may occur depending upon the pellet of homogenised material. Therefore, it is of great importance to investigate the degree of intra-pellet elemental variation such that necessary corrections may be applied when comparing the results for samples extracted from different positions within an organ. These corrections guarantee that any concentration differences noted are due to the position from where the sample is extracted or its size, rather than the homogenisation process.

The efficiency of the homogenisation procedure was investigated by the analysis of homogenised biological specimens in the form of pellets. These specimens included
liver, renal cortex, renal medulla, whole kidney (roughly 10% medulla, 90% cortex) and heart muscle (extracted from the right ventricle of heart 3). The IAEA standard reference material animal muscle (H-4) [MUR85] was also included in the study. Five to ten beam positions were taken on each pellet, the beam cross-sectional area being \((9.9 \pm 1.4) \times 10^{-5} \text{ m}^2\).

The approximate mass \(m\) of the sample probed by the proton beam can be calculated by the product of the beam cross-sectional area \(A\), proton range \(R\) and sample density \(\rho\) (equation 4.7)

\[
m = A R \rho \tag{Eq 4.7}
\]

The range may also be presented in the form of equation 4.8

\[
R = \frac{1}{\rho} \int_{E_0}^{0} \frac{dE}{S(E)} \tag{Eq 4.8}
\]

where \(E_0\) is the initial proton energy, and \(S(E)\) the mass stopping power of the sample upon the proton. Combination of equations 4.7 and 4.8 yields a further equation which is independent of sample density (equation 4.9).

\[
m = A \int_{E_0}^{0} \frac{dE}{S(E)} \tag{Eq 4.9}
\]

Therefore the factors that dictate the mass probed by the proton beam are the initial proton energy, proton beam cross-sectional area, and elemental composition of the sample. A description of the mass stopping power is given in section 2.3.3.2 and highlights that the integral illustrated in equation 4.9 may only be evaluated numerically. A program was written to perform this integration by Simpson's rule and employed Anderson and Ziegler's [AND77] semi-empirical fit with parameters to investigate the integral value for various biological matrices (animal muscle standard, liver, whole kidney and renal cortex and medulla and heart) and pure carbon. Very little difference existed between each of the values, and hence it may be concluded that the mass of a biological matrix probed by a
proton beam of 2 MeV is approximately given by equation 4.10.

\[ m = 8.5 \times 10^{-2} \mu \text{g} \]  

(Eq 4.10)

Therefore the replicate mass in the homogeneity studies is (8.4±1.2) μg. Typical intrapellet elemental relative standard deviations for the above tissues for this mass are presented in Table 4.2. The number of repeat measurements are given at the top of each column. The variance due to the analytical procedure was taken as that due to counting statistics only (equation 3.7). Measurements were made of the variance due to current integration fluctuations and a value of roughly 3.7% obtained. However, this value is not error corrected and if Gaussian statistics are assumed and applied to adjust counts collected, current integration fluctuation effects amount to a negligible contribution to the analytical variance.

Various features may be noted about Table 4.2. Firstly the elemental heterogeneity for the animal muscle standard is greater than those stated with certified concentrations (3.7%, 4.1% and 3.5% for K, Fe and Zn respectively [MUR85]). This is probably a consequence of larger sample sizes being employed in the certification of the standard. Secondly, liver and heart exhibit homogeneity values which are similar for each of the elements. Thirdly, elemental heterogeneity is greater for kidney medulla than

Table 4.2 Intra-pellet element relative standard deviations for various biological tissues. Replicate sampling mass (8.4±1.2) μg.

<table>
<thead>
<tr>
<th>Element</th>
<th>Animal muscle standard (H-4) [MUR85] (n=5)</th>
<th>Liver (n=10)</th>
<th>Kidney</th>
<th>Heart muscle (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=9)</td>
<td>medulla (n=5)</td>
<td>whole (n=10)</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>18.4%</td>
<td>22.4%</td>
<td>8.3%</td>
<td>22.8%</td>
</tr>
<tr>
<td>Ca</td>
<td>21.1%</td>
<td>18.2%</td>
<td>21.3%</td>
<td>5.9%</td>
</tr>
<tr>
<td>Mn</td>
<td>21.0%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>13.6%</td>
<td>21.3%</td>
<td>10.0%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Cu</td>
<td>21.8%</td>
<td>4.7%</td>
<td>17.3%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Zn</td>
<td>9.1%</td>
<td>24.5%</td>
<td>8.5%</td>
<td>21.5%</td>
</tr>
<tr>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>6.4%</td>
<td>-</td>
</tr>
</tbody>
</table>
cortex. The values for whole kidney lie between those for cortex and medulla, they however more closely relate to cortex values. This trend might be expected given the approximate whole kidney composition (10% medulla, 90% cortex).

Sampling factors were calculated for the above biological tissues (Table 4.3), for 1% and 5% variations due to elemental heterogeneity. These are denoted by $K_1$ and $K_5$ respectively. It is apparent that sampling factors at the 5% variation level are lower than those at 1%. This is a predictable feature as smaller sample weights will yield higher elemental variances. Values for $K_1$ vary from 180 µg to 5.1 mg, kidney cortex and whole kidney being the most homogenous biological materials of those studied. The sampling factors for animal muscle standard imply that analysis of roughly 3 mg of material will yield results which are negligibly affected by elemental heterogeneity. This is much lower than the 100 mg mass suggested by Muramatsu and Parr [MUR85].

It is obvious from Table 4.2 and Table 4.3, that the analysis of homogenised biological tissues in the form of pellets with a proton beam sampling only (8.4±1.2) µg of material will not yield representative trace element results if multiple sampling points are not employed. In fact, between 21 and 602 sampling points (Cu in kidney cortex and Zn in liver respectively) are needed, depending upon the tissue and element, to reduce elemental heterogeneities down to the 1% level. This number of sampling points is experimentally impracticable and hence large beam scanning areas were adopted in preference.

<table>
<thead>
<tr>
<th>Element</th>
<th>Animal Muscle Standard (H-4) [MUR85]</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cortex</td>
<td>medulla</td>
<td>whole</td>
</tr>
<tr>
<td>K</td>
<td>2834(113)</td>
<td>4223(169)</td>
<td>585(23)</td>
<td>4356(174)</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>3834(153)</td>
<td>2782(111)</td>
<td>3818(153)</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td>3823(153)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>1573(63)</td>
<td>3819(153)</td>
<td>836(33)</td>
<td>3858(154)</td>
</tr>
<tr>
<td>Cu</td>
<td></td>
<td>3907(156)</td>
<td>180(7)</td>
<td>2478(99)</td>
</tr>
<tr>
<td>Zn</td>
<td>686(27)</td>
<td>5060(202)</td>
<td>604(24)</td>
<td>3875(155)</td>
</tr>
<tr>
<td>Br</td>
<td></td>
<td>-</td>
<td>-</td>
<td>400(16)</td>
</tr>
</tbody>
</table>
A typical beam scanning area is \((3.7\pm0.5)\times10^{-6}\) m\(^2\) which accounts for sampling \((490\pm50)\) µg of a biological matrix. This scanning area was used to analyse liver samples extracted from a homogenised powder and in particular the variation of elemental concentrations between pellets. Liver was chosen as it is the most elementally heterogeneous biological powder of those studied. An attempt was made to calculate the sampling factors for each element between the 5 pellets. However, for each element, it was found that the analytical variation was greater than the variation between concentrations, and hence sampling factors could not be calculated. Instead the average concentrations for the intra-pellet variation measurements (multiple point method), and a single measurement from a large beam scan were compared to the average values for large beam scans between pellets. The results are shown in Table 4.4.

Table 4.4 Elemental concentrations in liver derived by the small area multiple point method and large area single point method. Values normalised to the average concentration between pellets are also shown.

<table>
<thead>
<tr>
<th>Element</th>
<th>Multiple point method ((5\times8.4) µg)</th>
<th>Single point method ((1\times490) µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm±SD(^\dagger)</td>
<td>normalised</td>
</tr>
<tr>
<td>S</td>
<td>6760±2600</td>
<td>1.27±0.61</td>
</tr>
<tr>
<td>Cl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>11110±3280</td>
<td>1.31±0.39</td>
</tr>
<tr>
<td>Ca</td>
<td>309±105</td>
<td>1.43±0.49</td>
</tr>
<tr>
<td>Mn</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>176±36</td>
<td>1.18±0.24</td>
</tr>
<tr>
<td>Cu</td>
<td>20.9±3.4</td>
<td>1.01±0.17</td>
</tr>
<tr>
<td>Zn</td>
<td>216±35</td>
<td>1.04±0.17</td>
</tr>
<tr>
<td>Br</td>
<td>2.6±1.0</td>
<td>0.32±0.14</td>
</tr>
<tr>
<td>Rb</td>
<td>20.1±3.8</td>
<td>1.38±0.28</td>
</tr>
<tr>
<td>Cd</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^\dagger\) standard deviation between results
\(^\ast\) standard deviation due to counting statistics
The concentrations presented in this table were calculated by the comparator method using animal muscle and Bowen's kale standards. This will be discussed further in the next section. The concentrations of the multiple point method show large standard deviations which are mostly due to the heterogeneous nature of the analysed material. These large standard deviations are one reason for the normalised values straying from unity, but another possible reason is the specimen damage from the ion beam which was noted visually by sample discolouration. This is an inevitability of decreasing the beam scanning area without sufficient decrease in the beam current. Approximate beam current densities for the small and large beam areas are $8 \times 10^{-14}$ A $\mu$m$^{-2}$ and $1 \times 10^{-15}$ A $\mu$m$^{-2}$ respectively. Valković et al [VAL74] note that Br and K may be lost from biological specimens through proton bombardment, whilst Kirby and Legge [KIR91] note the elements S and Cl. Other authors dispute the loss of any elements throughout PIXE analysis at all [JOH76].

Volatile element loss was investigated by the measurement of trace element levels in liver over 5 $\mu$C charge intervals. The current density was set to $1 \times 10^{-15}$ A $\mu$m$^{-2}$ and the beam scanning position kept static upon the pellet of homogenised material. At 5 $\mu$C charge intervals the spectra were stored such that the trace element variations due to sample damage could be noted with increasingly proton dose. A total of 25 $\mu$C was collected in all, and the results presented in Table 4.5.

Although an increase in concentration with proton dose is seen for K and Fe, no conclusive evidence exists for the volatilisation or concentration of elements at this beam current density as variations may adequately be explained by errors due to counting statistics. However, with the beam having a higher current density, effects of sample damage may be accentuated. Turning our attention back to Table 4.4, bromine is the only element for the small area scans that has a normalised value of less than unity. This may imply volatility of the element. However, the elements K and S take normalised values to be greater than one and hence it is assumed for elements other than Br, sample heating effects are negligible. As expected the concentrations derived from the single point method are close to the average values derived from the five single point large area scanned measurements.
Table 4.5 Investigation of the trace element levels in liver as a function of charge collected in PIXE analysis. Concentrations are shown in ppm with analytical errors.

<table>
<thead>
<tr>
<th>Element</th>
<th>Charge collected</th>
<th>Average concentration ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µC</td>
<td>10 µC</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>224±80</td>
<td>237±84</td>
</tr>
<tr>
<td>Fe</td>
<td>827±60</td>
<td>810±58</td>
</tr>
<tr>
<td>Cu</td>
<td>22.9±9.1</td>
<td>20.8±8.3</td>
</tr>
<tr>
<td>Zn</td>
<td>197±16</td>
<td>216±18</td>
</tr>
<tr>
<td>Br</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Mn</td>
<td>9.1±2.3</td>
<td>8.8±2.3</td>
</tr>
<tr>
<td>Cd</td>
<td>*</td>
<td>0.25±0.12</td>
</tr>
</tbody>
</table>

* below detection limit (see Figure 2.8 for typical values).

4.6.2 Comparison of analysis procedures

Prior to the presentation of trace elemental levels in biological samples it is essential to test the validity of the techniques employed to derive these concentrations. The most favourable approach is to compare values calculated in standard reference materials with certified concentrations. The comparative (section 2.4.1.2), single element comparative (section 2.4.1.1) and absolute (section 2.4.2) methods are employed in the analysis of Bowen's kale standard [MUR85]. Data may be found in Table 4.6. The standard used in both of the comparative approaches is IAEA animal muscle (H-4). All concentration values are derived with the aid of PIXAN [CLA86] (section 3.5.2). BATTY was used to calculate the experimental X-ray yield from collected spectra whereas THICK was used for theoretical X-ray yields, assuming unit concentration and charge.

It may be seen that elemental concentrations derived upon a comparative basis generally agree well with certified concentrations (excluding Cl). Much of this agreement may be attributed to the cancellation of detector efficiency, solid angle and charge
integration as indicated in equation 2.14. However, the main disadvantage of this approach is highlighted in that concentrations for Mn, Br and Sr could not be derived as these elements were either below detection limits in the standard spectrum, or not present within the standard at all.

The single element comparative approach circumvents the above problem in that only one element in the standard is used to calculate all elemental concentrations in samples. However, although this technique is attractive in that reliance upon charge integration and solid angle is removed, detector efficiency values must be employed in concentration calculations. Therefore, it was necessary to investigate the possibility of differences occurring in the detector efficiency model parameters [COH80, CLA86] used in PIXAN to those employed in our detector. Small but significant differences were found in efficiencies due to different gold contact and crystal dead layer thicknesses, and hence an energy dependent correction employed to account for this. A further measure which could be applied to minimise errors due to imprecise knowledge of the detector efficiency would be to select a comparator element with X-ray energy which is similar to that of interest. Two sets of data were found for concentrations in Bowen’s kale derived upon this basis i.e. one set using Zn as comparator, whilst the other K. These elements were chosen as comparators as their counts in the animal muscle spectrum were sufficiently large to ensure good counting statistics, and errors associated with certified values were also small (~4%). It may be seen that elemental concentrations derived by the single element comparator technique compare well to those derived by the comparative technique.

Calculation of elemental concentrations upon an absolute basis not only required correction of theoretical X-ray yields for variation due to detector efficiency, but also solid angle subtended to the detector. As the beam current was scanned over an area of roughly 6 mm², then point source dimensions could not be assumed. Calculation of solid angle was also complicated in that the scanned area plane was inclined at 45° to the detector axis. Hence, a Monte Carlo program was employed to calculate solid angle and thus concentrations in Bowen’s kale upon an absolute basis. These concentrations differ greatly from certified values. The fact that they differ by roughly the same factor implies the presence of a systematic error. This systematic error is most probably due to errors associated with the charge collection process and solid angle [JELY93], the latter could not be determined accurately and did not take into account a source centring offset which was
most likely present. A graph (Figure 4.5) of concentrations in Bowen's kale and IAEA animal muscle standards derived upon the absolute approach against certified values was used to ascertain this correction factor. A linear least square fit to the data yielded a value of 8.04 (correlation coefficient 0.994 n=16). This correction factor was applied to the absolute analysis data obtained from a typical homogenised heart muscle sample extracted from the right ventricle. The converted results are seen to compare well with those obtained by the comparator and single element comparative techniques (Table 4.6). However, the application of the correction factor defeats many of the attractions of the absolute method.

In conclusion, if concentrations are to be quoted, they will where possible, be calculated upon a comparative basis as these results are least affected by experimental set-up and parameters. If for reasons due to elements not being present below detection limits in the standard concentrations cannot be derived upon a comparative basis, they will be derived using the single element comparative technique.

![Graph showing concentrations derived upon an absolute basis against certified values for two standards to ascertain their correlation.](image-url)
Table 4.6 Elemental concentrations in Bowen’s kale and porcine heart muscle derived by the comparator, single element comparator and absolute methods in PIXE analysis.

<table>
<thead>
<tr>
<th>Element</th>
<th>Bowen’s kale (ppm) dry-weight [MUR85]</th>
<th>Heart muscle (ppm) dry-weight</th>
<th>Corrected Absolute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certified</td>
<td>Comparative</td>
<td>Single Element Comparator</td>
</tr>
<tr>
<td>Cl</td>
<td>3560±430</td>
<td>12830±5130</td>
<td>16690±2160</td>
</tr>
<tr>
<td>K</td>
<td>24370±1460</td>
<td>27610±1070</td>
<td>29630±2090</td>
</tr>
<tr>
<td>Ca</td>
<td>41060±2220</td>
<td>39600±10780</td>
<td>43450±3060</td>
</tr>
<tr>
<td>Mn</td>
<td>14.8±1.63</td>
<td>*</td>
<td>19.7±2.94</td>
</tr>
<tr>
<td>Fe</td>
<td>119.3±14.3</td>
<td>126.0±10.0</td>
<td>132.2±10.5</td>
</tr>
<tr>
<td>Cu</td>
<td>4.89±0.64</td>
<td>4.95±2.73</td>
<td>4.58±1.86</td>
</tr>
<tr>
<td>Zn</td>
<td>32.29±2.74</td>
<td>38.85±4.55</td>
<td>38.85±4.55</td>
</tr>
<tr>
<td>Br</td>
<td>24.9±2.47</td>
<td>*</td>
<td>19.5±5.3</td>
</tr>
<tr>
<td>Rb</td>
<td>53.4±5.3</td>
<td>56.4±23.1</td>
<td>53.6±11.6</td>
</tr>
<tr>
<td>Sr</td>
<td>75.7±29.5</td>
<td>*</td>
<td>103.3±17.2</td>
</tr>
</tbody>
</table>

* Below detection limit in IAEA Animal muscle standard (H-4), † Element not found in standard.
4.6.3 Intra-organ element variations

4.6.3.1 Heart

Initially, concentrations between the ventricles of each of the three hearts were compared. This was done by calculating the ratio of concentrations of the right ventricle to the left ventricle (RV/LV), a value significantly different from unity indicating some degree of variation. The average values between the three hearts for each element is shown in Table 4.7. It may be concluded that no significant difference between the two ventricles exists for the elements illustrated. Therefore, the average concentrations between the ventricles are calculated (RV+LV), and these used to compare to concentrations from other positions from within the heart, namely right atrium (RA), left atrium (LA), aortic valve (AV), mitral valve (MV) and pulmonary valve (PV). Again, the average ratios between the three hearts are given in Table 4.7. Typical concentrations in porcine heart muscle (extracted from the right ventricle), may be found in Table 4.6 and these may be used, if desired, in conjunction with the ratios in Table 4.7 to calculate typical concentrations in other areas of the organ.

Not surprisingly, the largest differences in concentrations are seen when comparing heart valves to ventricles, significant differences occurring for the elements Cl, K, Ca, Fe, Cu and Zn. For calcium only, the ratio exceeds unity and is roughly 2.5-3.5 times more concentrated in valves than muscle. Hypothesis testing on the above elements reveals that ratios are significantly different from unity (0.01 significance level) for K, Ca and Zn in all valves. For the remaining elements this level of difference exists for aortic and pulmonary valves apart from Br and Cl in the former. In the mitral valve, Fe strays from unity at the 0.05 significance level only, which is not satisfied for the remaining elements.

Turning our attention to the right and left atria, differences in ratios from unity are less defined. Differences are seen at the 0.01 significance level for K for both the right and left atria. This degree of significance is also seen for Fe in the left atrium, but at only the 0.05 level in the right atrium. Differences in concentrations of K and Fe between the atria and ventricles may be due to their slightly differing make-up. The ventricles are composed of thick myocardium (heart muscle) covered in a thin endocardium and epicardium, whilst the myocardium in the atria is much thinner and weaker.
Table 4.7 Ratios of concentrations from different positions in porcine heart to those derived from an average value between the ventricles.

<table>
<thead>
<tr>
<th>Element</th>
<th>RV/LV</th>
<th>RA/RV+LV</th>
<th>LA/RV+LV</th>
<th>AV/RV+LV</th>
<th>MV/RV+LV</th>
<th>PV/RV+LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>0.818±0.516</td>
<td>0.740±0.430</td>
<td>0.519±0.357</td>
<td>1.259±0.667</td>
<td>1.059±0.563</td>
<td>-</td>
</tr>
<tr>
<td>Cl</td>
<td>1.216±0.564</td>
<td>1.136±0.506</td>
<td>0.988±0.427</td>
<td>0.660±0.300</td>
<td>0.843±0.368</td>
<td>0.227±0.164</td>
</tr>
<tr>
<td>K</td>
<td>0.913±0.051</td>
<td>0.831±0.046</td>
<td>0.754±0.042</td>
<td>0.606±0.034</td>
<td>0.600±0.041</td>
<td>0.351±0.020</td>
</tr>
<tr>
<td>Ca</td>
<td>1.075±0.485</td>
<td>1.253±0.511</td>
<td>1.541±0.571</td>
<td>3.593±1.386</td>
<td>2.454±0.960</td>
<td>3.565±1.694</td>
</tr>
<tr>
<td>Fe</td>
<td>0.969±0.096</td>
<td>0.893±0.089</td>
<td>0.758±0.075</td>
<td>0.707±0.081</td>
<td>0.898±0.101</td>
<td>0.778±0.087</td>
</tr>
<tr>
<td>Cu</td>
<td>1.024±0.428</td>
<td>0.942±0.393</td>
<td>0.889±0.375</td>
<td>0.411±0.179</td>
<td>0.857±0.351</td>
<td>0.307±0.142</td>
</tr>
<tr>
<td>Zn</td>
<td>0.924±0.109</td>
<td>0.966±0.113</td>
<td>0.948±0.109</td>
<td>0.501±0.065</td>
<td>0.456±0.064</td>
<td>0.646±0.090</td>
</tr>
<tr>
<td>Br</td>
<td>1.398±0.828</td>
<td>1.342±0.750</td>
<td>1.168±0.667</td>
<td>0.811±0.491</td>
<td>1.239±0.693</td>
<td>0.308±0.223</td>
</tr>
<tr>
<td>Rb</td>
<td>1.121±0.639</td>
<td>1.016±0.573</td>
<td>0.784±0.473</td>
<td>1.105±0.608</td>
<td>0.878±0.509</td>
<td>-</td>
</tr>
</tbody>
</table>
4.6.3.2 Kidney

Trace and minor elemental concentrations in porcine kidney may be found in Table 4.8. Illustrated are values for whole kidney, cortex, medulla and sinus fat. The number of samples being utilised in the calculation for each area were 24, 8, 5 and 2 respectively. Samples were selected such that their composition was predominantly of the tissue type of interest.

Initial features to note about Table 4.8 is that concentrations of Cu, Zn, Mn and Cd in cortex exceed those in medulla, whilst for K, Ca and Fe the values are approximately the same. These are essentially the same conclusions drawn by Tanaka et al [TAN87] and Livingstone [LIV71] although Tanaka et al also note preferential collection of Rb in the cortex. With respect to adipose, trace element levels are mainly at lower concentrations than in cortex, although there seems to be an increased calcium and iron content in this tissue fraction.

Rutherford backscattering data (see section 7.5) yields concentrations in cortex and medulla for C, N and O to be 68%, 11% and 15% respectively. Corresponding elemental concentrations in predominantly fat samples are 62%, 11% and 14%. Slight differences in matrix composition are to be expected given the data for fat presented in ICRP-23 [ICR75].

4.6.3.3 Liver

Mean elemental concentrations with standard deviations in each of the three livers are shown in Table 4.9. It may be seen that apart from Zn and possibly Cu, there are large differences in elemental compositions between organs. A possible explanation for this could be the different ages of the slaughtered animals. Therefore is was not possible to pool data to test for differences in sample composition extracted from various positions within the organ, and hence the data from each liver had to be examined individually. Data showing the mean concentration (± analytical error) for each of the sampling areas for liver 1 are illustrated in Table 4.10. Significance testing of this data reveals that differences are found when comparing the iron, copper and zinc content of the left lobe to either of the sampling positions of the right lobe (99% confidence level for all comparisons apart from zinc in the right lobe peripheral, which has roughly a 98% confidence level). In fact, zinc is found also to be significantly different when comparing
Table 4.8 Elemental concentrations in porcine whole kidney, renal cortex, renal medulla and sinus fat (ppm-dry weight).

<table>
<thead>
<tr>
<th>Element</th>
<th>Arithmetic mean±standard deviation (range) ppm-dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>whole kidney</td>
</tr>
<tr>
<td>K</td>
<td>10300±1170 (7890-12290)</td>
</tr>
<tr>
<td>Ca</td>
<td>398±88 (292-724)</td>
</tr>
<tr>
<td>Fe</td>
<td>220±114 (146-649)</td>
</tr>
<tr>
<td>Cu</td>
<td>11±3 (7-17)</td>
</tr>
<tr>
<td>Zn</td>
<td>61±9 (45-77)</td>
</tr>
<tr>
<td>Br</td>
<td>35±10 (19-51)</td>
</tr>
<tr>
<td>Rb</td>
<td>16±3 (10-22)</td>
</tr>
<tr>
<td>Mn</td>
<td>4.5±1.5 (2.4-6.8)</td>
</tr>
<tr>
<td>Sr</td>
<td>10±3 (6-14)</td>
</tr>
<tr>
<td>Cd</td>
<td>0.28±0.05 (0.20-0.39)</td>
</tr>
<tr>
<td>Pb</td>
<td>1.8±0.1 (1.8-1.9)</td>
</tr>
<tr>
<td>Cr</td>
<td>0.03±0.03 (0.01-0.10)</td>
</tr>
<tr>
<td>As</td>
<td>1.5±0.5 (0.9-2.2)</td>
</tr>
<tr>
<td>Se</td>
<td>3.3±1.5 (1.2-6.7)</td>
</tr>
<tr>
<td>Ni</td>
<td>0.50±0.22 (0.34-0.65)</td>
</tr>
</tbody>
</table>
Table 4.9 Mean elemental compositions and standard deviations for the three porcine livers employed in sampling studies.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean concentration ± Standard deviation (ppm - dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver 1</td>
</tr>
<tr>
<td>Cl</td>
<td>2440±340</td>
</tr>
<tr>
<td>K</td>
<td>7090±380</td>
</tr>
<tr>
<td>Ca</td>
<td>262±36</td>
</tr>
<tr>
<td>Mn</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>Fe</td>
<td>344±22</td>
</tr>
<tr>
<td>Cu</td>
<td>24.9±2.9</td>
</tr>
<tr>
<td>Zn</td>
<td>223±22</td>
</tr>
<tr>
<td>Br</td>
<td>5.8±1.1</td>
</tr>
<tr>
<td>Rb</td>
<td>10.9±1.4</td>
</tr>
</tbody>
</table>

Table 4.10 Arithmetic mean concentrations for each of the main sampling sites in liver 1.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration ± analytical error (ppm - dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left lobe</td>
</tr>
<tr>
<td>Cl</td>
<td>2620±340</td>
</tr>
<tr>
<td>K</td>
<td>6920±160</td>
</tr>
<tr>
<td>Ca</td>
<td>271±39</td>
</tr>
<tr>
<td>Mn</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>Fe</td>
<td>317±12</td>
</tr>
<tr>
<td>Cu</td>
<td>20.8±1.1</td>
</tr>
<tr>
<td>Zn</td>
<td>204±7</td>
</tr>
<tr>
<td>Br</td>
<td>5.9±1.7</td>
</tr>
<tr>
<td>Rb</td>
<td>11.9±1.6</td>
</tr>
</tbody>
</table>
both of the positions in the right lobe. These relationships however could not be verified in the remaining two livers mainly because of analytical errors being too large.

The above discussion implies that the trace element levels for Fe, Cu and Zn determined in liver will be dependent to some extent upon where in the organ the sub-samples are extracted. For iron, this agrees with the findings of Perry et al [PER77], and may be attributed to the differing blood content of the sub-samples. Concerning the elements Cu and Zn, literature is split regarding the presence of positional content variation. We would expect no positional dependency for these elements as the physiological function of this organ is the same throughout. However, the above findings agree with those of Schicha et al [SCH70], and to an extent Koenig et al [KOE79].

4.6.4 Organ homogeneity and sampling factors

Table 4.11 and Table 4.12 contain data for the elemental inhomogeneity (see section 4.3.1) and sampling factors (see section 4.3.2) respectively for whole and different tissue components for liver, kidney and heart. Sampling factors are presented at 1% and 5% variation levels in grams dry weight. Both of these quantities are calculated assuming that apart from elemental heterogeneity, X-ray peak count statistics are the only significant source of peak area variation. For the reason why charge integration fluctuations are not deemed significant, see section 4.6.1.

At first glance, the inhomogeneity values in Table 4.11 for whole kidney imply that this organ is by far more elementally heterogeneous than for example whole heart or whole liver. However, it must be noted that the sub-samples extracted to derive these inhomogeneity values were roughly one tenth to one twentieth of the weight (0.03 g) of those to derive values in liver (0.31 g) and heart (0.57 g). Inhomogeneity values for kidney are greater than those for heart or liver mostly due to this. Hence, it is more logical when wishing to compare the heterogeneity of organs that have been analysed using differing sub-sample weights, to consider sampling factors.

When comparing the sampling factors between whole kidney, liver and heart, no organ is obviously more elementally homogeneous or heterogeneous than another. Concentrating upon whole kidney, the elements K and Ca possess sampling factors that are small in comparison to many of those for other elements. This is an expected feature given the findings by Tanaka et al [TAN87] who show no preferential collection site for
these elements. Tanaka et al also note that the concentration of iron is independent of where sub-samples are extracted to derive it. This is contradicted by the relatively large sampling factor (36 g at 1% variation level) for this element as seen in Table 4.12. This may be the result of differing residual blood contents of sub-samples. Zinc also has a relatively small sampling factor in whole kidney which is unexpected given that it preferentially collects within the cortex [TAN87,LIV71] as do Mn, Cu and Rb these however exhibiting some of the largest sampling factors in whole kidney.

If we compare sampling factors for renal medulla and cortex to whole kidney, it is apparent that in most cases (apart from K and Ca), values are smaller in individual composite tissues than the organ as a whole. This is a consequence of the medulla and cortex having different elemental compositions as indicated in section 4.6.3.2. Hence it is more logical when reporting elemental concentrations in kidney, to report those for cortex and medulla individually as the sample mass needed to be extracted to derive reliable concentrations is smaller than that needed to be extracted to derive results about the organ as a whole.

As the liver is essentially composed of one cell type [PER77], it was expected that this organ would be the most elementally homogeneous of those studied. This initial postulation seems untrue as it possesses sampling factors that are similar to those for whole kidney. Sampling factors for whole liver range from 6.3 g for K to 42 g for Ca. Significance testing in section 4.6.3.3 revealed that differences in concentrations for Fe, Cu and Zn occurred between the three sampling sites. This is reiterated in Table 4.12 in that sampling factors for these elements in whole liver are of the largest, although this is not as obvious for Fe.

Sampling factors in heart were calculated for whole heart muscle (atria and ventricles), and ventricle muscle only. When comparing values for whole heart to ventricles only, apart from Cl, they are smaller in the latter. This implies that there is a difference in concentrations between ventricles and atria for K, Fe and Zn. Hypothesis testing has shown that differences in concentrations are significant for K and Fe (see section 4.6.3.1). Heydom [HEY84] and Aalbers et al [AAL87] note good homogeneity of copper in heart muscle which is seen in Table 4.12, a value of 1.5 g (1% variation level) being attained in whole heart. Variations of copper in ventricle only, fell below the analytical variance and hence could not be determined.
Table 4.11 Typical percentage elemental inhomogeneity levels, $\sigma_\beta(\%)$ in porcine liver, kidney and heart.

<table>
<thead>
<tr>
<th>Element</th>
<th>Liver</th>
<th></th>
<th></th>
<th></th>
<th>Kidney</th>
<th></th>
<th></th>
<th></th>
<th>Heart</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL</td>
<td>RLC</td>
<td>RLP</td>
<td>WHOLE</td>
<td>CORTEX</td>
<td>MEDULLA</td>
<td>WHOLE</td>
<td>VENTRICLE</td>
<td>WHOLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>5.3%</td>
<td>9.5%</td>
<td>-</td>
<td>-</td>
<td>11.6%</td>
<td>-</td>
<td>3.6%</td>
<td>20.5%</td>
<td>17.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>3.9%</td>
<td>4.2%</td>
<td>4.5%</td>
<td>1.1%</td>
<td>7.0%</td>
<td>8.1%</td>
<td>9.4%</td>
<td>16.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>-</td>
<td>1.8%</td>
<td>19.4%</td>
<td>11.6%</td>
<td>7.1%</td>
<td>8.6%</td>
<td>15.2%</td>
<td>-</td>
<td>8.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>-</td>
<td>1.9%</td>
<td>7.4%</td>
<td>6.1%</td>
<td>-</td>
<td>40.8%</td>
<td>34.0%</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>-</td>
<td>5.2%</td>
<td>-</td>
<td>4.9%</td>
<td>16.1%</td>
<td>16.6%</td>
<td>34.8%</td>
<td>2.8%</td>
<td>12.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>-</td>
<td>2.4%</td>
<td>&lt;0.1%</td>
<td>10.2%</td>
<td>9.5%</td>
<td>-</td>
<td>21.7%</td>
<td>-</td>
<td>1.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>11.8%</td>
<td>3.3%</td>
<td>9.8%</td>
<td>9.4%</td>
<td>-</td>
<td>13.8%</td>
<td>12.2%</td>
<td>3.9%</td>
<td>4.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20.9%</td>
<td>35.9%</td>
<td>27.7%</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>4.4%</td>
<td>-</td>
<td>6.5%</td>
<td>-</td>
<td>13.3%</td>
<td>-</td>
<td>18.7%</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LL left lobe, RLC right lobe central, RLP right lobe peripheral.
- Variation due to analytical procedure greater than that due to elemental heterogeneity.
Table 4.12 Typical sampling factors (grams) for porcine liver, kidney and heart on a dry weight basis. Values are shown at the 1% and 5% (in parentheses) levels.

<table>
<thead>
<tr>
<th>Element</th>
<th>Liver</th>
<th></th>
<th></th>
<th></th>
<th>Kidney</th>
<th></th>
<th></th>
<th></th>
<th>Heart</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>8.6</td>
<td>28</td>
<td>-</td>
<td>0.39</td>
<td>-</td>
<td>240</td>
<td>168</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>4.6</td>
<td>5.5</td>
<td>6.3</td>
<td>0.39</td>
<td>3.7</td>
<td>2.5</td>
<td>50</td>
<td>157</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>1.0</td>
<td>117</td>
<td>42</td>
<td>2.5</td>
<td>1.4</td>
<td>6.1</td>
<td>41</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>1.1</td>
<td>17</td>
<td>12</td>
<td>-</td>
<td>55</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>8.5</td>
<td>-</td>
<td>7.3</td>
<td>7.0</td>
<td>8.0</td>
<td>36</td>
<td>4.6</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>1.7</td>
<td>6.9</td>
<td>32</td>
<td>2.7</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>1.8</td>
<td>3.4</td>
<td>30</td>
<td>-</td>
<td>5.7</td>
<td>4.4</td>
<td>8.5</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>39</td>
<td>23</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>6.1</td>
<td>13</td>
<td>-</td>
<td>6.3</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See notes for Table 4.11
Sampling factor ranges (\(K_i\)) for whole liver, renal cortex, renal medulla, whole kidney and heart muscle are 6.3g (K) - 42g (Ca), 0.39g (K) - 13g (Br), 1.4g (Ca) - 55g (Mn), 2.5g (K) - 36g (Fe) and 1.5g (Cu) - 168g (Cl) dry weight basis respectively. Converted to a fresh weight basis, these translate to values of 23g - 152g, 2.3g - 80g, 8.4g - 330g, 15g - 220g and 7.4g - 828g respectively. In most situations, the upper limit of the sampling factor range exceeds the total fresh weight of that organ these being for reference man 1400 -1800 g for liver, 140 -155 g for kidney and 200 -400 g for heart [ICR75]. Organ weights for pig are similar. This implies that the whole organ is required to derive representative elemental concentrations with 1% variation upon results. In any case, sampling factors at the 1% level are on the whole impractically large, and thus if sampling from an organ, a higher level of elemental heterogeneity would have to be settled for e.g. 5% (K\(_3\)) as shown in Table 4.12 in parentheses.

Of the limited sampling factor data presented in the literature, \(K_i\) takes a value of 200 g for Br, Co, Mn, Rb and Zn (Fe is a little higher whilst Se is a little lower) in liver [HEY84], 194 g and 208 g for Se and Zn respectively in renal medulla [DAM82] and upper limit 10 g for Cu in heart muscle [HEY84]. Corresponding \(K_i\) values from this work are 43 g, 97 g and 26 g for Mn, Zn and Fe in liver, 34 g for Zn in renal medulla and 7.4 g for Cu in heart muscle, all on a wet weight basis. These values are lower than those indicated above. Heydorn [HEY84] indicates that a reasonable sampling factor value for soft tissue is about 200 g wet weight and although this would encompass most of the sampling factors presented in Table 4.12 (apart from, for example Cl and K in heart muscle), it is felt that this value, for the organs studied, is slightly excessive. A wet weight value of 150 g (corresponding weight for K\(_3\) being 6 g) may be more applicable as this covers 80% of the sampling factors listed.

4.6.5 Correlation coefficients

Correlation coefficients (linear regression) for kidney and liver and heart at the 95%, 98% and 99% confidence levels are presented in Tables 4.13 and 4.14 respectively. Data for kidney is split into that for renal medulla, renal cortex and kidney as a whole.

Correlation coefficients in renal cortex are significant for the relationships between K-Rb, Fe-Cd, Cu-Zn and Cu-Mn. The correlation between K-Rb is interesting as a negative correlation was obtained by Tanaka et al [TAN87] which was explained by an
exchange effect between the elements. A positive correlation between the elements Cu and Zn was expected due to the presence of metallothionein [BRE90,TAN87,LIV71], although positive correlations for Cu-Cd and more importantly Zn-Cd were also predicted for the same reasons, but not seen. The negative correlation between the elements Fe and Cd agrees with the findings of Bunn and Matrone [BUN66], they however provide no reason for this relationship.

Table 4.13 Elemental correlation coefficients for renal cortex, renal medulla and whole kidney at the 95%, 98% and 99% confidence levels.

<table>
<thead>
<tr>
<th>Renal cortex (n=8)</th>
<th>Renal medulla (n=4)</th>
<th>Whole kidney (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Element</strong></td>
<td><strong>Correlation</strong></td>
<td><strong>r</strong></td>
</tr>
<tr>
<td>K-Rb</td>
<td>0.732*</td>
<td>K-Br</td>
</tr>
<tr>
<td>Fe-Cd</td>
<td>-0.910*</td>
<td>Ca-Br</td>
</tr>
<tr>
<td>Cu-Zn</td>
<td>0.718*</td>
<td>Ca-Zn</td>
</tr>
<tr>
<td>Cu-Mn</td>
<td>0.730*</td>
<td>Ca-Rb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca-Mn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca-Rb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Zn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Rb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe-Mn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zn-Rb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rb-Mn</td>
</tr>
</tbody>
</table>

*≥95% confidence level, †≥98% confidence level, ‡≥99% confidence level.
Table 4.14 Typical correlation coefficients for liver and heart at the 95%, 98% and 99% confidence levels.

<table>
<thead>
<tr>
<th>Element Correlation</th>
<th>Liver (n=14) r</th>
<th>Heart (n=7) r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl-Rb</td>
<td>0.619*</td>
<td>Cl-Ca</td>
</tr>
<tr>
<td>K-Mn</td>
<td>0.805†</td>
<td>Cl-Br</td>
</tr>
<tr>
<td>K-Fe</td>
<td>0.681†</td>
<td>K-Ca</td>
</tr>
<tr>
<td>K-Zn</td>
<td>0.892†</td>
<td>K-Cu</td>
</tr>
<tr>
<td>Mn-Fe</td>
<td>0.464†</td>
<td>Ca-Fe</td>
</tr>
<tr>
<td>Mn-Zn</td>
<td>0.686†</td>
<td>Ca-Br</td>
</tr>
<tr>
<td>Fe-Cu</td>
<td>0.552*</td>
<td></td>
</tr>
<tr>
<td>Fe-Zn</td>
<td>0.761†</td>
<td></td>
</tr>
<tr>
<td>Cu-Zn</td>
<td>0.636†</td>
<td></td>
</tr>
</tbody>
</table>

*≥95% confidence level, †≥98% confidence level, ‡≥99% confidence level.

All correlations between the elements in renal medulla are positive. There is a highly significant correlation between the elements Ca, Fe, Zn and Rb. A possible reason for this may be the enhancement of these elements in medulla by blood.

With respect to whole kidney, Ca-Fe, Ca-Rb and Fe-Rb are the only significant correlation coefficient relationships that are also seen in renal medulla. This may be for the reasons stated earlier. A positive correlation is seen for Cu and Zn which again is most probably due to metallothionein.

A positive correlation between Cu and Zn in liver may be due to the formation of metallothionein, as this organ is the main site of the protein formation [BRE90]. Significant positive correlations are also seen between the elements K-Mn, K-Fe, Mn-Zn and Fe-Zn. These correlations may be due to the presence of blood or the formation of ferritin or hemosiderin.

Of the element correlations in whole heart muscle, two are highly significant, namely Cl-Br and K-Ca, the former being a positive relationship whilst the latter negative.
With respect to Cl and Br, one would expect their relationship to be negatively correlated due to their chemical similarities such that one may substitute for the other in tissues [UND71]. The fact that they are positively correlated may suggest the presence of other physiological effects.

4.7 Summary of conclusions

Trace element concentrations were derived for samples extracted from different positions in porcine liver, kidney and heart. These concentrations were utilised in the assessment of organ heterogeneity which are expressed in terms of percentage standard deviations and sampling factors. Comparison of percentage standard deviations between organs did not prove a reliable method for comparing the elemental heterogeneity between the liver, heart and kidney, as different sample weights were used. As the weight of kidney samples was smaller than those for liver and heart, percentage standard deviations for the former organ gave the impression that it was by far the most elementally inhomogeneous tissue of those studied. The calculation of sampling factors proved that this was not the case and that the heterogeneity of each organ was similar. This was surprising for liver as it was initially thought that because this organ was essentially composed of one tissue type, it should be relatively homogeneous. Comparison of derived sampling factors to the few values found in the literature showed that those derived in this work were smaller. Heydorn [HEY84] suggests a sampling factor weight of 200 g for most elements in biological tissues, whilst the sampling factors derived here suggest a value that is lower.

Significance testing concentrations in heart between the ventricles and valves not surprisingly found differences for Cl, K, Ca, Cu and Zn. When comparing the ventricles and atria, differences were also found for the elements K and Fe. The most probable reason for this being the variation of the amount of myocardium between sites. The amount found in the ventricles is larger and hence the requirement for blood in this area of heart is greater than that for the atria. Significant differences in Cu, Zn and Fe were found in liver depending upon where sub-samples were extracted. This was unexpected due to the homogeneous physiological function of this organ. With respect to the kidney, findings were very much as expected, K, Fe and Ca showing no preferential collection whilst Cu, Zn, Mn and Cd accumulated within the renal cortex.
Chapter 5
Analysis of Thick Biological Sections

5.1 Introduction

Sub-samples from various tissues and organs were analysed for their trace and minor element contents in chapter 4. Analysis demonstrated that different element levels could be obtained which were to an extent due to the position from where the sub-samples were extracted. However, as the sub-samples were analysed in the form of manufactured pellets, a minimum mass of material (roughly 10 mg) had to be extracted which limited the spatial resolution in the quantification of trace element distributions. This is unfortunate as a high degree of spatial resolution is one of the merits of proton induced X-ray emission analysis. An approach to optimise this merit in the determination of elemental distributions in biological tissues would be to employ specimen sections in preference to homogenised samples in the form of pellets.

Biological sections fall into one of the categories of thin, intermediate or thick targets as explained in section 2.3. Realistically, criteria for thin target classification are not experimentally satisfied and hence comparison between intermediate and thick biological sections will only be considered. Disadvantages of intermediate sections are related to the proton beam passing through the target and include contamination of the PIXE spectra from sample backing materials [RUS81] which are used for sample support and the added complication of quantifying specimen thickness so that quantitative trace element concentration determination may be carried out (see section 2.3.2). Also, as there is no Faraday cup incorporated into the University of Surrey PIXE system, there is a potential for the proton beam to strike the current integration plate and to create a spurious signal in the X-ray spectra. The preferred scenario is to employ a thick biological section that may be mounted directly onto an aluminium target plate. As the proton beam is completely halted within the sample, no risk of X-ray contamination from target backing materials or the plate is introduced. However, the utilisation of thick sections is not without problem. These are discussed in depth by Campbell and Cookson [CAM84] (see also section 2.3.3) and many have been addressed in chapters previous to this. The most problematic to this work though have not yet been discussed and include
requirements for the geometrical and surface specifications of targets.

The proton induced X-ray emission analysis carried out in this work assumes that the ion beam enters the sample perpendicular to the surface plane, and X-rays are detected at an angle of 135° to the incident beam direction. However, if the specimen surface is tilted with respect to the incident beam direction (i.e. no longer at 90°), then depending on the orientation of the tilt, X-ray signals in spectra may be either enhanced or deteriorated. The degree of tilt is dependent upon the quality of the specimen surface preparation technique which also affects how rough the sample is. Sample surface roughness also dictates the degree of X-ray enhancement or deterioration in spectra but is further complicated in that biological specimens contain, for example, blood vessels and bile ducts which will be present even if sample surface preparation techniques are perfect. A simple stylised model has been developed to estimate the degree of significance of sample surface roughness effects to X-ray yield in biological samples. Results are presented in the following sections accompanied with an investigation of the effect of sample surface tilt upon PIXE analysis. These are discussed in relation to the quantification of the distribution of trace elements across the biological sections. Plots of element levels over specimens highlight the heterogeneous nature of the studied tissues and support many of the findings of chapter 4.

5.2 Sample selection, extraction and preparation

As an in-depth description of sub-sample selection, extraction and preparation was presented in section 4.4, only a brief discussion will be provided here. Sample sections to be studied were extracted from porcine kidney, liver and lung. Samples of kidney were taken in the form of a coronal section through the organ by a stainless steel blade, so that a surface similar to that illustrated in Figure 4.2 was obtained. A stainless steel blade was also used to extract sections from a liver (right lobe extending from outer surface and encompassing an hepatic artery) and a pair of lungs, the sampling sites being illustrated in Figure 5.1. Note that samples of bronchi were extracted in addition to lung tissue (samples 5 and 10). All biological samples were bathed in de-ionised water to remove blood and other fluids, dried using filter papers and were then frozen to be ready for lyophilisation. Freeze-drying took the order of 40 hrs for each slice, this length of time being accredited to the large mass of each sample. Average dry to wet ratios for liver and
kidney were 0.28 and 0.20 respectively. These compare favourably with the experimental values presented in Table 4.1. With respect to the lungs, the right and left had different water contents, dry to wet ratios being 0.18±0.01 and 0.12±0.01 (mean ± standard deviation) respectively. Corresponding ratios for the right and left bronchi were 0.22 and 0.14 respectively. This is an unexpected feature as the lungs were taken from the same animal and prepared for freeze-drying at the same time.

Following lyophilisation, the specimen sections were prepared in the manner described next. A stainless steel blade was initially cleaned with acetone and then bathed in a Decon 90° solution and rinsed in de-ionised water. After this cleaning process, the blade was dried and used to shave successive layers of the specimen surface away until

Figure 5.1 The sampling sites employed in the study of elemental distributions in a porcine lung pair.
the correct thickness of section was achieved and a suitably flat specimen surface obtained. Initially it was thought that a microtome could be used to cut sections of tissue. However, calculation of the range of protons in the lyophilised biological sections revealed that they could reach depths which were far greater than the maximum setting of commercial microtomes. Hence to avoid interference of the X-ray spectra from the section backing material and plate, a sample thickness of the order 2-3 mm was employed. Once sectioned, samples were mounted upon an Al backing plate using double sided adhesive tape, making certain that prepared surfaces were parallel to the plate surface plane. Carbon dag was then applied to their sides for the reasons presented in section 4.4. Target plates were then stored in a desiccator ready for 2 MeV proton induced X-ray emission analysis.

5.3 Quantification of elemental homogeneity and distributions in sections

In the quantification of trace or minor element levels across biological sections, one must be aware that these concentrations will be affected by several sources of variation. Section 4.3.1 identifies a number of these sources to be fluctuations in proton energy, charge integration and photopeak counting statistics. The relative variances for these are denoted by $\sigma_E^2$, $\sigma_C^2$ and $\sigma_S^2$ respectively. However, Campbell and Cookson [CAM84] state that a high degree of geometrical accuracy and sample surface smoothness is required in the analysis of thick samples by PIXE. These requirements are largely satisfied when samples are in the form of pellets. They are however, more difficult to satisfy when analysing thick specimen sections as sample surface preparation techniques dictate the degree of geometrical accuracy and surface roughness obtained. Hence, in the quantification of elemental homogeneity across a sample section by calculation of standard deviations, a slightly modified version of equation 4.3 must be used which takes into account errors from geometrical set-up and surface roughness. The standard deviations accredited to these effects are denoted by $\sigma_G$ and $\sigma_R$ respectively and may be found in equation 5.1.

$$\sigma_a = \sqrt{\sigma_C^2 + \sigma_E^2 + \sigma_S^2 + \sigma_G^2 + \sigma_R^2}$$

(Eq 5.1)
Little work has been carried out upon the effects of sample surface tilt and roughness upon the yield of X-rays from thick targets in PIXE analysis. From the limited data available, conclusions imply that the above effects may, in some cases, prove significant in the proton induced X-ray emission analysis of thick sample sections. This will be discussed in detail next.

5.3.1 Sampling factors

The concept of sampling factors has been introduced in depth in section 4.3.2. However, a number of points which are relevant to this type of sample will be clarified. That is, the standard deviation that is accredited to the analytical procedure will no longer take the form of equation 4.3 but the above equation 5.1. Also, the replicate mass of sample probed by the proton beam is predicted by equation 4.10. This mass is dependent upon the initial proton energy, the beam cross-sectional area and the elemental composition of the specimen.

5.4 The effect of sample surface roughness upon X-ray yield

5.4.1 Development of a simple stylised model

A small number of studies have been carried out to investigate the effect of structured surfaces of thick targets upon X-ray yield in PIXE analysis. Ahlberg and Akselsson [AHL76] analysed thick sections of teeth using a 2.5 MeV proton beam. Close examination of the tooth surface structure revealed its rough nature which was a product of the surface preparation technique. A saw-tooth model was adopted, which represented closely the tooth surface structure, to investigate the effect of surface roughness upon X-ray yield from the various elements present. Application of this model demonstrated that errors in X-ray yield could be significant, especially when photon energies were sufficiently low (<4 keV). Cookson and Campbell [COO83] also adopted a stylised model to investigate this effect which was in the form of a groove and ridge geometry. Much of their work centred upon the study of the variation of X-ray yields from trace elements present within various metallic matrices whilst parameters of the groove model were varied. However, their results have only a limited application to this work as only a small volume of data is presented for trace elements in a biological equivalent matrix (carbon) and in any case, all analysis is conducted assuming a 3 MeV proton beam energy whereas
all experimental analysis in this work is performed at 2 MeV. Dahlmann et al [DAH84] investigated the dependency of 400 keV energy PIXE measurements upon a metallic curved relief which may be typically found upon the surface of an archaeological artifact. Results suggest that correct analysis will be conducted as long as the radius of the curvature of the surface structure is larger than 3 to 4 times of the ion beam radius used.

Examples of the effects of surface roughness upon PIXE analysis in thick samples presented above have been directed toward specific stylised situations. Campbell et al [CAM85] however concentrate upon X-ray yield modification as a result of a random surface roughness. The conception of their model was based upon previous work by Edge and Bill [EDG80], who modelled and made measurements of the effects of surface topology upon Rutherford backscattering spectra. Campbell et al [CAM85] derive a general formula for estimating the surface roughness effects for any matrix. This however is only applicable to 3 MeV proton induced X-ray emission analysis, and it is expected from the data presented within this paper that the effects of analysis by 2 MeV protons are by far greater.

It is proposed that the effect of sample surface roughness upon X-ray yield in PIXE analysis be investigated by the employment of a simple stylised model similar to that adopted by Cookson and Campbell [COO83]. It is felt that this model is the most applicable when quantifying specimen surface effects that originate from the ducts and vessels present within biological sections.

5.4.2 Mathematical formalisation

Figure 5.2 represents a schematic of the stylised single groove model adopted in the investigation of surface roughness effects upon PIXE analysis. The view shown is a cross-section through the groove which is assumed infinitely long, the axis of which is perpendicular to the plane of the paper. The groove has a maximum depth D and is symmetrically inclined around this point with angle $\alpha$ to the flat sample surface. When a proton enters the sample at point $x$, an X-ray is produced at a depth under the surface which may leave the sample at an angle $\theta_{to}$ and be recorded by a detector that is placed a large distance from the specimen. The angle $\theta_{to}$ is defined as that between the exit path of the X-ray photon and the plane of the flat specimen surface. A Cartesian set of coordinates has been adopted in this model, the origin (0,0) being the position at the left
Figure 5.2 Schematic illustrating the stylised groove model used to investigate the effect of surface roughness upon X-ray yield in PIXE analysis.
hand side of the groove. Positive x and y directions are to the right and up respectively. The groove is split into three surfaces, namely S1, S2 and S3 as indicated in Figure 5.2.

As a proton transverses the sample, X-rays may be produced along the length of its path down to a maximum depth which is denoted by its range R. The position at which an X-ray is created is denoted by R'. This X-ray may leave the sample via differing routes depending upon under which surface it was created, the depth it was produced and the relationship between the X-ray take-off angle \( \theta_{\text{to}} \) and the groove inclination angle \( \alpha \) (e.g. proton 1). This will be discussed later. Also, it should be noted that there is a value of \( x \) such that protons entering at a point beyond this will produce X-rays that are not affected by the groove at all. This co-ordinate will be denoted by \( x_{\text{max}} \) (proton 2).

The X-ray yield from an element Z present within a matrix that completely halts the proton beam within it (thick specimen) and possesses a flat specimen surface is given by equation 2.7. This may be re-written in the form:

\[
Y_Z^s = \text{const}.N_p C_Z \int_0^{\theta_{\text{to}}} \frac{\sigma_z(E) T(Z|E)}{S(E)} dE
\]

where

\[
\text{const} = \left( \frac{N_A Y_{\text{Av}} Z \varepsilon_{Z | E}}{A_Z} \right)
\]

and the superscript S denotes that it is the X-ray yield from a thick target with smooth surface. Definitions of the symbols employed in equations 5.2 and 5.3 are the same as those in equation 2.7. If the integral in equation 5.2 is represented by \( I^s \) then it may be re-written in the form:

\[
Y_Z^s = \text{const}.N_p C_Z I^s
\]

and the corresponding equation for the yield of X-rays from a rough surface is:
\[ Y^R_Z = \text{const.} N_p C_Z I^R \]  
(Eq 5.5)

where it is assumed that in each of the rough and smooth surface cases, the number of protons and concentration of element Z in identical matrices are the same. Now the ratio of equation 5.5 to 5.4 is given by equation 5.6 i.e. the rough to smooth surface X-ray yield ratio.

\[ \frac{Y^R_Z}{Y^S_Z} = \frac{I^R}{I^S} \]  
(Eq 5.6)

However, if the above mathematical treatment applies to an infinitely small diameter proton beam, then we must integrate the X-ray yield over each of the specified surfaces i.e. from \( x=0 \) to \( x=x_{\text{max}} \). This is because in reality, we will have a proton beam of finite diameter. Now the yield of X-rays from smooth and rough surface samples is given by equations 5.7 and 5.8 respectively.

\[ Y^S_Z = \text{const.} N_p C_Z \int_0^{x_{\text{max}}} \frac{\sigma_z(E)T_z(E)}{S(E)} dE \]  
(Eq 5.7)

\[ Y^R_Z = \text{const.} N_p C_Z \left( I^R_{S1} + I^R_{S2} + I^R_{S3} \right) \]  
(Eq 5.8)

The integrals \( I^R_{S1}, I^R_{S2} \) and \( I^R_{S3} \) denote the X-ray yields from the proton beam falling upon the surfaces S1, S2 and S3 respectively. The X-ray yields from the integrals are further complicated in that they have to split further depending upon how the excited X-rays leave the sample (discussed next). Now the ratio of the rough to smooth X-ray yield is given by equation 5.9.

\[ \frac{Y^R_Z}{Y^S_Z} = \frac{\left( I^R_{S1} + I^R_{S2} + I^R_{S3} \right)}{\int_0^{x_{\text{max}}} I^S dx} \]  
(5.9)
Figure 5.3 illustrates the five possible scenarios which an X-ray may leave the sample once created along the path of the proton. It is apparent from this that the type of route that the X-ray takes is dependent upon under which surface it was created, at what depth and the relationship between $\alpha$ and $\theta_{\text{to}}$. e.g. a case iii exit may only occur if $\theta_{\text{to}}<\alpha$.

Hence, when calculating the X-ray yield at a specific value of $x$, the X-rays that are produced along the length of the proton path will be subject to a different magnitude of attenuation from the matrix depending upon the depth at which they are created. Of course, this is the same as the smooth surface case, but is far more complicated in that the X-ray may depart from the sample via five different escape scenarios whilst the smooth surface case has only one.

The integral in equation 5.2 may only be evaluated numerically. Hence rather than perform a double numerical integration as indicated in equation 5.7, it was preferred to evaluate the rough surface X-ray yield at a discrete number of points denoted by NIRR, and to calculate the effect of the groove from this. As long as a reasonably large number of irradiation points were employed then this approach should yield a result that approximates the integrated approach closely.

Appendix 3 contains a flow chart that illustrates the methodology behind the calculation of the effect of the groove upon X-ray yield. This methodology is essentially as follows. Initially all model parameters should be stated i.e. $D$, $E_0$, $\alpha$, $\theta_{\text{to}}$, matrix density, $\rho$, and composition, trace elements of interest and their respective characteristic photon energies and atomic numbers. Also, it should be decided upon the value of NIRR. This procedure is covered in the section on results. Given this information it is now possible to calculate the maximum proton range $R$ within the matrix and the maximum value of $x$ ($x_{\text{max}}$) that needs to be considered. Hence an interval step width, INTVW, may now be determined.

The procedure now is to begin with an irradiation point at $x=0$ (I=1 if I represents the current number of irradiation points evaluated), and to move along the groove with steps dictated by INTVW evaluating the rough X-ray yield at each point until $x=x_{\text{max}}$ is reached (I=NIRR). At each point, it is decided on which surface the proton beam is incident, and given the information about $\theta_{\text{to}}$ and $\alpha$, only certain exit scenarios may occur e.g. if the proton beam is incident upon surface 3 when $\theta_{\text{to}}<\alpha$ then exit cases i, ii, iv and v may only occur (see Figure 5.3). There will be discrete lengths of the proton path
Figure 5.3 Illustration of the five possible escape scenarios how an X-ray may leave the specimen once created along the proton path.
that each of these effects will exclusively occur. Hence the depth below the surface (and thus corresponding energy limits) for each effect have to be evaluated e.g. the case i effect may occur between depths $R$ to $R'$ (corresponding integration limits $E_{ul}$ (upper energy limit) = 0 to $E_{ll}$ (lower energy limit) = $E_i$ respectively), then effect iii may occur between $R'$ to $R''$ (corresponding integration limits $E_{ul}'$ = $E_i$ and $E_{ll}'$ = $E_2$ respectively where $E_2$ = $E_i$) and so on until a situation is reached when $E_{ll}$ = $E_0$. When the yield of X-rays has been calculated by numerical integration along the length of the proton path, this value is summed with INT that records the present sum of X-ray yields from each of the irradiation points before. Once $x = x_{max}$ has been reached and evaluated, an average rough surface X-ray yield may be calculated and compared to a single point smooth yield evaluation. This will provide a measure of the effect of the groove upon X-ray yield. There are however certain problems associated with the employment of this approach that will be discussed later.

The flow chart in appendix 3 was coded into a computer program written in FORTRAN 77. Values for the proton ionisation cross-section (section 2.2.2.1), mass stopping powers (section 2.3.3.2) and mass attenuation coefficients were taken from Johansson and Johansson's [JOH76] fit and parameters, Anderson and Ziegler's [AND77] semi-empirical fit and parameters, and Storm and Israel's [STO70] extensive tabulation respectively. Numerical integration was carried out using Simpson's rule and a maximum of $2 \times 10^4$ integration steps employed. A large number of integration points were needed to ensure good precision of the integration procedure and also to calculate the integration limits accurately. The effect of the groove upon PIXE analysis is investigated by varying many of the parameters of the model. However, it is always assumed that the proton beam enters the sample at an angle perpendicular to the flat surface plane. Any variation of this angle will be addressed in the section upon specimen tilt. Also it is assumed that the proton current is the same across the integrated x direction.

5.4.3 Results and discussion

Initially the relationship between the number of irradiation points and the calculated rough surface effect was investigated. Figure 5.4 represents a plot of the ratios of the estimated rough surface yield to precise yield as NIRR is increased. The parameters employed in the model for this investigation are 2 MeV, 45°, 30°, 50 μm, 220 kg/m³
representing $E_0$, $\theta_{10}$, $\alpha$, $D$ and $\rho$ respectively for Cl in a pure carbon matrix. It is apparent that the estimate to precise yield ratio oscillates rapidly to unity as we approach 30 irradiation points. A small degree of oscillation is still present after this value of NIRR, however the magnitude is extremely small. Hence, it is safe to conclude that $\text{NIRR} \geq 30$ should represent the effect of the groove upon PIXE analysis adequately. A value of $\text{NIRR}=50$ is adopted throughout this work to avoid any possible errors as a result of inadequate irradiation points being taken as model parameters may marginally affect the rate at which the curve in Figure 5.4 approaches unity.

![Graph showing the relationship between the ratio of estimate to precise yield and number of irradiation points.](image)

**Figure 5.4** The relationship between the ratio of the estimate and accurate rough surface yield and NIRR.

Additionally the precise rough surface yields (normalised to smooth surface yield) for Cl in a carbon matrix and a typical porcine kidney matrix were compared. Chlorine was chosen as the energy of the K$_\alpha$ X-ray is relatively small (2.621 keV) and hence differences should be more pronounced than if a higher Z element were employed. Less than 0.05% difference was observed between the two $Y_p/Y_s$ ratios and hence a pure carbon matrix will be used to represent a biological matrix in the rest of the groove work.
Figure 5.5 represents plots of $Y_R/Y_S$ as a function of $x$ (i.e. across the groove) for the model parameters stated above. Figure 5.5a shows plots for three $K_\alpha$ lines from low to medium $Z$ elements (Cl, Fe, Br) whilst Figure 5.5b shows plots for three $L_\alpha$ lines from medium to high $Z$ elements (Ag, Gd, Pb). The $L_\alpha$ line energies were chosen such that they were close to the $K_\alpha$ line energies. Elements up to and including $Z=45$ will be classified using their $K_\alpha$ energies and $K$-shell ionisation cross-sections whilst those elements with $Z>45$ by their $L_\alpha$ energies and $L$-shell ionisation cross-sections.

Little difference is observed when comparing Figure 5.5a and Figure 5.5b. However, of the differences that do occur, the greatest is seen when comparing the plots for Cl and Ag. This is mostly due to $K_\alpha$ and $L_\alpha$ photon energies for these elements not coinciding accurately, although there may be an effect due to the $Z$ dependence on mean ionisation depths which will be discussed later. The shape of the plots in Figure 5.5 may be explained as follows. As the proton irradiation point moves in a positive direction from $x=0$, the photon attenuation length (the distance the X-ray has to transverse before leaving the matrix) is increased. Hence the $Y_R/Y_S$ ratio is seen to decrease until it reaches its minimum point at $x=D/tan\alpha$ (roughly $x=87\ \mu m$ given the above parameters). As $x$ increases further, photon attenuation lengths shorten and a maximum $Y_R/Y_S$ value is attained at $x=2D/tan\alpha$ ($x=173\ \mu m$). At this point, a proportion of the X-rays produced along the path of the proton transverse a distance to reach the matrix surface which is smaller than that of corresponding X-rays in the smooth surface situation. Also, no corresponding X-ray has a photon attenuation length that is larger than the smooth surface case. Hence, at $x=2D/tan\alpha$, $Y_R/Y_S$ will always take a value that is greater than unity. As $x$ increases toward $x_{max}$, we approach the smooth surface situation again until ultimately a value of $Y_R/Y_S=1$ is reached.

Initial conclusions that may be drawn from Figure 5.5 are that serious effects may be caused on PIXE analysis if the incident proton beam (which has dimensions that are smaller than that of the groove) is positioned upon or in the vicinity of a crack and the elements of interest produces an X-ray that is of sufficiently low energy. Also, as the energy of the X-ray photon increases i.e. as the atomic number of the element increases, the effect of the groove lessens. If we look at the average $Y_R/Y_S$ value for Fe ($K_\alpha=6.398$ keV) we find that it takes a value indistinguishable from 1.000. That is, the groove has no effect upon the yield of X-rays from this element. Even the average effect of the
Figure 5.5 Plots of rough to smooth X-ray yield ratios ($Y_r/Y_s$) for a) three differing $K_{\alpha}$ X-ray transitions, b) three differing $L_{\alpha}$ X-ray transitions.
groove upon the yield of X-rays from Cl is small \((Y_r/Y_g=0.997)\). Hence, for this situation anyway, as long as the proton beam encompasses the area integrated then the effect of the groove upon X-ray yield in PIXE analysis is insignificant.

The effects of differing incident proton energies upon \(Y_r/Y_g\) ratio was investigated and results may be found in Figure 5.6. The above model parameters were assumed apart from \(\theta_{i0}\) in Figure 5.6b where \(\theta_{i0}=25^\circ\) was taken (Figure 5.6a still assumes \(\theta_{i0}=45^\circ\)) and plots drawn for Cl in a pure carbon matrix at initial proton energies of 1 MeV, 2 MeV, 3 MeV and 4 MeV. These energies were chosen as they are regarded as providing best experimental scenarios in PIXE analysis [JOH76,MIT81] and their use frequently found in proton induced X-ray emission analysis publications [YIN87,TAN87,HEC87,MEI79].

Figures 5.6 a and b reveal that the X-ray yield ratio \((Y_r/Y_g)\) differs depending upon the energy of the incident proton beam. When the proton beam is incident upon surface 1 whilst \(\theta_{i0}>\alpha\) (Figure 5.6a), the X-ray yields are modified in a differing manner which is dependent upon the incident proton energy. That is, the greater the value of \(E_0\), the lower the value of \(Y_r/Y_g\) although this becomes less pronounced between \(E_0=3\) MeV and \(E_0=4\) MeV. The reason for this is the manner that X-rays are produced along the path of the proton with differing energies. Figure 5.7a shows the relationship between the proportion of the total range that 99% of all X-rays are produced and element atomic number. As can be seen, when the incident proton energy increases, the proportion of the total range needed to encompass 99% of the total X-rays produced increases. However, if the X-ray distribution is corrected for attenuation in the matrix as illustrated in Figure 5.7b for a smooth surface case with \(\theta_{i0}=45^\circ\), then we are now concentrating upon the proportion of the total range for which 99% of the total X-rays are detected \((D_{99}/R)\). Now the relationship for incident proton energy is not as simple as that seen in Figure 5.7a as for low Z elements, the higher the value of \(E_0\), the less the proportion of the total range needed to produce 99% of all X-rays that are detected. This is due to X-ray production at lower depths as the incident proton energy increases. However these X-rays are attenuated more highly due to the longer length of matrix they have to transverse. Given the range of protons to be roughly 130 \(\mu\)m, 390 \(\mu\)m, 770 \(\mu\)m and 1260 \(\mu\)m for 1 MeV, 2 MeV, 3 MeV and 4 MeV incident energy respectively, it is obvious that the large proportion of X-rays are produced nearer the surface as \(E_0\) decreases. Hence, for a 1 MeV
Figure 5.6 The rough to smooth X-ray yield ratios ($Y_{R}/Y_{S}$) for Cl in a pure carbon matrix at 1 MeV, 2 MeV, 3 MeV and 4 MeV incident proton energies where a) $\theta_{T0}>\alpha$ ($\theta_{T0}=45^\circ$, $\alpha=30^\circ$) and b) $\theta_{T0}<\alpha$ ($\theta_{T0}=25^\circ$, $\alpha=30^\circ$).
proton beam, more X-rays may leave the sample via surface 1 than for a higher energy proton beam of let's say 3 MeV. Therefore, as X-rays from a 3 MeV proton beam are more likely to leave via the flat surface to the left of x=0 and hence transverse longer distances, they attenuate more highly and thus produce values of \( \frac{Y_R}{Y_S} \) that are smaller than those for 1 MeV proton induced X-rays. For the model case where \( \theta_{TO} < \alpha \) (Figure 5.6b), all X-rays leave to the left of x=0, and thus exhibit the same function of attenuation which is given by equation 5.10.

\[
\frac{Y_R}{Y_S} = \exp\left(-\frac{\mu}{\rho}x\rho \tan \alpha \sin \theta_{TO}\right)
\]  

(Eq 5.10)

The relationship of \( \frac{Y_R}{Y_S} \) with differing proton energies as the irradiation point is incident upon surfaces 2 and 3 may be explained by the discussion given above.

Figure 5.6 highlights the problem associated with using the average \( \frac{Y_R}{Y_S} \) value, which is calculated from x=0 to x=\( x_{max} \), as a measure of the effect of the groove upon PIXE analysis. That is, for each incident proton energy, there will be a different \( x_{max} \) such that it is impossible to compare yield ratio values from each. Also, as \( \theta_{TO} \) changes the maximum x-value will alter (compare the inset graph in Figure 5.6b to Figure 5.6a). Many other factors may affect the value of \( x_{max} \) and the larger it becomes, the more it masks the true effect of the crack. Take for instance the curves for 1 MeV and 4 MeV incident protons in Figure 5.6a. The average \( \frac{Y_R}{Y_S} \) values for each are 0.987 and 1.000 respectively. However, it is obvious from the plots that the effect from the 4 MeV protons is greater than that from 1 MeV protons. The reason for the average yield ratio not reflecting this is that most \( \frac{Y_R}{Y_S} \) data points are on surface 3 which tends to ‘wash out’ the effect of the groove. Hence some other method of evaluating the effect of the crack should be developed. By far the most realistic criteria would be to set a value of \( x_{max} \) and evaluate the average value of \( \frac{Y_R}{Y_S} \) for differing model parameters over this. This approach will be taken when calculating the effect of the groove for specific proton irradiations of the studied biological samples. However the ratio of the maximum value of \( \frac{Y_R}{Y_S} \) to the minimum value of \( \frac{Y_R}{Y_S} \) will be used for the investigation of the groove effect with differing model parameters.
Figure 5.7 Plots of the elemental dependency of the proportion of the total range that
a) 99% of all Kα X-rays are created b) 99% of all Kα X-rays are detected.
Relationship at four different proton energies are shown.
Figure 5.8 shows, for the above model parameters, the effect of the groove upon different elements. X-rays from Kα and Lα transitions are illustrated which are created from four different initial proton irradiation energies. The effects from 4 MeV, 3 MeV and 2 MeV are similar and hence only two curves may be seen. For the Kα transitions, the curves fall from an initial high ratio at Z=11 (Na) rapidly to a low ratio with negligible effects at around Z=20 (Ca). The Lα X-ray transitions shown never reach values over approximately 1.2 although they probably would if lower Z elements were plotted. However, maximum to minimum ratios for Z ranges are illustrated for either Kα or Lα X-rays depending upon the manner in which elements are usually studied in PIXE analysis.

The dependence upon X-ray yield effect with groove depth for 2 MeV protons is illustrated for three different elements in Figure 5.9. As expected, the greater the value of D, the greater the effect of the groove upon X-ray yield. Also as Z decreases the effect of the groove increases (as illustrated in Figure 5.8). For Br even large values of D affect the X-ray yield negligibly.

Matrix density increments are seen to increase the effect of the groove, the level of which is dictated by the element studied (Figure 5.10). A relatively large increase in X-ray yield ratio is observed for Cl upto 1000 kg/m³ whereas a trend of similar magnitude for Fe and Br is not seen. The range of density illustrated in Figure 5.10 was chosen as this encompasses the mass per unit volume for various lyophilised tissue sections to pelletised biological samples. The trend of the curves may mostly be attributed to changes in \((Y_R/Y_s)_{\text{min}}\) and \((Y_R/Y_s)_{\text{max}}\) essentially remains static throughout density increments. This behaviour may be linked to X-ray production being closer to the sample surface and an increase in the linear attenuation coefficient of the material.

An interesting trend was noted when varying the detector take-off angle \(\theta_{\text{ROI}}\) and observing the X-ray yield ratio (Figure 5.11). That is, when \(\theta_{\text{ROI}}\) is set to 90° the effect of the groove is zero. This is because X-rays leave the sample along a route of the incident protons and hence X-ray attenuation paths are the same for any position on or around the groove and for the smooth surface situation. Hence, this suggests that the most favourable geometrical configuration to minimise the effects of sample surface roughness would be to position the detector at an angle which is as near 90° as practically possible.
Figure 5.8 The effect of the groove in relation to target atom atomic number (Z). 
$K_\alpha$ and $L_\alpha$ X-ray transitions are illustrated.

Figure 5.9 The dependence of the X-ray yield upon groove depth (\(\mu\text{m}\)) for Cl, Fe and Br.
Figure 5.10 The dependence of X-ray yield upon matrix density for Cl, Fe and Br.

Figure 5.11 The relationship between the X-ray yield ratio and X-ray take-off angle ($\theta_{10}$) for three different elements.
5.4.4 Conclusions

Conclusions concerning the effect of sample surface roughness upon PIXE analysis that have been drawn from the stylised single groove model are as follows. As the atomic number of the element of interest increases, the effect upon their X-rays from a rough sample surface decreases. Hence for K_alpha X-rays emanating from elements within a pure carbon matrix with roughly Z ≥ 20, the degree that photons are affected ranges from small to insignificant. Given that L_alpha X-rays that are of similar energy to K_alpha X-rays are affected by corresponding extents, the yield of L_alpha X-rays from elements Z ≥ 50 are only mildly modified from the flat surface situation.

The dependence of incident proton energy upon rough surface X-ray yield revealed that for surface 1 and 2 (Figure 5.6), little difference exists between E = 4 MeV, 3 MeV and 2 MeV. However, if these are compared to Y_r/Y_s ratios from incident proton energy 1 MeV, significant differences are found. When the proton beam is positioned upon surface 3, larger differences in X-ray yield ratio are observed between proton energies 4 MeV, 3 MeV and 2 MeV, the latter showing the greatest Y_r/Y_s value and falling most rapidly to unity. Campbell et al [CAM85] identify that the lower the incident proton energy, the greater the effect of surface roughness upon X-ray yield. This trend was drawn from a randomly generated rough surface and is not identifiable from the above stylised groove model.

Effects upon the X-ray yield ratio from the increment of the groove depth D and matrix density p were investigated. As expected, an increase in D or p resulted in larger X-ray yield ratios. These were significant increases for low Z elements (Cl), whilst the effect was small or insignificant for medium Z elements (Fe,Br). Also as \( \theta = 90^\circ \), the effect of the groove disappears. This is in agreement with findings by Campbell et al [CAM85] and Cookson and Campbell [COO83].

5.5 The effect of specimen surface tilt upon X-ray yield

The formula (equation 2.7) used to predict the yield of X-rays in thick target proton induced X-ray emission analysis assumes that the geometrical configuration of proton bombardment and X-ray detection is accurately known. In the analysis of thick biological sections, there is an ambiguity involved in the sample surface plane being inclined at a predisposed angle to the incident proton beam (perpendicular in the case of
this work). This uncertainty arises from imperfections in the sample surface preparation
techniques and results in the surface plane being inclined at an angle that is different to
the one employed in PIXE calculations. Campbell and Cookson [CAM84] and Duerden
et al [DUE79] have shown that the effect of sample surface tilt upon X-ray yield in PIXE
analysis may be significant, especially in the case of low Z elements. Hence, it was
decided to investigate the effects of sample surface inclination to the yield of X-rays from
elements in a carbon matrix, which are produced as a result of PIXE.

Two worst case scenarios of sample surface tilt are considered (Figure 5.12). Scenario a) assumes that the surface is inclined away from the detector whilst scenario b) toward the detector. The relative X-ray yield (relative to the X-ray yield from a proton beam entering the sample perpendicular to the sample surface plane) from elements in a pure carbon matrix for 2 MeV proton bombardment are shown for both scenarios in Figure 5.13. Curves illustrate inclinations at \( \alpha = 5^\circ \) and \( \alpha = 10^\circ \). The plot at \( \alpha = 10^\circ \) demonstrates the severity of sample surface tilt at this angle especially for low Z (Z<20) elements. However, ambiguity of angle of ±10° is deemed too severe for the biological sections analysed in this work and \( \alpha = \pm 5^\circ \) thought more likely to be representative of the uncertainty in tilt angle involved. This is the same uncertainty in angle that Duerden et al [DUE79] considered in the analysis of their thick obsidian samples.

The plot of \( Y_y/Y_s \) for \( \alpha = \pm 5^\circ \) as expected is less extreme and converges to unity faster than that for \( \alpha = \pm 10^\circ \). Again, the effect of sample surface tilt is greater as Z decreases and is small, although not insignificant (\( Y_y/Y_s = 1.015 \)) at around Z=20 (Ca). Hence, one should be aware that in the determination of element levels in biological sections, the variability of concentrations for Z<20 may partly be due to surface tilt effects. Conversely, as long as sample surface tilt is not great, variance in trace element results for Z>20 will be small and in most cases insignificant and the effect may be neglected.
Figure 5.12 Diagram illustrating the two worst case surface tilt scenarios employed in the estimation of the effects of surface inclination ambiguity upon PIXE analysis.

Figure 5.13 Tilt to normal X-ray yield ratio as a function of element for $\alpha=5^\circ$ and $\alpha=10^\circ$. 

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5.6 Results and discussion
5.6.1 Elemental distributions across selected biological sections

The elemental distributions across sections of porcine kidney, liver and lung were determined by 2 MeV proton induced X-ray emission analysis using IAEA animal muscle (H-4) as the comparator. Derivation of elemental distributions across two kidney sections was as follows. The sampling positions across one such section are illustrated in Figure 5.14. Note that 12 sampling points were taken over the section which was approximately 22 mm in length and 2 mm thick. At each analysis point, the beam was scanned over a surface area of $(3.5 \pm 0.6) \times 10^{-2}$ m$^2$ which accounts for a replicate sampling mass of approximately $30 \pm 5 \mu g$ (equation 4.9). Sampling positions were chosen so that the presence of concentrations gradients could be identified and elemental data for the various composite tissues determined. Thus, analysis points 1-7 inclusive lie in the cortex (although this varies from subscapular to juxtamedullary portions) and positions 8-12 inclusive lie in the renal pyramid (medulla), which is indicated by the dotted line in Figure 5.14.

Plots of concentrations across the kidney sections may be seen in Figure 5.15. The data points displayed in each graph may easily be correlated with the sampling positions in Figure 5.14. One should be aware that in some instances data for certain sampling positions is unavailable e.g. Cl concentration for position 4 as this was below minimum detection limits due to the low count rate associated with this peak. The data illustrated in Figure 5.15 is broken into discrete graphs. Figure 5.15a represents plots for elements that should exhibit no preferential collection site within the kidney, whilst Figure 5.15b preferential accumulation within the renal cortex [TAN87, LIV71].

An initial feature to note about Figure 5.15 is that sampling point 4 (cortex) has an unusually relatively high value in each of the elemental plots (excluding Br) which is contrary to any of the trends exhibited. Possible explanations could be that the proton beam was positioned upon a ridge or sloping surface (towards the detector) or source of contamination e.g. as a result of surface preparation, dust particle or coagulated blood. Close examination of the surface with a scanning electron microscope (SEM) (Figure 5.16) revealed that the proton irradiation position showed damage and its surrounding was
rough and close to a surface structure. However, this surface structure was orientated in a manner that would not affect X-ray measurement. In any case, sections 5.4 and 5.5 have demonstrated that X-ray enhancements due to inadequate surface preparation procedures negligibly affect medium Z elements i.e. little effect should be seen for Zn, Cu, Br, Rb even if the surface area were not flat. Hence, this implies that high concentration values at position 4 are as a result of some source of contamination, sample burning or are a real artifact of the specimen.

It is possible to evaluate if these high concentrations arise from a spurious source of contamination by looking at element $K_{\beta}/K_{\alpha}$ X-ray yield ratios [AHL75b]. That is, as $K_{\beta}$ and $K_{\alpha}$ X-rays are attenuated in a matrix to varying extents due to their different energies, we would expect $K_{\beta}/K_{\alpha}$ X-ray yield ratios to be different depending upon from where photons originate. Hence, as $K_{\beta}$ X-rays have higher energies than $K_{\alpha}$ we would expect $K_{\beta}/K_{\alpha}$ to be higher if photons originate within the matrix than if they were created at the surface. Obviously factors such as geometrical analysis configuration and element

Figure 5.14 Position of sampling points employed in the study of elemental distributions across a lyophilised porcine kidney section.
Figure 5.15 Plots of concentrations of various elements across a lyophilised porcine kidney section.
Figure 5.16 Scanning electron microscope photographs of a) a coarse view of kidney sample position 4 and surrounding area, b) magnified view of the beam damage at sampling point 4.
atomic number dictate the degree of difference that the $K_p/K_\alpha$ ratios exhibit. That is, small $\theta_{to}$ and low $Z$ will yield largest differences. However Ahlberg [AHL75b] comments that $K_p/K_\alpha$ ratios may be calculated precisely due to a reduction in uncertainty from the cancellation of errors in solid angle and charge integration process. Hence if X-rays originate from the surface at sampling position 4, then this will be a strong indicator that there is a source of contamination at this point.

X-ray yield $K_p/K_\alpha$ ratios are presented in Table 5.1. Shown for each element are three sets of data. The first column represents the ‘true’ yield ratio [JOH88], the second, the $K_p/K_\alpha$ ratios from X-rays produced upon the surface and adjusted for detector efficiency and attenuation in the mylar filter [COH80, CLA86] and column three $K_p/K_\alpha$ X-ray yield ratios for photons produced from an equal distribution of the elements of interest in the matrix (carbon) which also have been adjusted for the detector efficiency and filter. Data employed in the calculation of the values in column three (stopping powers, mass attenuation coefficients, proton ionisation cross-sections) are the same as those used in the groove mode program. It is evident from Table 5.1 that as $Z$ number

Table 5.1 Non-modified, surface produced (detector efficiency and mylar filter adjusted) and depth produced in a carbon matrix (also detector efficiency and mylar filter adjusted) $K_p/K_\alpha$ X-ray yield ratios.

<table>
<thead>
<tr>
<th>Element</th>
<th>Non-modified $K_p/K_\alpha$ ratio</th>
<th>Surface produced $K_p/K_\alpha$ ratio</th>
<th>Depth produced $K_p/K_\alpha$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>0.0862</td>
<td>0.5194</td>
<td>0.5643</td>
</tr>
<tr>
<td>K</td>
<td>0.1211</td>
<td>0.3253</td>
<td>0.3420</td>
</tr>
<tr>
<td>Ca</td>
<td>0.1230</td>
<td>0.2603</td>
<td>0.2703</td>
</tr>
<tr>
<td>Mn</td>
<td>0.1348</td>
<td>0.1662</td>
<td>0.1679</td>
</tr>
<tr>
<td>Fe</td>
<td>0.1355</td>
<td>0.1601</td>
<td>0.1613</td>
</tr>
<tr>
<td>Cu</td>
<td>0.1388</td>
<td>0.1410</td>
<td>0.1520</td>
</tr>
<tr>
<td>Zn</td>
<td>0.1409</td>
<td>0.1513</td>
<td>0.1518</td>
</tr>
<tr>
<td>Br</td>
<td>0.1682</td>
<td>0.1732</td>
<td>0.1734</td>
</tr>
<tr>
<td>Rb</td>
<td>0.1780</td>
<td>0.1817</td>
<td>0.1819</td>
</tr>
</tbody>
</table>
Table 5.2 The measured and surface and depth produced predicted peak areas for unresolved X-ray photopeaks in the spectrum collected at position 4.

<table>
<thead>
<tr>
<th>X-ray line</th>
<th>Measured X-ray peak area</th>
<th>Predicted surface produced X-ray peak area</th>
<th>Predicted depth produced X-ray peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kα</td>
<td>62991±253</td>
<td>27314±777</td>
<td>28113±795</td>
</tr>
<tr>
<td>Ca Kα/Ca Kβ</td>
<td>26147±167</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca Kβ</td>
<td>1776±50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn Kα</td>
<td>324±25</td>
<td>12078±990</td>
<td>11988±983</td>
</tr>
<tr>
<td>Fe Kα/Mn Kβ</td>
<td>11159±108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe Kβ</td>
<td>1925±47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu Kα</td>
<td>1485±41</td>
<td>3996±208</td>
<td>4001±208</td>
</tr>
<tr>
<td>Zn Kα/Cu Kβ</td>
<td>4171±67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn Kβ</td>
<td>573±25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of element increases, differences between the surface produced and depth produced Kp/Kα yield ratios decreases. Hence, this implies that in testing from where X-rays originate, low Z elements should prove the most appropriate.

To test the point of X-ray origination at sampling position 4, one needs to extract the number of counts in Kα and Kβ photopeaks from the collected PIXE spectra. Comparison of experimental Kp/Kα ratios with theoretical values shown in Table 5.1 should indicate the nature of the X-rays. However, this approach is limited by the Si(Li) detector resolution, and in most cases there is an overlap of Kβ and Kα X-ray lines from adjacent Z elements (K Kp/Ca Kα, Mn Kp/Fe Kα, Cu Kp/Zn Kα etc). Therefore an alternative approach is adopted where the above ratios are used to calculate the unresolved photopeak counts from the two resolved, and this is compared to that extracted from the sampling point 4 PIXE spectrum. Results are shown in Table 5.2. Peak area errors are calculated from equation 3.7.

It may be seen from Table 5.2 that errors associated with peak areas are
sufficiently large such that the number of predicted counts from surface X-ray production and depth X-ray production are undifferentiable. However, predicted peak areas are generally in agreement with measured i.e. to a standard deviation. Larger differences between expected peak counts and measured occur for the unresolved Ca Kα/K Kβ line as difficulties in area measurement occurred from K Kα and Ca Kβ lines not being fully separated from the overlapping peak. In conclusion, comments regarding the position of X-ray production at sampling point 4 cannot be made due to the accumulation of the PIXE spectra over insufficient charge (5.01 µC). The high elemental concentrations at this sampled position are unlikely to be the result of contamination from the surface preparation technique as expected enhancements from the stainless scalpel would be much lower and not affect the elements Cl, K, Ca, Br and Rb [VER73,BEH80]. Without doubt, the most probable reason for high concentrations at position 4 is the presence of severe sample burning although there is an outside possibility that high concentrations actually originate from the specimen itself, or as a result of the presence of blood [UND71].

The elemental concentrations shown in Figure 5.15 are generally in good agreement with those presented in Table 4.8. As the elements illustrated in Figure 5.15 have been demonstrated to show no preferential collection site within the kidney [TAN87], then we would expect their plots to be relatively flat exhibiting only statistical and inhomogeneity fluctuations. For Cl this is the case, but only because of the large uncertainties at sampling points which are due to the low count rate. Elemental concentrations for K, Ca and Fe are elevated at sampling positions 1 and 2 (subscapular cortex), but however remain more or less constant over the remaining points although there may be a slight concentration gradient for Ca. It may be noted that the concentrations at position 12 are slightly lower than at previous sampling points. The most probable explanation is the presence of a hole, which was noted when examined under the SEM (Figure 5.17) and accounts for approximately 50% of the beam scan area. The effect of this was to decrease concentrations of low Z elements (K, Ca) whereas medium Z elements (Fe, Zn, Cu, Br, Rb) should be relatively unaffected.

Figure 5.15b illustrates the distribution of elements that should exhibit preferential site collection. As expected a concentration gradient exists for Zn and Cu which fall from initial high concentrations of 260 ppm and 39 ppm (dry weight) respectively in the subscapular cortex to relatively constant values of roughly 60 ppm and
Figure 5.17 SEM photograph of kidney sampling point 12 illustrating the presence of a crack which was thought to modify low Z X-ray signals at this position.

12 ppm respectively in the renal pyramid. Section 4.6.3.2 revealed that Mn may preferentially reside within the cortex. This is not evident from Figure 5.15b as the Mn concentration plot along the kidney section length is indistinguishable from that when no preferential site is expected. Tanaka et al [TAN87] identifies Rb to collect within the cortex. This trend cannot be identified mainly because of the large errors associated with concentrations. Bromine should remain at roughly constant levels across the specimen slice (section 4.6.3.2). However there is a possibility that a concentration gradient exists with peak value that is found within the renal pyramid.

Apart from the unexpected high concentrations at sampling point 4, the above trends are essentially those noted in the analysis of the second lyophilised porcine kidney section.

Figure 5.18 illustrates the sampling positions employed to study the distribution of elements across a section of freeze-dried porcine liver. A beam scan area of (8.4±2.1) $10^{-7}$ m$^2$ was used which equates to a replicate sampling mass of roughly 72±18 μg. The
liver section was extracted from the right lobe and includes part of the capsule and hepatic vein as illustrated.

Plots of elemental distributions may be found in Figure 5.19. As the liver is composed of essentially one cell type [PER77], it is expected that no concentration gradients exist across the specimen section, and for most of the elements displayed, this is the case. However, a degree of variability does exist which is partly due to statistical fluctuations from, for example, the number of counts collected, but also real heterogeneity of the biological sample. There may be greater surface roughness effects in the analysis of liver owing to the surface being more difficult to prepare due to the sinusoids (Figure 5.20). Iron levels at sampling positions 1 and 2 are higher than at other points along the sample. This may simply be due to an increased blood content at this position but reinforces the findings of section 4.6.3.3 that Fe levels between peripheral and central positions differ.

Elemental concentrations at sampling point 8 are relatively low whereas for most elements they return to their normal values at position 9. The reason for this dip in elemental levels is unknown. However, the high Ca concentration seen after sampling point 8 is most probably due to the positioning of sample spot 9 in the vicinity of the hepatic vein wall. Analysis of a section of the vein wall revealed high Ca concentrations (230±40 ppm). Concentrations for other elements were close to those found in hepatic tissue and hence levels seen at position 9 are close to those found at 1-7 inclusive.

A 92±12 \( \mu \)g proton sampling mass was used to investigate the elemental distributions across freeze-dried lung section 1 which was approximately 50 mm in length and 4 mm thick. The result of this investigation is illustrated in Figure 5.21. Fourteen sampling positions distributed from near the base of the lung (distance =0.0 mm) to a region well within the organ (rough distance = 46.0 mm) were taken. There is an absence of concentration gradient for any of the element plots which on the whole tend to be flat but subject to a varying degree of fluctuation. This fluctuation is a culmination of analytical variance (peak count statistics, surface roughness etc) and biological sample elemental heterogeneity. Concentration variance for K is seen to be the most significant whilst that for Zn and Cu exhibit the least fluctuation. One may postulate that sample surface roughness is a major culprit of the large concentration variation seen for K as when the section was viewed under the SEM, the surface was grossly pitted (Figure 5.22).
Figure 5.18 Sampling positions employed in the study of the distributions of trace elements across a lyophilised porcine liver section.

Figure 5.19 Plots of concentrations of various elements across a lyophilised porcine liver section.
Figure 5.20 SEM photograph illustrating the surface of the liver section.

This is expected due to the presence of alveoli, the functional compartments of the lung at which gases are exchanged with the blood (see section 4.2.4). However, if the sample surface effect is significant, one might expect the Ca concentration distribution to be affected in the same manner as K, as these elements lie close in the periodic table. Levels for Ca are relatively constant and any fluctuations non-parallel with those in K. Also as the matrix density is relatively low (roughly 100 kg/m³), it is expected that the effect of surface roughness upon Ca and K levels is small (see Figure 5.10).

It may be seen that the iron content at sampling position 11 (37.8 mm) is small in comparison to levels at other points. This is thought to be due to the presence of an observed terminal bronchiole. Analysis of bronchi (sample 5 and 10) revealed that Fe levels are in fact lower than in lung tissue as is Zn and K (see section 5.6.2). These trends are not easily identifiable for Zn and K at position 11 as differences in concentration between lung tissue and bronchi are not as great as those for Fe, and also the large variance seen for K probably masks the effect for this element.
Figure 5.21 Plots of concentrations of elements across freeze-dried lung section 1.

Figure 5.22 SEM photograph of a portion of the freeze-dried lung section analysed by PIXE analysis illustrating the heterogeneous physical nature of the tissue.
5.6.2 Intra-organ element variations

5.6.2.1 Kidney

Mean elemental concentrations and total standard deviations (dry-weight) for subscapular cortex/cortex, juxtamedullary cortex and medulla (kidney section studied in section 5.6.1, excluding data points 4 and 12) are presented in Table 5.3. General conclusions are as follows. No difference may be demonstrated for Cl and K between the three different sampling areas. Calcium and iron should exhibit no preferential collection site [TAN87]. This is narrowly satisfied for Fe, whereas the concentrations of Ca between the sampling areas are significantly different. A large standard deviation seen in the subscapular cortex/cortex reflects the concentration gradient seen across this region (Figure 5.15a). A similar trend is also noted for Zn and Cu which are seen to be elevated in the subscapular cortex. Concentrations for Mn, Br and Rb are essentially constant across the kidney slice which is unexpected for Mn and Rb at least given the discussion by Tanaka et al [TAN87].

Table 5.3 Mean concentrations and total standard deviation for different areas of porcine kidney.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean concentration ± SD (ppm) - dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subscapular cortex/cortex (n=3)</td>
</tr>
<tr>
<td>Cl</td>
<td>6600±2150</td>
</tr>
<tr>
<td>K</td>
<td>8780±2140</td>
</tr>
<tr>
<td>Ca</td>
<td>437±128</td>
</tr>
<tr>
<td>Mn</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>212±61</td>
</tr>
<tr>
<td>Cu</td>
<td>31.3±9.1</td>
</tr>
<tr>
<td>Zn</td>
<td>110±21</td>
</tr>
<tr>
<td>Br</td>
<td>10.6±3.9</td>
</tr>
<tr>
<td>Rb</td>
<td>28.7±3.2</td>
</tr>
</tbody>
</table>
5.6.2.2 Lung

Table 5.4 shows the concentration data from each of the sub-samples extracted from the lung pair. Given are the mean sub-sample concentrations with their associated analytical errors. The analytical errors comprise of peak counting statistics and variances associated with the standard (IAEA animal muscle H-4) concentration certification [MUR85]. Uncertainties linked with surface roughness have been estimated to range from small to negligible given $\delta \geq 20$ and a matrix density of 100 kg/m$^3$ (see Figures 5.8 and 5.10). However, sample surface roughness is thought to be significant for Cl, as are the effects from sample surface tilt (Figure 5.13). Therefore the analytical errors for Cl seen in Table 5.4 are to a degree underestimated. This underestimation may range from ±4% upwards (based upon ±5° tilt and no surface roughness effects). Charge integration fluctuations were also measured and found to be negligible.

Given the discussion presented above, no difference between Cl content of each of the lung sub-samples may be proved reliably. With respect to potassium levels, these are similar for each area apart from bronchi wall samples, the level in which is approximately 64% of that seen in lung tissue. The constant K level seen throughout the lung is in agreement with Barisch et al [BAR82]. Calcium should also exhibit similar levels throughout the lung pair, however observations imply that concentrations of this element are greater in the left lung. Significance testing reinforced this presumption as differences in mean lung Ca contents (left lung 436±76 ppm, right lung 310±83 ppm) were found to differ at the >99% significance level. This degree of significance was also satisfied when testing Ca levels between lung tissue and bronchi, the latter possessing higher levels. Differences in calcium content between the lung pair is probably an artifact which is backed-up by the different dry/wet ratios of the lungs (section 5.2) and the large variation of Fe content in the left lung, whilst that of the right lung remains relatively stable, as is expected [BAR82]. Note that iron levels in bronchi are significantly lower than in lung tissue.

Copper, zinc and rubidium levels should remain stable across the lung tissue and for Cu and Rb, this is true. However a significant degree of variation for Zn is seen between sampling sites. Zinc levels in areas 2 and 3 are found to be significantly higher (99% confidence level) than areas 1 and 4. Similarly the levels in areas 8 and 9 are higher than areas 6 and 7. One may postulate that higher levels of Zn are found in areas that dust
Table 5.4 Mean concentrations and analytical errors (dry weight ppm) for the ten sampling sites in the freeze-dried porcine lung pair.

<table>
<thead>
<tr>
<th>Element</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>9740±</td>
<td>4680±</td>
<td>4670±</td>
<td>6050±</td>
<td>7420±</td>
<td>9710±</td>
<td>6340±</td>
<td>4540±</td>
<td>6130±</td>
<td>7710±</td>
</tr>
<tr>
<td></td>
<td>2490</td>
<td>1340</td>
<td>1200</td>
<td>1760</td>
<td>2100</td>
<td>2470</td>
<td>1770</td>
<td>1480</td>
<td>1860</td>
<td>2240</td>
</tr>
<tr>
<td>K</td>
<td>8730±324</td>
<td>9160±341</td>
<td>9140±342</td>
<td>9280±346</td>
<td>5990±229</td>
<td>9050±336</td>
<td>9250±345</td>
<td>8890±337</td>
<td>8040±305</td>
<td>5410±207</td>
</tr>
<tr>
<td>Ca</td>
<td>350±112</td>
<td>308±55</td>
<td>262±50</td>
<td>272±51</td>
<td>648±103</td>
<td>412±71</td>
<td>369±63</td>
<td>615±105</td>
<td>574±98</td>
<td>695±117</td>
</tr>
<tr>
<td>Fe</td>
<td>203±9</td>
<td>221±11</td>
<td>194±10</td>
<td>218±11</td>
<td>53±3</td>
<td>243±12</td>
<td>153±7</td>
<td>135±7</td>
<td>105±5</td>
<td>63±3</td>
</tr>
<tr>
<td>Cu</td>
<td>4.7±1.1</td>
<td>4.5±1.1</td>
<td>4.4±1.1</td>
<td>4.2±1.1</td>
<td>8.6±1.3</td>
<td>4.7±1.1</td>
<td>3.6±0.9</td>
<td>4.8±1.1</td>
<td>5.2±1.2</td>
<td>3.9±1.0</td>
</tr>
<tr>
<td>Zn</td>
<td>73±4</td>
<td>90±5</td>
<td>92±5</td>
<td>78±4</td>
<td>51±3</td>
<td>80±5</td>
<td>78±4</td>
<td>91±5</td>
<td>90±5</td>
<td>54±3</td>
</tr>
<tr>
<td>Br</td>
<td>20.9±6.4</td>
<td>18.9±6.0</td>
<td>14.8±4.5</td>
<td>17.4±5.1</td>
<td>18.7±5.6</td>
<td>26.7±8.0</td>
<td>17.3±5.2</td>
<td>20.1±6.0</td>
<td>21.3±6.7</td>
<td>15.5±5.1</td>
</tr>
<tr>
<td>Rb</td>
<td>16.0±5.6</td>
<td>14.5±5.4</td>
<td>15.7±5.2</td>
<td>16.7±5.6</td>
<td>-</td>
<td>14.9±5.6</td>
<td>16.6±5.3</td>
<td>16.9±5.6</td>
<td>14.6±5.6</td>
<td>11.2±4.8</td>
</tr>
</tbody>
</table>
is predominately deposited (1 μm diameter aerosols may typically contain between 152-118000 μg/g Zn [VAN82]).

5.6.3 Elemental homogeneity and sampling factors for biological sections

Percentage elemental inhomogeneity ($\sigma_n$) and sampling factors for 1% and 5% variation levels for the studied biological sections may be found in Table 5.5 and 5.6 respectively. Presented are values for lung (sample 1, sample 6 and whole left and right), whole kidney, cortex and medulla and liver. Note that for whole kidney, the number of sampling points in each constituent tissue are proportioned as Kratochvil and Taylor [KRA81] suggest in the analysis of striated materials, i.e. roughly 70% of sampling points are positioned within the cortex and the remaining in the medulla. This is essentially the proportion that these tissues are found within the kidney [ICR75]. This treatment is not required for other tissues.

In the calculation of $\sigma_n$ and sampling factors, analytical errors must be considered. For the purpose of this work, analytical errors were essentially those originating from photopeak counting statistics. For lower atomic number elements, basing analytical variations upon this is not wholly correct as sample surface roughness and tilt significantly modify the X-ray signal. In fact, it can be seen (Figures 5.16, 5.20 and 5.22) that the volume of the biological sections contains physical inhomogeneities and thus even in the event of positioning the beam upon a perfectly prepared sample surface, the sample has the potential to distort X-ray signals from the smooth surface situation. Also if the beam current is set too high for the beam scanning area, then sample damage may occur which will lead to the falsification of results. This was seen at sampling point 4 on the kidney section when the beam was positioned in the vicinity of a blood vessel, and hence a portion of the surface was destroyed. However, excessive current density may also create problems even in the absence of physical anomalies of the sample. Figure 5.23 shows an SEM photograph of a cross-section through a kidney cortex sample that has been irradiated with a proton beam of current density approximately $2 \times 10^{14}$ A.μm⁻². Figure 5.23a shows a coarse view through the section and illustrates the relatively flat sample surface which is the product of a satisfactory preparation technique, but is interrupted by a groove which has resulted from the proton beam positioning. A magnified view of the sample surface beam position may be seen in Figure 5.23b which illustrates the created
Table 5.5 Percentage inhomogeneities (σ, %) at the 30-100µg level for various elements in lyophilised porcine lung, kidney and liver sections.

<table>
<thead>
<tr>
<th>Element</th>
<th>Lung</th>
<th></th>
<th></th>
<th>Kidney</th>
<th></th>
<th></th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Whole</td>
<td>Left</td>
<td>Cortex</td>
<td>Pyramid</td>
<td>Whole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 6</td>
<td>Whole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>13.2%</td>
<td>37.3%</td>
<td>17.2%</td>
<td>26.8%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>11.7%</td>
<td>10.8%</td>
<td>16.2%</td>
<td>12.3%</td>
<td>23.0%</td>
<td>23.9%</td>
<td>24.1%</td>
</tr>
<tr>
<td>Ca</td>
<td>-</td>
<td>7.6%</td>
<td>-</td>
<td>19.6%</td>
<td>28.4%</td>
<td>13.5%</td>
<td>42.7%</td>
</tr>
<tr>
<td>Mn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>28.0%</td>
<td>21.3%</td>
<td>18.4%</td>
<td>37.0%</td>
<td>62.2%</td>
<td>15.5%</td>
<td>29.2%</td>
</tr>
<tr>
<td>Cu</td>
<td>19.8%</td>
<td>2.4%</td>
<td>-</td>
<td>16.7%</td>
<td>19.2%</td>
<td>-</td>
<td>38.9%</td>
</tr>
<tr>
<td>Zn</td>
<td>2.7%</td>
<td>20.8%</td>
<td>18.8%</td>
<td>16.9%</td>
<td>21.6%</td>
<td>3.1%</td>
<td>32.0%</td>
</tr>
<tr>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>24.2%</td>
<td>23.7%</td>
<td>-</td>
<td>12.7%</td>
<td>14.9%</td>
</tr>
<tr>
<td>Rb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.6%</td>
<td>21.4%</td>
</tr>
</tbody>
</table>
Table 5.6 Sampling factors for porcine lung, kidney and liver sections. Values are shown in mg at 1% ($K_1$) and 5% ($K_2$) variation levels, the latter in parentheses.

<table>
<thead>
<tr>
<th>Element</th>
<th>Lung</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Cortex</td>
</tr>
<tr>
<td></td>
<td>Sample 1</td>
<td>Whole</td>
<td>Sample 6</td>
</tr>
<tr>
<td>Cl</td>
<td>16 (0.64)</td>
<td>140 (5.6)</td>
<td>30 (1.2)</td>
</tr>
<tr>
<td>K</td>
<td>13 (0.50)</td>
<td>12 (0.47)</td>
<td>26 (1.1)</td>
</tr>
<tr>
<td>Ca</td>
<td>=</td>
<td>5.8 (0.23)</td>
<td>=</td>
</tr>
<tr>
<td>Mn</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Fe</td>
<td>72 (2.9)</td>
<td>45 (1.8)</td>
<td>34 (1.3)</td>
</tr>
<tr>
<td>Cu</td>
<td>36 (1.4)</td>
<td>0.60 (0.024)</td>
<td>=</td>
</tr>
<tr>
<td>Zn</td>
<td>34 (1.4)</td>
<td>43 (1.7)</td>
<td>35 (1.4)</td>
</tr>
<tr>
<td>Br</td>
<td>=</td>
<td>=</td>
<td>59 (2.3)</td>
</tr>
<tr>
<td>Rb</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>
groove which is approximately symmetrical in shape with diameter ~400 μm and maximum depth ~100 μm. It may be noted that at this point there is a compacting of material, under which is the presence of sample burning. If the beam is assumed to cover only the area of the groove, and if no current density gradient exists across this feature, then the model developed in section 5.4 may be employed to ascertain the degree of X-ray yield modification from the smooth surface situation. Average rough to smooth yield ratios for Cl, K, Ca, Fe and Rb are 0.933, 0.960, 0.970, 0.994 and 1.000 respectively. A large degree of variation exists for Cl as expected, while significant differences are also seen for K and Ca, uncertainties being 4% and 3% respectively. Hence it is essential to limit proton beam current density and thus inhibit sample surface damage so as not only to restrict falsification of results which may arise from the volatilisation of various elements, but also to guard against surface geometry modification which is predicted to particularly affect the X-ray yields from low atomic number elements.

Table 5.5 contains data for percentage inhomogeneities of various elements in the above mentioned tissues. Data for the lower Z elements are probably smaller than those shown due to the features discussed previously. In the right lung, Zn exhibits greater heterogeneity when considering all sampling positions than a single area. This is not apparent when looking at σh values in the left lung, however Fe mimics this trend. Confirmation of these observations are found in section 5.6.2.2.

Percentage elemental inhomogeneities in kidney reiterate many of the findings of section 4.6.4. That is, values for Ca, Fe, Cu and Zn in renal cortex are greater than in medulla due to expected concentration gradients in the former tissue type which remain constant in the latter [TAN87,LI71]. Also, due to the large vascular supply to the cortex which is far less in the medulla [DAR80], one might expect substantial heterogeneity of Fe in the cortex as a result of differing blood content. Regarding liver, inhomogeneities of all elements studied are similar which was confirmed in the chapter previous to this. When comparing the magnitude of percentage inhomogeneities in Table 5.5 for kidney and liver with those in Table 4.11, one finds that values derived here are on the whole greater than those found in chapter 4. This is expected due to a smaller sampling mass being employed in the analysis of biological sections [LIE84]. Sampling masses used here were of the order 30-100 μm, whereas in Table 4.11, typical masses were in the gram range. However, the degree of elemental variations seen in Table 5.5 are
Figure 5.23 Cross-section through a lyophilised porcine kidney section illustrating sample damage as a result of excessive beam current density. SEM photograph a) represents a coarse view of the damage whilst b) a magnified view of the beam spot.
insufficient to yield sampling factors that are of a similar level to those in Table 4.12. In fact sampling factors in Table 5.6 vary from those in Table 4.12 by roughly $10^3$ (Table 5.6 is in mg dry weight, whereas those in Table 4.12 are in g dry weight). As the sampling factors in chapter 4 agree with the limited published data, then it is assumed that values presented in Table 5.6 are misleading.

A possible explanation for sampling factors being at a level that is deemed excessively low is the breakdown of the sampling theory at this replicate mass as suggested by Ingamells and Switzer [ING73]. Although their work is directed toward geochemical analysis, they discuss that as sampling masses become smaller, there is a decreased probability that inhomogeneities are included within the aliquot. The example they present is a mineral grain which has low frequency in a matrix and hence as sample weights are decreased, it is less likely for that grain to be included and thus high concentrations are encountered more seldomly. This scenario may or may not have direct correlations with biological section analysis. Ingamells and Switzer [ING73] state that this situation may be identified by observation of concentration frequency plots, these showing a positively skewed relationship rather than the more expected symmetrical Gaussian curve which is the distribution that sampling factors are based upon. The frequency distribution of elemental concentrations in the studied biological sections was investigated, two such plots are seen in Figure 5.24. These plots represent Ca (a) and Zn (b) in the whole right lung. The frequency graph for Ca is asymmetric and exhibits positive skewing whereas Zn is symmetrical in shape. As sampling factors for each of these elements is in the mg range, it is difficult to conclude if the above reason is the major cause for the lower sampling factor weights encountered. Without doubt, more work is required to investigate the relationship between sampling factors and sampling weight in the $\mu$g to mg range to ascertain where the derivation of the representative sample mass becomes valid.

5.7 Conclusions

Proton induced X-ray emission analysis was used to investigate the elemental distributions across thick biological specimens. A number of trends were noted due to the physical and physiological functions of the studied tissues. Random fluctuations were observed which were not solely due to analytical uncertainties, but elemental
Figure 5.24 Concentration frequency plots for Ca (a) and Zn (b) in the whole right lung.
heterogeneities of the biological material. To quantify these inhomogeneities, it was necessary to take account of all sources of analytical variation. In the analysis of thick biological sections this is complicated in that geometrical requirements of proton irradiation and X-ray detection orientations are poorly defined. Hence stylised models were developed to estimate the effects of sample surface roughness and tilt upon X-ray yield. Results from the surface roughness model are numerous, however this effect is found to fall off rapidly with increasing X-ray energy, although matrix density plays an important role in this relationship. The effect upon sample surface tilt upon X-ray yield is also highly correlated with proton energy although the degree is independent of the physical density.

Elemental distributions across sections of porcine kidney, liver and lung were investigated. Concentration plots along the length of the kidney sample revealed the presence of concentrations gradient for Zn and Cu and their preferential collection in renal cortex which was identified in chapter 4. Unexpected high concentrations were observed at a sampling point. The most probable reason for this was sample burning at this position which was seen when examined under an scanning electron microscope.

Concentrations across the liver section did not exhibit trends but fluctuated due to the analysis procedure and heterogeneous nature of the tissue. Calcium concentrations were seen to increase at sampling position 9 which was close to a hepatic vein wall. Elemental analysis of a section of such a sample of vein wall was carried out revealing high Ca levels.

Ten samples in all were extracted from a porcine lung pair. Concentration plots along one such sample identified a situation similar to that in liver i.e. no concentration gradients but fluctuations accredited to the analytical procedure and heterogeneous nature of the tissue. However, comparison of average concentrations between sampling areas revealed differences. As expected, the concentration of various elements varied when comparing bronchi wall to lung tissue. On the whole, little variation of concentrations were noted in the lung themselves, however Zn did exhibit higher levels in sampling areas that were in the vicinity of the apex of the organ. A possible reason could be the deposition of aerosols at these sites.

Sampling factors and percentage inhomogeneities were calculated for each of the tissues studied. Values for lower atomic number elements are probably lower due to
the effects of sample surface roughness and tilt. Also, in extreme cases uncertainties may be introduced from excessive proton beam current density. Percentage homogeneities calculated here are greater than those found for corresponding tissues in chapter 4. This is an expected feature due to smaller sampling weights being employed in the measurements on lyophilised biological sections. However, we would expect sampling factors for each type of sample to be similar which is not the case, a factor difference of roughly $10^3$ observed. Ingamells and Switzer [ING73] offer an explanation which may or may not be applicable to biological specimens. This is linked to an asymmetry of the concentration distribution, Gaussian statistics are assumed in the calculation of sampling factors. A concentration frequency plot for Ca in the whole right lung exhibited positive skewing whereas Zn a symmetrical distribution. Thus conclusions about the type of distribution governing the derivation of sampling factors cannot be drawn. More data are required over the $\mu$g to mg sampling weight range for these specimens in order to test the validity of sampling factors at this level.
Chapter 6
Photon Transmission Tomography

6.1 Introduction

Photon transmission tomography is a technique that allows the mapping of the linear attenuation coefficient, $\mu$, through a slice of an object whilst still preserving its integrity i.e. the technique is non-destructive. The enormous capabilities of photon transmission tomography were not realised until the development of the first commercial scanner by Hounsfield in 1973 [HOU73]. Previous to this development, early tomography involved various physical methods for producing images of sections through objects. These images were however of low quality which was a result of inadequate reconstruction procedures. It was not until 1963 that Cormack [COR63] independently developed an accurate mathematical theory and computational method for reconstructing images and demonstrated that tomographs of simple phantoms could be derived from a set of projections from a discrete energy radioactive source. Considering the revolutionary impact of tomography upon medicine, it is no surprise that Hounsfield and Cormack received the Nobel Prize for Medicine in 1979 for their work [GIL84]. Further information about photon transmission tomography may be found in numerous amounts in the literature, those by Brooks and DiChiro [BR076], Gilboy and Foster [GIL84] and Kouris et al [KOU82a] are to name but a few.

When a well collimated mono-energetic photon beam passes through a length $L$ of an homogeneous material of single element composition, the photon beam is attenuated with respect to an exponential function, the intensity $I$ of the X- or $\gamma$-rays emerging from the sample being predicted by equation 6.1. The incident photon intensity is given by $I_0$.

$$\frac{I}{I_0} = \exp(-\mu L) \quad \text{(Eq. 6.1)}$$

The term $\mu$ is the linear attenuation coefficient of the material and is the quantity imaged in photon transmission tomography. The linear attenuation coefficient is given by the product of $n_2$ and $\sigma$, $n_2$ representing the number of atoms per unit volume and $\sigma$ the total atomic cross-section [KOU82b]. It has been shown that the approximate total atomic
cross-section may be written in the form of equation 6.2 [JAC81].

$$\sigma(Z,E) = Z^j K^{coh}(E) + Z^{l} K^{KN}(E) + Z^{m} K^{ph}(E)$$  \hspace{1cm} (Eq. 6.2)

The right hand side of equation 6.2 contains three terms describing the effects of coherent scattering (coh), incoherent scattering (KN) and photoelectric absorption (ph) upon the photon beam. Note that the above equation is valid only for energies below 1.022 MeV as pair production is not taken into account. Also, equation 6.2 is accurate to varying degrees depending upon the photon energy and target element atomic number. This is because the exponents j, l and m are derived by fitting to experimental data which varies with E and Z and also the assumption that each separate cross-section may be factorised into a function of E and a function of Z is not wholly consistent with fundamental quantum theory of photon-electron interactions [JAC81]. An accurate parameterisation of the total cross-section is derived by Jackson and Hawkes [JAC81], however this is omitted here.

Coherent scattering, incoherent scattering and photoelectric absorption vary with incident photon energy and target material atomic number, and each becomes predominant over certain ranges of E and Z. Photoelectric absorption involves the ejection of a bound electron from a parent atom and hence occurs only when the photon energy is greater than the binding energy of the electron. The effect predominates for low energy photons and high atomic number elements and is complicated by absorption edges. Coherent or elastic scattering implies that the energy of scattered photons is unchanged from that before the process occurred. When coherent scattering involves bound electrons, it is known as Rayleigh scattering, whereas when it involves essentially free electrons, it is known as Thomson scattering. Although coherent scattering is generally regarded as insignificant as a photon attenuation process due to the effect rapidly falling off with increasing X- or γ-ray energy, it is a significant process at low energies (tens of keV) especially in high Z materials [KOU82b]. Incoherent or Compton scattering involves the momentum transfer from an incident photon to a free electron and results in an energy loss to the X- or γ-ray the degree of which is a function of the scattering angle. This is predicted by the Klein Nishina differential cross-section. Coherent scattering increases with increasing atomic number and decreases with higher photon energy. However, it forms the dominant X- or γ-ray attenuation mechanism when the attenuating medium consists of low atomic number elements and the photon energy is sufficiently high. The above discussion upon radiation

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interaction with matter is obviously very brief and further and more involved reading matter may be found in the literature, examples being by Davisson and Evans [DAV52] and Jackson and Hawkes [JAC81].

The above discussion has shown that the linear attenuation coefficient of a particular material at a specific energy is dependent upon the elemental composition of that material and also its density ($n_2$ is the number of atoms per unit volume). Therefore by imaging the distribution of $\mu$ through a slice of a sample, it may be possible to quantify the degree of elemental heterogeneity of that specimen and to locate regions of interest or abnormality such that these may be selected for examination by other physical or analytical techniques. The relevance of this will become apparent later. One of the first applications of tomography to the study of sample heterogeneity and region of interest identification was in the area of dendroanalysis i.e. the analysis of trees. At the time, neutron activation analysis was being investigated for its use in dendrochronology i.e. to indicate historical occurrences of pollution by elemental analysis of individual tree rings [TOU77]. It was discovered that one tree ring containing high Mg, Ca, Sr and Ba levels, when imaged using a medical computerised tomography (CT) head scanner, was shown to be clearly visible as a highly attenuating area. This was one of the first indications of the value of tomography in providing a measure of sample homogeneity and identifying regions of interest whilst preserving the integrity of the sample [SPY77].

Chapter 4 and chapter 5 have highlighted the heterogenous nature of biological tissues and that differences in elemental concentration may be derived which is partly due to the positioning of the sub-sampling site. If photon transmission tomography proves capable, it may be employed to initially scan tissues or organs to provide information concerning where best to extract a sub-sample that is representative or make aware to us the presence of some region of interest. It would also give a measure of the degree of heterogeneity of the sample. The application of such a procedure could prove advantageous when, for some reason, only a small quantity of the whole sample can be extracted for either analysis or storage as in the case of biological and environmental specimen banks. Specimen banks have been established to provide baseline environmental data and trends, the opportunity for retrospective analysis of samples from the past, and the evaluation of the performance of storage procedures [WIS89]. As these facilities have a limited capacity, sample size has to be restricted.
This chapter is dedicated to the introduction of photon transmission tomography and experimental considerations for the technique. The following sections include information pertaining to the formation of projections, methods of image reconstruction, limitations of the data and the experimental scanning rig employed for measurements. This will then be used in chapter 7 to assess the capabilities of the technique to provide a measure of sample homogeneity and identify regions of interest under differing analysis and specimen preparation conditions.

6.2 Formation of projections

Mathematically, when reconstructing an image of a slice through an object, the slice is assumed infinitely thin so that 2 dimensions may be assumed. All points upon this plane are indicated by a Cartesian set of co-ordinates (x, y) (see Figure 6.1), where f(x, y) represents the density function at a specific point i.e. the linear attenuation coefficient in the case of photon transmission tomography. The direct path from source to detector (which are assumed of point dimensions) is described by polar co-ordinates, the variables r and φ being defined as the distance between the line and origin and angle between the line and y axis respectively. Integration of f(x, y) along the ray path (equation 6.3) yields a quantity known as the ray-sum (s represents the distance between source and detector).

$$p(r, φ) = \int_{r,φ} f(x, y) \, ds \quad \text{(Eq. 6.3)}$$

If the photon beam consists of monoenergetic X- or γ-rays then equations 6.1 and 6.3 may be combined thus yielding the relationship between the ray-sum and initial and transmitted photon intensity (equation 6.4).

$$p(r, φ) = -\ln\left(\frac{I}{I_0}\right) \quad \text{(Eq. 6.4)}$$

A complete set of ray-sums at a given angle is called a projection, and theoretically an infinite number of projections at differing angles are required for image reconstruction as the 2-dimensional function f(x, y) is continuous. In practice, the object plane is broken into a coarse set of squares or picture elements formally known as pixels.
Hence, now a finite number of projections at equally spaced angles over 180° are needed for solution of the values of the pixels. The methods for doing so are mentioned next.

6.3 Image reconstruction from projection data

Methods for reconstructing an image of a slice of an object from a finite number of projections may be grouped into three categories, namely simple back projection, iterative techniques and analytical techniques. Simple back projection formed the first attempts of image reconstruction and is the least complex to implement. The technique is generally regarded the crudest of the approaches and produces images of low quality. However, simple back projection highlighted the need for the development of accurate mathematical treatments for high quality image reconstruction producing tomograph parameters e.g. linear attenuation coefficient, that are truly representative of the scanned
object. This is satisfied by iterative and analytical image reconstruction approaches. Iterative reconstruction functions by successive modification of pixel elements by various techniques until satisfactory agreement is obtained between predicted and measured projection values, whereas analytical reconstruction relies upon the direct solution of equation 6.3 to yield the density function \( f(x,y) \). A detailed description of these techniques may be found by Brooks and DiChiro [BRO76], Kouris et al [KOU82a] and Herman [HER80].

6.4 Noise in reconstructed images

The explanations regarding images and their data not fully correlating with the scanned object may be grouped under the headings limitations in the data and algorithm and experimental system deficiencies. The latter category includes factors such as approximations in the reconstruction algorithms, inaccuracies in projection angle and ray-sum spacing and noise that arises from the detector system and associated electronics. Many of the sources of variations due to the detector system and associated electronics are hardware specific and will be discussed, where needed, in the sections describing the experimental set-up.

Limitations in the data arise from inadequate ray-sum and projection data accumulation and statistical fluctuations from the random nature of the number of photons collected for each ray-sum. The number of ray-sums required in each projection is dependent upon the desired spatial resolution of the image. Kouris et al [KOU82a] state that ray-sum spacing should be less than the pixel size (between 0.4 to 0.7) whereas Brooks and DiChiro [BRO76] and Sanders [SAN82] equal to the pixel size, especially when detector and source collimation is of the same order. In the brief theory presented upon tomography, it was stated that the detector and radioactive source were of point dimensions. In reality, this is of course untrue, and it is found that the collimation employed in the accumulation of data in tomography is the limiting factor upon spatial resolution unless deconvolving techniques are employed.

A number of equations exist predicting the number of projections required for the solution of each pixel density value. These are essentially of the same form, they however differ by a factor of two i.e. \( m=n\pi/4 \) [BRO76] as opposed to \( m=n\pi/2 \) [KOU82a] where \( m \) represents the number of projections required, and \( n \) the number of independent
data values in a projection. Hence as data is collected over angles 0 to \( \pi \) radians (transmission tomography), \( \pi/m \) yields the angular spacing to provide the minimum number of projections for pixel density determination. The relationship for \( m \) being equal to \( \pi n/2 \) is more widely accepted, whereas in practice it has been found that the required number of projections for reconstruction is substantially less than that predicted by the equation above [SAN82,GIL84,CHO87].

Due to the random nature of radioactive quanta, a degree of fluctuation in the image may be attributed to this. In the case of employing a polychromatic X-ray tube as photon source, the contribution to tomograph noise from this limitation of data may be reduced to negligible levels, as long as doses to the imaged body are not limited. However, as pixel density values i.e. linear photon attenuation coefficient, are of great importance to this and many other studies, the employment of polychromatic photon sources is not always viable. This is because different energy photons are attenuated to varying levels making the accurate quantification of \( \mu \) in a heterogeneous sample difficult [KOU82a]. Therefore we are limited to the utilisation of discrete energy sources typical intensities being substantially lower than X-ray tubes and hence fluctuations in the image due to limited photon counting statistics may be significant.

If the standard deviation of the counts of all ray-sums is the same i.e. \( \sigma_p \), then it may be shown [GIL82,KOU82a] that the total image standard deviation \( \sigma_i \) in convolution filtered back projection is given by:

\[
\sigma_i = \frac{\sigma_p \pi k}{dm^{1/2}}
\]

(Eq. 6.5)

where \( d \) is the ray-sum spacing and \( k \) a factor that depends upon the filter employed e.g. \( k=0.289 \) for the Ramachandran and Lakshminarayanan algorithm. The value of \( \sigma_p \) is given by \( 1/C^m \), where \( C \) is the number of counts in each projection.

6.5 **Experimental aspects for tomography**

The tomography scanner employed in this work was originally devised by Mossop [MOS88] and built at the University of Surrey. A schematic of the scanning system and associated electronics is illustrated in Figure 6.2. The sample is rotated and translated upon a scanning bed to build up projection data, the transmitted intensity in
each ray-sum being measured by a low energy HpGe semi-conductor detector. Data from
the detector is initially processed by an amplifier which may be sent to a number of single
channel analysers so that the count for specific energy windows may be recorded. Ray-
sum data is stored upon a BBC micro-computer ready for reconstruction. Aspects of the
scanning rig and detector pulse processing modules will be discussed in the next sections.

6.5.1 Scanning rig

In many tomographical scanners, the object of interest remains static whilst the
photon source(s) and detector(s) is/are rotated [KOU82a]. As the samples to be scanned
are relatively small, Mossop [MOS88] designed the scanner so that projection data is built
up by movement of the object instead. This is achieved by two stepper motors that rotate
and translate the sample through the single pencil beam geometry photons (Figure 6.3).
Projection data is constructed by the photon intensity being measured at discrete positions
across the sample, points being set by translation of the sample through the beam. Once
data for a complete projection are accumulated, the sample is rotated to the next
projection angle and then moved back into the beam. Hence, projection data is collected
in a positive direction for odd projections and a negative direction for even projections.
Mossop refers to this method of accumulation as ‘no swap’ and it is obvious that this
mode of operation is more time efficient than the collection of data in one direction only
or ‘swap’.

Control of the stepper motor is by the stepper motor controller which may
function in manual or automatic mode. In the accumulation of tomographical data, the
controller is left in automatic mode, and the BBC micro-computer dictates the length of
translation or angle of rotation that the scanner bed is moved. Signals from the computer
user port pass directly to the stepper motor controller which moves the motors with linear
precision of 0.0125 mm or rotational precision 0.02°.

The single pencil beam geometry for the photons in the experimental set-up is
achieved by suitable collimation of the detector and radioactive source. The crystal is
surrounded by a steel shield that sits on top of a semi-circular support platform. At the
front of this steel shield is an aperture into which fit brass collimators with different bore,
namely 1 mm and 2 mm diameter parallel holes. Directly opposite is a circular support
into which the sealed radioactive sources are housed. These radioactive sources are of

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Figure 6.2 Schematic representation of the scanning apparatus and electronics involved in photon transmission tomography.
Figure 6.3 Top view of the single pencil beam geometry used in the acquisition of data for tomography.

High activity i.e. 3.7 GBq/7.4 GBq (100 mCi/200 mCi), and emit photons of low energy ($^{241}$Am). The high activity ensures that sufficient counts may be collected for each ray-sum in a short period of time, thus limiting the total scan period, whilst the low energy photons ensure good image contrast between areas of similar but different composition and density [KOU82a]. However, if the linear attenuation coefficient of the sample is too large, little of the initial beam intensity will be transmitted through the sample. At the front of the source shielding fits lead collimators, the bore of which is chosen to match that of the detector. The collimation size limits the spatial resolution achievable in a tomographical scan. The detector and source supports allow vertical movement which enables tomographical scanning of the object of interest at differing levels. Alignment of collimation bore is carried out with the aid of a laser or maximisation of count rate.
6.5.2 HpGe detector specifications

The detector employed in this work is a low energy hyper-pure germanium (HpGe) planar semiconductor detector supplied to the University of Surrey by Princeton Gamma-Tech. The manufacturer's specifications of the detector may be found in Table 6.1.

Table 6.1 Manufacturers specifications of the HpGe detector employed in tomographical scans.

<table>
<thead>
<tr>
<th>Description</th>
<th>Manufacturers specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>HpGe crystal: thickness</td>
<td>10 mm</td>
</tr>
<tr>
<td>active front face area</td>
<td>200 mm$^2$</td>
</tr>
<tr>
<td>voltage bias</td>
<td>-2000 V</td>
</tr>
<tr>
<td>Crystal front face to Be window distance</td>
<td>unspecified</td>
</tr>
<tr>
<td></td>
<td>(11.0±0.5 mm)$^*$</td>
</tr>
<tr>
<td>Be window: thickness</td>
<td>unspecified</td>
</tr>
<tr>
<td>diameter</td>
<td>19.0±0.5 mm</td>
</tr>
<tr>
<td>Energy resolution: @ 5.9 keV ($^{55}$Fe)</td>
<td>204 eV</td>
</tr>
<tr>
<td>@ 122 keV ($^{57}$Co)</td>
<td>490 eV</td>
</tr>
</tbody>
</table>

* measured by Mossop [MOS88].

Note that the active front face area is given as 200 mm$^2$. Measurements by Mossop [MOS88] involving scanning of the crystal by a highly collimated point source revealed that the active front face area is energy dependent and in fact not a circular response as would be expected, but more square. The measured active front face area is presented as 170±5 mm$^2$ at 60 keV, whilst this falls to 100±10 mm$^2$ at 6 keV (FWHM values). This he attributes to the dead layer of the crystal. As the distance of the planar crystal behind the Be window is not specified by the manufacturer, Mossop measured this in a similar
fashion to Arshed [ARS91] and Gooding [GOO89] for the Si(Li) detector discussed in section 3.3.3. The result is presented in Table 6.1.

The hyper-pure germanium crystal is housed within a vacuum cryostat that serves to inhibit thermal generation of electrons in the semiconductor material and thus degrade the energy resolution of the detector [KNO89]. In close vicinity to the crystal is the pulse-optical feedback pre-amplifier which is also housed within the cryostat package. This has the advantage that capacitive loading upon the detector is minimised and that the input stage into the pre-amplifier may be cooled along with the detector to reduce electronic noise. The pulsed optical feedback pre-amplifier is different from other pre-amplifiers in that the feedback resistance is eliminated from the electronic configuration, thus improving the noise level. Thus current pulses from the detector are accumulated upon the feedback capacitor and the output voltage is seen as a step configuration, each step corresponding to a separate detection pulse. Just before the voltage reaches a saturation level, the capacitor is discharged by momentarily illuminating the drain-gate junction of the input stage FET. Hence the output voltage signal from the pre-amplifier is seen as a saw-tooth configuration, the reset rate being dictated by quanta energy and count rate and the leakage current across the detector crystal.

The manufacturers specifications stipulate that the leakage current is less than 10 pA which accounts for 4 resets per second when no source is present. Throughout the use of the detector, the pre-amplifier reset rate was monitored so that any increase could be used to indicate contamination build-up upon the detector crystal surface. Following a long period of inactivity of the detector, which was maintained with the crystal at liquid nitrogen temperatures at all times, the reset was found to be 3200 s⁻¹. This indicated a high level of crystal surface contamination and the need for a thermal cycle of the detector. The process of thermal cycling involves draining the cryostat of liquid nitrogen and allowing the crystal to warm up to room temperature, in this case the detector being left for three days. After the dewar was refilled with liquid nitrogen, the detector was left for a further day before application of high voltage and the reset rate was measured to be 4 s⁻¹. With excessive leakage current, the resolution of the HpGe detector is severely degraded, the Mn Kα and Mn Kβ peaks from an ⁵⁵Fe source (5.9 keV and 6.5 keV) being unresolved before the thermal cycle and which should be easily defined under normal working conditions.
6.5.2.1 Calibration and linearity

The energy calibration and linearity testing of the HpGe detector and amplifier was carried out using a set of point sources of approximately 37 kBq (10 μCi) activity each. Information pertaining to these sources may be found in Table 6.2, most information being taken from Browne and Firestone [BRO86]. It should be noted that the energy calibration and linearity testing is conducted up to energies and including 136.0 keV ($^{60}$Co) only. However data above this is used in the calculation of detector resolution and intrinsic photopeak efficiencies.

A graph of channel against energy was obtained, and a linear least square fit performed upon the data. The calculated intercept and gradient coefficients may be found in equation 6.6, where $c$ represents the channel number and $E$ the photon energy in keV.

$$E = (0.04042 \pm 0.00003)c - (0.48450 \pm 0.05813) \text{ keV} \quad \text{(Eq. 6.6)}$$

A correlation coefficient of 1.00000 was obtained from the first order fit which indicates excellent linearity of the response of the detector over this energy range.

6.5.2.2 Energy resolution

The concept of energy resolution was introduced in section 3.3.3.2 and indicates the ability of the detector to differentiate between two closely spaced photopeaks. Energy resolution is quantified by the measurement of a photopeak width at half its maximum height (FWHM), the total spread of the peak $W_T$ being linked to other factors by equation 3.2,

$$W_T^2 = W_E^2 + W_D^2 + W_X^2 \quad \text{(Eq. 3.2)}$$

where $W_E$, $W_D$, $W_X$ are the FWHM contributions from electronic noise, statistical spread of the charge carriers and detector leakage and charge integration respectively [KNO89].

The photopeak spread due to the electronic noise may be quantified by the application of a pulse from a precision pulse to the test input of the pre-amplifier. The output signal from the pre-amplifier is processed by the amplifier and the FWHM of the peak determined by a Gaussian fit to the data acquired upon a multi-channel analyser (MCA). A noise FWHM of 204 eV was obtained, which should remain constant across the energy range.
Table 6.2 Source data used in the calibration, linearity testing, resolution determination and intrinsic photopeak efficiency calculation of the HpGe detector.

<table>
<thead>
<tr>
<th>Source</th>
<th>Line</th>
<th>Photon energy ( E ) (keV)</th>
<th>Photons/decay ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{55}\text{Fe})</td>
<td>Mn K(_{\alpha})</td>
<td>5.894</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>Mn K(_{\beta})</td>
<td>6.489</td>
<td>0.0286</td>
</tr>
<tr>
<td>(^{241}\text{Am})</td>
<td>Np L(_{\beta})</td>
<td>17.8</td>
<td>0.194±0.004*</td>
</tr>
<tr>
<td></td>
<td>(\gamma)</td>
<td>26.36</td>
<td>0.0236±0.0001*</td>
</tr>
<tr>
<td>(^{133}\text{Ba})</td>
<td>Cs K(_{\beta_1})</td>
<td>34.981</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Cs K(_{\beta_2})</td>
<td>35.815</td>
<td>0.0439</td>
</tr>
<tr>
<td>(^{241}\text{Am})</td>
<td>(\gamma)</td>
<td>59.54</td>
<td>0.357</td>
</tr>
<tr>
<td>(^{133}\text{Ba})</td>
<td>(\gamma)</td>
<td>81.0</td>
<td>0.3738</td>
</tr>
<tr>
<td>(^{57}\text{Co})</td>
<td>(\gamma)</td>
<td>122.1</td>
<td>0.8563±0.0015(^*)</td>
</tr>
<tr>
<td></td>
<td>136.0</td>
<td></td>
<td>0.1069</td>
</tr>
<tr>
<td>(^{133}\text{Ba})</td>
<td>(\gamma)</td>
<td>176.4</td>
<td>0.0709</td>
</tr>
<tr>
<td></td>
<td>302.9</td>
<td></td>
<td>0.1840</td>
</tr>
<tr>
<td></td>
<td>356.0</td>
<td></td>
<td>0.6220</td>
</tr>
<tr>
<td></td>
<td>383.9</td>
<td></td>
<td>0.0892</td>
</tr>
<tr>
<td>(^{22}\text{Na})</td>
<td>(\gamma)</td>
<td>511.0</td>
<td>1.80</td>
</tr>
</tbody>
</table>

All data taken from [BRO86] except * [COH80], † [CAM86]

The charge collection process in germanium detectors is inherently slow. A large reverse bias across the crystal allows higher electron drift velocities, however a saturation value being achieved at 1 \(10^5\) Vm\(^{-1}\) where a corresponding saturation drift velocity for holes is achieved at a field of approximately 3 \(10^5\) Vm\(^{-1}\) [KNO89]. The HpGe detector employed in this work functions with an applied electric field of 2 \(10^5\) Vm\(^{-1}\). The reverse bias may
be increased in order for holes to travel at their saturation drift velocity. This however may not diminish the photopeak spread due to this effect as the leakage current may be increased across the surface of the crystal. Due to the relatively large charge collection time, ballistic deficit may occur when amplifier shaping times are inadequately short. Ballistic deficit occurs when amplifier shaping constants are smaller than pre-amplifier pulse rise times, and hence the voltages of the shaped pulses are less than they would be when integrated over larger time periods. In a detector with constant charge collection, the deficit may occur. However it may be irrelevant due to a constant fraction of amplitude being lost from each pulse. In HpGe detectors, especially those of large crystal dimension, the total charge collection time varies with position of photon interaction and hence ballistic deficit effects may contribute significantly to the spread seen in photopeaks. Therefore, the relationship between FWHM (59.5 keV line from $^{241}$Am) was

Figure 6.4 Relationship between FWHM of a photopeak at 59.5 keV ($^{241}$Am) and amplifier shaping constant.
investigated with amplifier shaping time (1 μs, 2 μs, 4 μs, 8 μs, 12 μs) and the results shown in Figure 6.4. It may be seen that the total FWHM of the photopeak decreases as the amplifier shaping constant becomes larger, and the resolution takes its minimum value at 8 μs shaping although that at 12 μs shaping constant is not significantly different. Hence it is apparent that for the minimisation of \( W_p \), a shaping constant of 8 μs or 12 μs should be chosen. However, this is assuming that photon count rates are low, as high count rates when large shaping constants are employed may lead to higher photopeak resolutions due to pulse pile-up effects. As the count rates encountered in this work are relatively low (see section 6.5.5 for typical values), a shaping constant of 8 μs is used throughout.

The resolution of the HpGe detector was determined for most of the photon energies listed in Table 6.2. These were calculated by obtaining a Gaussian fit to the data. Comparison of experimental values at 5.9 keV and 122.1 keV to the manufacturers data and previous published resolutions showed close agreement although being slightly higher i.e, 240 eV and 508 eV at 5.9 keV and 122.1 keV energy respectively as opposed to the manufacturers data of 204 eV and 490 eV and Mossop's [MOS88] data 207 eV and 480 eV. Resolution data for all energies may be found in Figure 6.5. The total FWHM, \( W_T \), the noise contribution \( W_n \) and the statistical spread from the charge carriers \( W_p \) are shown. Note that the latter is calculated using equation 3.3 where the energy to create an electron-hole pair \( \epsilon \) is taken as 2.96 eV [KNO89], and the Fano factor (for germanium at 77K) \( F \) is 0.058 [STR71]. Now equation 3.2 may be solved to evaluate the contribution to the total FWHM from the charge integration and leakage and its dependency over the illustrated energy range. This is seen to be significant and predominates over fluctuations due to electronic noise whilst being of similar magnitude to the statistical spread of the charge carriers. This is a similar trend to that illustrated by Knoll [KNO89].

The energy resolution of the HpGe has been found to be far superior to other detectors e.g. typically better than at least a factor of ten when compared to NaI(Tl) scintillation detectors [SAN82]. This is one of the major reasons for this type of detector being chosen for this specific imaging application. A significant factor contributing to the degradation of image spatial resolution is scattered photons from the sample reaching the detector [SAN84]. To a large degree, this may be inhibited by detector and source collimation which is employed in the tomographical scanning system (section 6.5.1).
Figure 6.5 The energy dependence of the HpGe resolution $W_T$ and its contributions from electronic noise $W_{el}$, charge carrier statistics $W_D$, and charge integration and leakage current $W_X$.

However, forward scattered photons may still reach the detector. A further measure may be taken to reduce incoherent scattered photon contribution to the image where an energy window is set around a photopeak, and only counts falling within this window are included in ray-sum measurements. However, at low photon energies, forward scattered energies are little changed from their initial value, thus making energy discrimination from detectors with poor photopeak resolution difficult. Scattered photon discrimination by HpGe detectors is far easier due to their superior energy resolution, and are essential when high spatial resolution is required within reconstructed tomographical images [MOS88].

6.5.2.3 Energy efficiency

Intrinsic photopeak efficiencies for the HpGe detector were calculated for most of the energy range shown in Table 6.2 and are illustrated in Figure 6.6. These
efficiencies were calculated using the set of listed points sources (initial activity roughly 37 kBq/10 μCi), which apart from $^{57}$Co and $^{22}$Na were placed 300±10 mm from the front face of the detector (310±20 mm from the crystal front face). Source to detector distances were 50±5 mm and 100±5 mm for $^{57}$Co and $^{22}$Na respectively, and are smaller than that stated above because of low count rates. This was due to the radioactive decay of $^{57}$Co (it had undergone decay over nearly 6 half lives), and the poor detection efficiency of gamma-rays emitted from $^{22}$Na. Errors upon efficiencies are included in Figure 6.6 and take into account uncertainties in peak area count (equation 3.7), source activity (stated by the manufacturer, Amersham International, plc, UK and roughly 5%) and solid angle.

When compared with data calculated by Mossop [MOS88] and Barlow [BAR90], the intrinsic photopeak efficiencies compare favourably. However, that at 59.54 keV ($^{241}$Am) is somewhat larger than expected (1.19±0.15), Mossop and Barlow obtaining values at this energy of 0.9±0.1 and 0.841±0.035 respectively. A non-linear least square fit of the form of equation 6.7 was obtained from the data (excluding the value at 59.54 keV) where the fitted coefficients take values $A_0=-1.9495 \times 10^6$, $A_1=-3.0843$, $A_2=-1.4965 \times 10^5$ and $A_3=-1.8530$.

$$
\epsilon_f = (1 - \exp(A_0E^{A_1})) \exp(A_2E^{A_3})
$$

(Eq. 6.7)

The terms $\epsilon_f$ and $E$ represent the intrinsic photopeak efficiency and photon energy (keV) respectively. The first term in equation 6.7 is analogous to the attenuation of photons in the Ge crystal. Initially values of $A_0$ and $A_1$ for energies greater than 100 keV were based upon data for the mass attenuation coefficient of germanium [STO70] and the manufacturers data for the detector. However, this produced an unsatisfactory fit for this energy range mainly due to unaccounted factors e.g. insufficient charge collection [HAN73]. The second term describes the decrease in efficiency toward lower energies, and may be likened to an attenuating factor from the crystal surrounding material. However, photon attenuations from the Be window, gold contact and crystal dead layer are predicted to become significant only below roughly 6 keV and thus the fall in intrinsic efficiency must be attributed to other factors e.g. decrease in the crystal active volume with lower energy as measured by Mossop [MOS88].

The inclusion of intrinsic photopeak efficiencies upon the same graph that have been calculated by the placement of standard sources at differing distances from the
Figure 6.6 Intrinsic photopeak efficiencies for the HpGe detector employed in tomographical scans.

detector is not wholly correct [HAN73]. This is not because of the differing air thickness between source and detector, as for most purposes the photon attenuation from this may be assumed negligible. Rather, the efficiency is found to vary with solid angle which may be attributed to the escape of photons from the sides of the detector crystal. However, using the geometrical correction factor supplied by Cohen [COH80] and the linear attenuation coefficients of germanium, the ratio of this effect at 50 mm and 300 mm source to detector spacing at 136.0 keV is 1.054 and hence relatively small. Given the limited time available to carry out the efficiency measurements, it was deemed advantageous to maximise the photopeak counts by placing the standard sources ($^{27}$Co, $^{22}$Na) closer to the detector, although, where possible a source-detector spacing of 300 mm was preferred in order to minimise errors in distance measurements.

Figure 6.6 illustrates the rapid fall-off of the HpGe intrinsic photopeak efficiency after a peak value at roughly 60 keV. However, given that tomographical imaging is of low density and low atomic number objects, energies of 60 keV and below
are required to obtain maximum image contrast whilst providing high count-rates in the measurement of ray-sums. This coupled with high energy resolution makes HpGe detectors an ideal candidate for this imaging application.

6.5.3 Electronics

As can be seen in Figure 6.2, the electronics involved in the tomographical scanning system consist of a Canberra 2022 amplifier, two Canberra 2030 single channel analysers (SCA) and an in-house built duel counter. Gain settings upon the amplifier were coarse=30, fine=8.00 allowing calibration equation 6.6 to be obtained. A shaping constant of 8 µs was employed for the reasons presented in section 6.5.2.2. An amplifier such as the Canberra 2022 is required due to the active base-line restoration facility. This ensures conversion of the pre-amplifier step configuration output (section 6.5.2) into unipolar output pulses which are fed to both single channel analysers.

The single channel analysers are set to output logic pulses when their linear unipolar inputs fall into specific voltage amplitude ranges (energy ranges), namely photopeak and scatter windows. Discussion regarding these energy windows and their establishment may be found in the following sections. Logic output pulses from the SCAs are relayed to the 16-bit binary counter. This module was built at the University of Surrey [HAR85], and is based upon an MC6840 Motorola programmable timer. The counter is interfaced to the BBC micro-computer by the 1 MHz data bus.

6.5.4 Computer and software

As previously indicated, scanner control and data collection is provided by a BBC micro-computer that runs under BBC basic programs written by Mossop [MOS88]. The suit of programs functions upon a menu format, J.DEV_2 controlling the main menu which allows the execution of six options. Option 1 (J.AVER_2) provides the opportunity to measure the counts in each energy window over a preset time, the data being taken from the dual counter. This option is utilised when deciding upon the ray-sum counting period. Option 2 (J.INIT_2) concerns the specification of the scan parameters and requires input data for the ray-sum step length, number of ray-sums in a projection, projection angular spacing and if this is taken over 180° (transmission) or 360° (emission) and the preset counting time which should be derived using option 1. This data is stored in a
separate file (RUNDATA) and is called up when a scan is begun using option 4 (J.SCAN_A). Option 3 (J.MOVE_3) allows linear and rotational movement of the scanner bed by the stepper motor controller which is interfaced to the computer by the user port. This option is employed mainly when centring the scanner bed, an important tomographical scan prerequisite which will be discussed in the following section. A single line scan i.e. data collection over a single projection is performed using option 5 (J.LIN_1) and option 6 allows exit from the main menu.

Data from tomographical scans is collected upon disc filenames SCANj and SCANj+1 denoting that from duel counter input 1 and input 2 respectively. The integer variable j is specified by the user when executing option 2. The duel counter thus allows two sets of data to be collected simultaneously which may represent the transmission of photons at differing energies, or the transmission of photons at a single energy, the second channel providing an estimate of scattered or background radiation.

Reconstruction of data was performed upon the BBC micro-computer using the convolution filtered back projection algorithm presented by Brooks and DiChiro [BRO76] in a program written by Davies [DAV89]. This program assumes parallel source-detector geometry and the collection of an odd number of ray-sums. Input data to the program includes the number of projections and ray-sums in each projection, the pixel size, the total scan angle (180° for transmission tomography), specification of preset time or counts and the format in which data was collected i.e. ‘swap’ or ‘no swap’ as discussed in section 6.5.1. It must be noted that a maximum of 75×75 pixels may be calculated, this being limited by the memory capacity of the BBC micro-computer. During reconstruction, background and scattered radiation may be subtracted from the photopeak window, which may improve image quality. This will be discussed in depth later. At the end of reconstruction, the image is stored in an array of data on disk, pixel values being in mm⁻¹ (linear attenuation coefficient), which are calculated taking \( I_0 = (I_0^1 + I_0^2)/2 \) where n is the number of ray-sums in projection k. That is, the first and last ray-sum in each projection are used to calculate an average initial photon intensity (see equation 6.4), and thus these positions should always only contain negligibly photon attenuating air.

Image data is transferred to the mainframe, namely MARIE for display and data analysis upon the X-window terminals via the commercial package Xv and self written FORTRAN 77 programs.
6.5.5 Procedure for tomographical scanning

The procedure for preparing the experimental rig for the acquisition of tomographical images begins with centring the scanning bed. Failure to do this results in the incorrect reconstruction of the image due to back projection of data to wrong positions. Table centring is performed with the aid of a 2 mm diameter brass pin that fits upright into the centre of the scanning bed. With the source and detector collimated by 1 mm diameter bores, the pin may be translated through the photon beam until a minimum count rate is identified which is essentially straddled by three closely spaced distances. One such scan is seen in Figure 6.7 where the peak area counts from the 59.54 keV line of $^{241}$Am was employed to centre the table, an accuracy of between 0.5-1.0 mm being obtained.

![Figure 6.7](image.png)

Figure 6.7 Plot illustrating the 59.54 keV ($^{241}$Am) energy peak area count-rate as the brass pin is translated through the photon beam to centre the scanning bed.

The SCA windows were set with the aid of a Canberra series 35+ multi-channel analyser (MCA). A typical procedure for the photopeak window is as follows. The linear output from the amplifier is split, one signal being routed directly to the ADC input of
the MCA, whilst the other to the input of the SCA. The logic output from the SCA is sent to the MCA gate which is set in coincidence with the amplifier linear output. Hence, in theory as long as linear pulse heights fall within the upper and lower level discriminator settings, a pulse will be recorded upon a specific MCA channel. However in practice, this does not occur, the reason being the manner in which SCA logic pulses are generated. That is, in the event of discriminator criteria being satisfied, a logic pulse is output when the linear input decays through the lower discriminator level [CAN76]. As the linear gate is open for a finite period only (around 250 ns) [CAN86], linear pulses will be accepted solely when they are just greater in amplitude than the lower level discriminator (LLD) voltage of the SCA. Hence with the upper discriminator (ULD) set to its maximum value, a number of channels of data just above the LLD will only be collected by the MCA. However, due to the excellent energy resolution of the HpGe detector, this is never a problem as the number of channels encompassing photopeaks is far less than that limited by the MCA gate width. When using detectors with poor energy resolution, problems may arise which may be remedied by the introduction of a micro-second delay amplifier, such as the Ortec 427, between amplifier linear output and MCA ADC input.

The photopeak window is set by initially obtaining count data around the energy of interest. Using this data, the channels relating to the full width at tenth maximum (FWTM) of the photopeak are found as suggested by Sanders [SAN82] and the LLD and ULD adjusted to set a window around this. Sanders found that superior image spatial resolution could be obtained by setting the photopeak window around the full width at half maximum (FWHM). This however was derived using detectors of poorer energy resolution than the HpGe and hence it was thought more appropriate to maximise the number of counts rather than minimise the negligible number of scattered photons included in the FWTM window. A scatter window was set directly to the left (lower energy) of the photopeak window which was of the same number of channel as suggested by Sanders.

Parameters required by J.DEV_2 for tomographical scanning were set as follows. The step length was taken as equivalent to the source and detector collimation bore, and the number of steps enough to cover the whole object at energy projection angle with at least one ray-sum at the beginning and end of each projection free from photon attenuation. Angular spacing was calculated using \( \pi/4 \) (see section 6.4) typical
projections being separated by 4°-6°. The preset time for each ray-sum was derived using option 2 of the scanning program, this being dictated by the detection of a minimum of 2000 counts (2.2% error) from the transmission of photons through the most attenuating area of the object. Two sources were employed in scanning, both $^{241}$Am, but differing in activity namely 3.7 GBq and 7.4 GBq. Obviously, it is advantageous to employ the source with higher activity for all measurements so to decrease the total scan time. However, low energies are screened on this source, hence the smaller activity source is employed for lower energy measurements. Typical photon intensities ($I_0$) at 59.54 keV using 1 mm and 2 mm collimation are roughly 25 s$^{-1}$ and 530 s$^{-1}$ respectively and at 17.8 keV with 2 mm collimation 30 s$^{-1}$. Due to the low transmission of photons at 17.8 keV, measurements were not made with 1 mm collimation due to the extremely low count-rate for ray-sums. Obviously, the preset time is dependent upon the sample to be imaged, however, this ranged from 30 seconds (low attenuating object imaged with 59.54 keV photons with 2 mm collimation) to 120 seconds (higher attenuating object imaged with either 59.54 keV photons with 1 mm collimation or 17.8 keV photons with 2 mm collimation). Total scan times ranged from 7.75 hrs to 4.2 days, these long periods highlighting a disadvantage of discrete energy radionuclide sources over polychromatic X-ray generators, the photon intensity in the latter being considerably greater. However, for accurate quantitative determination of sample linear attenuation coefficients, discrete energy sources are required.
Chapter 7
Tomography of Biological Samples

7.1 Introduction

The basis for photon transmission and the experimental apparatus required to perform the technique upon biological specimens has been discussed in chapter 6. Photon transmission tomography is to be tested for its ability to provide information regarding the elemental heterogeneity and also to locate areas of interest or abnormality in the above mentioned category of samples. Knowledge of these parameters may aid in the selection of sub-samples and could be particularly useful in the event of being limited to a small number of aliquots which are used for elemental analysis by a technique such as PIXE or storage as in the case of biological specimen banks.

The scanning apparatus and reconstruction algorithm are initially tested to evaluate their performance in determining sample linear attenuation coefficients. This is achieved by employing a phantom containing solutions of known $\mu$ values. Sub-samples are then extracted from porcine lung, liver and kidney and these imaged at 59.5 keV and 17.8 keV photon energies under different sample and scanning conditions. Tomographs are presented and compared and their salient features identified e.g. the presence of regions of interest. Data extracted from scans are compared to published and experimentally derived (RBS and PIXE data from chapter 4 and chapter 5) values, and employed in the estimation of the elemental inhomogeneity of samples.

7.2 Testing scanning rig and reconstruction algorithm performance

It is essential before tomographically scanning biological samples to ascertain that data collection and image reconstruction is performed accurately. The method by which this was achieved involved the employment of a perspex phantom which was constructed at the university [LAC90] and its specifications may be found in Figure 7.1. The phantom contains three circular holes of dimensions shown, into which are placed phials holding aqueous solutions of Pb(NO$_3$)$_2$ of differing concentration. The phials are made of polyethylene and have walls of approximately 1mm thickness. They are arranged in a non-symmetrical manner for reliable evaluation of image reconstruction such that
symmetrical placement may result in the formation of a suitable image even if the reconstruction procedure is inadequate.

Aqueous solutions containing differing concentrations of Pb(NO$_3$)$_2$ were prepared and theoretical linear attenuation coefficients calculated using the mixture rule [KOU78] and employing tabulated mass attenuation coefficients [STO70] and measured solution densities. Linear attenuation coefficients were chosen to range widely (for a fixed energy) but to encompass expected values in biological samples although this could not be achieved for the low $\mu$ coefficients anticipated for higher photon energies in low density materials. A tomographical scan at 59.5 keV photon energy of the phantom containing phials of three different solutions of Pb(NO$_3$)$_2$ is illustrated in Figure 7.2. These solutions are of lead nitrate concentration 45.47% (top left), 11.72% (top right) and 20.10% (bottom). The image is reconstructed upon a 1mm×1mm pixel scale from rays-sums taken at intervals of 1mm, 57 of these contributing to each projection which were angularly spaced at 4°.

The image is circular in shape as expected, and each of the phials are clearly visible with higher photon attenuations exhibited in ascending concentration of Pb(NO$_3$)$_2$. 

Figure 7.1 Illustration of the perspex phantom utilised in quality assurance of the tomography scanner and reconstruction algorithm.
Figure 7.2 Tomographical scan at 59.5 keV of the perspex phantom and test solutions with 1mm×1mm pixel size.

Figure 7.3 Line scan across Figure 7.2 which intercepts the centres of the upper two phials.
A degree of fluctuation exists upon the pixel values in the image, observation of the perspex phantom which is predicted to have constant $\mu$ throughout illustrating this the most considerably i.e. 17% variation. This may be attributed to the relatively poor counting statistics in each ray-sum, which is due to the low photon transmission through the phantom. Fluctuations in the tomograph may be seen more clearly when a plot of linear attenuation coefficient is obtained across the image as in Figure 7.3. This line scan is taken directly across Figure 7.2 and intersects the upper two phials approximately through their centres. Pixel values across this line are indicated by circular points and the phials and phantom easily definable. Interfaces between perspex phantom and the polyethylene containers with solutions are not sharp, the reason being an averaging of constituent material linear attenuation coefficients at these points. That is, the pixel walls have linear attenuation coefficients at 60 keV of between 18-19 m$^{-1}$ [HUB82, WEA86] which when averaged with, for example, the $\mu$ values (1.61±0.06 m$^{-1}$ theoretical) of the 45.47% lead nitrate solution reduces pixel values which is observed on either side of the fluctuating inner phial plateau. A dip in linear attenuation coefficients for pixels between the phantom and phial walls is observed this being accredited to the above mentioned averaging effect between perspex ($\mu$ value 23 m$^{-1}$) and polyethylene and any air gap present between the two materials. However, although material interfaces are not sharp, they represent features of a correct size, namely 15 mm and 50 mm for the diameters of the phial insert holes shown and perspex phantom respectively.

The mean linear attenuation coefficients, obtained by taking regions of interest from tomographical scans of the phantom, are compared to theoretical values in order to ascertain the accuracy of the scanning process. This is achieved by plotting a graph of theoretical $\mu$ against experimental $\mu$ (Figure 7.4), perfectly correlating data producing a linear plot of gradient unity and intercept zero. Theoretical linear attenuation coefficient errors include uncertainties in the weight and densities of aqueous solutions of Pb(NO$_3$)$_2$, whilst experimental errors are the standard deviation of pixels over a region of interest which represents a particular value of $\mu$. These regions of interest were taken over as many pixels as possible to obtain a mean linear attenuation coefficient which is least affected by measurement errors [BAN86] e.g. photon counting statistics. A linear least square fit to the data illustrated in Figure 7.4 yields a straight line of gradient 0.932 and intercept 3.272 m$^{-1}$ with correlation coefficient 0.998, this being significant at greater than
the 99.95% confidence level. Hence there is high correlation between theoretical and experimental values of linear attenuation coefficient although the relationship between the two is only approximately unity. However, it is felt that this may be improved by greater accumulation of counts in each ray-sum which should be achievable in the analysis of biological samples due to their relatively lower linear attenuation coefficients. In any case, the errors shown in Figure 7.4 are enough for the data points to be lying upon the $\mu_E = \mu_T$ line.

7.3 Sample selection and preparation

Samples of porcine liver, lung, adipose and kidney were chosen for investigation by photon transmission tomography. Kidney samples were analysed in fresh and dried states to ascertain differences in image between the two and to decide the most favourable analysis mode for tomography. A 15mm thick fresh slice of kidney was obtained through the centre along the length of the organ which was freeze-dried, the dry to wet sample ratio being 0.167 (see Table 4.1). Two circular sub-samples of diameter 50mm were

Figure 7.4 Plot of theoretical $\mu$ against experimentally derived $\mu$ to test the accuracy of the scanning rig and reconstruction algorithm.
extracted from this freeze-dried slice (see Figure 7.14), the sub-samples containing portions of cortex, medulla and fat. These were stored in 50mm inner diameter air-tight polystyrene containers (1mm wall thickness) ready for analysis by tomography. Air-tight containers were chosen to inhibit the uptake of moisture by the samples thus enabling long scan times (see section 7.4 for typical values). If this precaution were not taken, the re-absorption of water may lead to the degradation of the freeze-dried kidney sample a result being the change in the linear attenuation coefficient. Fresh kidney and adipose samples were also chosen for analysis by photon transmission tomography, the kidney samples containing cortex and medulla. Due to the rapid degradation of fresh samples stored at room temperature e.g. due to dehydration, scanning times were required to be limited. This was achieved by their storage in polyethylene containers of smaller diameter than that stated above i.e. 20mm with 1mm wall thickness, and the restriction of ray-sum spacing to an interval of 2mm. These restrictions not only reduced the number of ray-sums per projection but also the counting time for each due to the large (2mm diameter) source and detector collimation being employed.

A 15mm section of liver was extracted from the right lobe of liver 2 (see section 4.4) and freeze-dried giving a dry/wet weight ratio of 0.279 which correlates well with values presented in Table 4.1 and published values by Maenhaut et al [MAE84] and that in ICRP-23 [ICR75]. Two 50mm diameter sub-samples were extracted from this slice and stored in polystyrene containers for the reasons given above. Sample 1 contains two visible large blood vessels whilst sample 2 a portion of fat which should be visible when imaged. Two samples of lung were prepared as described above, lung sample 1 being extracted from a slice of the lower lobe of the right lung, lung sample 2 from a slice of the lower lobe of the left lung, the slices being roughly 40mm in thickness. Dry/wet weight ratios were found to be 0.198 and 0.189 for lung sample 1 and lung sample 2, respectively, these agreeing well with data presented in section 5.2 (0.18±0.01). Both samples contain a number of large visible bronchi.

7.4 Biological sample tomographs

7.4.1 Fresh specimens

As previously stated, the fresh samples were contained within 20mm inner diameter polyethylene containers and ray-sum data were accumulated at 2mm intervals,
19 of these making up each projection. Using \( \pi/4 \) to predict the number of projections required for image reconstruction, this was found to be 15. Due to the 2mm detector and source collimation, good counting statistics for the 59.5 keV photopeak could be accumulated in a relatively short time (count rate approximately 120 s\(^{-1}\) through the centre of the sample) and hence in most cases ray-sum count times of 20 seconds were employed giving a total scan time of approximately 1.67 hrs. Tomographic scans at 17.8 keV energy could not be made due to the low transmission of these photons through the samples because data accumulation would be on a time scale too large and hence specimen degradation may occur.

Tomographical scans at 59.5 keV photon energy, of fresh kidney sample 1 and fat sample 2 may be viewed in Figure 7.5a and Figure 7.6a respectively. These images were constructed using photopeak data only, no subtraction of the scatter window counts being undertaken (see section 6.5.5). To ascertain the effect of scatter subtraction from photopeak data upon the tomographical images, reconstruction was performed with and without this process and the data from each were compared. Data for this comparison is presented in Table 7.1, mean linear attenuation coefficients and standard deviations being given for a rectangular region of interest. This region of interest is represented by two coordinates, one for the upper left hand corner and the other for the lower right hand corner, the top left hand pixel of the image taking the co-ordinate (1,1). It is evident, that there is negligible difference between the region of interest mean linear attenuation coefficients and standard deviations from images reconstructed with and without scatter correction. This is reiterated in the line scans through the images (Figure 7.5b and Figure 7.6b), and the photopeak to scatter ratios also presented in Table 7.1. Line scans were taken across the tomographs, through the centre of the specimens. The ratios were calculated to give a photopeak window to scatter window estimate of points external to the scanned samples and through the most attenuating position. The former ratio is derived by calculating an average ratio from ray-sums at the beginning and end of each projection (\( n=30 \) given the above scanning parameters) whilst the latter ratio from the ray-sum that passes through the centre of the scanned object (\( n=15 \)). It is predicted that little difference exists between transmission of photons through the sample and their detection by the HpGe detector for the photopeak and scatter windows i.e. the mean energy of the photopeak window is 59.5 keV whilst that of the scatter window is 59.1 keV. If a significant number of incoherently
Figure 7.5 Tomographical scan (a) at 59.5 keV of a porcine kidney sample and plot (b) of the linear attenuation coefficient across the scan, through the centre of the specimen. The pixel size is 2mm×2mm.
Figure 7.6 Tomographical scan at 59.5 keV (a) of a porcine adipose sample and plot (b) of the linear attenuation coefficient across the scan, through the centre of the specimen. The pixel size is 2mm×2mm.
Table 7.1 The region of interest mean and standard deviation of the linear attenuation coefficient in fresh kidney and fat for the images reconstructed with and without scatter subtraction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Region of interest</th>
<th>Linear attenuation coefficient m⁻¹ (µ±σₘ)</th>
<th>Photopeak/scatter ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No scatter subtraction</td>
<td>Scatter subtraction</td>
</tr>
<tr>
<td>Kidney sample 1</td>
<td>(8,7)-(13,13)</td>
<td>20.09±1.79</td>
<td>20.08±1.81</td>
</tr>
<tr>
<td></td>
<td>n=42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney sample 2</td>
<td>(6,8)-(13,13)</td>
<td>20.27±1.48</td>
<td>20.28±1.46</td>
</tr>
<tr>
<td></td>
<td>n=48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose sample 1</td>
<td>(8,8)-(14,14)</td>
<td>17.20±1.47</td>
<td>17.15±1.51</td>
</tr>
<tr>
<td></td>
<td>n=49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose sample 2</td>
<td>(7,7)-(13,13)</td>
<td>17.49±1.55</td>
<td>17.49±1.62</td>
</tr>
<tr>
<td></td>
<td>n=49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scattered photons are reaching the detector, we would expect the peak/scatter ratio to be less for raysums passing through the sample than photons that do not pass through the specimen at all. It may be seen that the two ratios are indifferentiable, and hence we may conclude that an insignificant number of incoherently scattered photons reach the detector which explains why no improvement in image quality is seen for scatter subtraction.

The tomographical image (Figure 7.5a), line scan (Figure 7.5b), and data presented in Table 7.1 highlight the significant variation of the linear attenuation coefficient in the kidney sample. This variation is a product of image noise and density and composition deviations throughout the specimen. However, note that the area of low attenuation in the lower right hand side of the image is due to the sample not completely filling the polyethylene container. The kidney sample consists of a portion of cortex and a portion of medulla. The medulla stretches from the vacant area within the container to a central position. This tissue is not discernible from the cortex although they differ in composition (see chapters 4 and 5) and density i.e. the cortex has density 1.049 g/cm³.
whilst medulla 1.044 g/cm³ in man [ICR75]. This is the same conclusion drawn by Curtis et al [CUR80]. However, the mean linear attenuation coefficient of this sample (20.09m⁻¹) compares well with data from the literature, values of 21.9m⁻¹ (experimental) [RAO75] and 21.5m⁻¹ (theoretical) [ICR92] at 60 keV being cited. Linear attenuation coefficients derived here may be slightly lower than literature values due to a smaller sample density, the mean value calculated from data in Table 7.1 and Table 7.12 being 0.97 g/cm³.

The variation of μ in the fat sample image (Figure 7.6a) is also significant, the line scan across the tomograph (Figure 7.6b) highlighting this effect. The mean linear attenuation coefficient of the region of interest though takes a value of 17.49m⁻¹ which correlates well with published data i.e. experimental value 18.8m⁻¹ [RAO75] and theoretical values 18.3m⁻¹ [WHI77] and 18.7m⁻¹ [ICR92].

Table 7.2 Linear attenuation coefficients at 59.5 keV photon energy at three positions upon the porcine kidney and adipose samples derived by the single point transmission method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative position (mm)</th>
<th>Linear attenuation coefficient μ (m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney 1</td>
<td>1</td>
<td>21.0±0.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>22.9±0.7</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>21.8±0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean = 22.2±0.7</td>
</tr>
<tr>
<td>Kidney 2</td>
<td>1</td>
<td>22.7±0.7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>21.9±0.7</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>22.3±0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean = 22.3±0.7</td>
</tr>
<tr>
<td>Adipose 1</td>
<td>1</td>
<td>18.7±0.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>18.0±0.6</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>18.5±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean = 18.4±0.6</td>
</tr>
<tr>
<td>Adipose 2</td>
<td>1</td>
<td>19.2±0.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>18.5±0.6</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>19.4±0.6</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>18.0±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean = 18.8±0.6</td>
</tr>
</tbody>
</table>
As the fresh samples were mostly present between the polyethylene container inner bottom surface and inner top surface (i.e. the lid) without the presence of areas not containing the specimens, kidney and adipose heights were fixed and could be measured to a high degree of accuracy i.e. \((2.53\pm0.05) \times 10^{-2}\) m as determined with a travelling microscope. Hence, with the containers with samples rotated through 90° such that container sides are parallel to the detector and source collimator bores (Figure 7.7), it is possible to obtain a measure of specimen linear attenuation coefficients by transmission of photons through the samples. The polyethylene containers with kidney and adipose specimens were mounted upon the scanning bed as described above, and the transmission of 1mm collimated 59.5 keV photons measured with the aid of a multi-channel analyser (MCA) at a number of positions. The initial photon intensity was derived by the placement of an identical empty polyethylene container in the gamma-ray beam. The linear attenuation coefficients were calculated using equation 6.1, photopeak area counts being employed in derivations.

![Diagram](image)

**Figure 7.7** The experimental set-up (side-view) used for single point linear attenuation coefficient measurements of the fresh biological samples.
A degree of variation exists between \( \mu \) values at the points upon each sample (Table 7.2), single point measurements being taken at an interval spacing of 1mm. These variations may be partly described by photon counting statistics (typically 1% standard deviation upon \( I \) and \( I_0 \)), but also heterogeneity of the samples themselves. Mean linear attenuation coefficients are relatively close to those presented in Table 7.1, although they are slightly elevated and bear more resemblance to the data given in the literature. The literature values are either derived using the single point photon transmission technique or theoretically from biological elemental compositions which do not take into account the inhomogeneous nature of these samples. A possible reason for \( \mu \) values derived by tomography being lower than those from the single point measurements is the averaging of the former over a larger volume of an essentially heterogeneous matrix.

### 7.4.2 Freeze-dried specimens

Freeze-dried samples of porcine kidney, liver and lung were analysed by photon transmission tomography at energies 17.8 keV and 59.5 keV. These were scanned in 50mm inner diameter air-tight polystyrene containers to inhibit specimen uptake of water so that total scanning times could be extended and good counting statistics obtained for each ray-sum. Two collimators were employed of different size i.e. 1mm and 2mm diameter, so that images could be reconstructed with 1mm\( \times \)1mm and 2mm\( \times \)2mm pixel dimensions. Using the smaller collimation, a typical number of ray-sums in a projection is 61, 50 projections employed at 3.6° angular spacing in all. Corresponding typical numbers of ray-sums for 2mm source and detector collimation are 31 in each of the 30 projections which are angularly spaced at 6°. Given that counting times for each ray-sum for the most attenuating sample (liver) with 1mm collimation and least attenuating sample (lung) with 2mm collimation are 120 seconds and 30 seconds respectively, this gives total scan times of approximately between 4.24 hrs and 7.75 days.

A 1mm\( \times \)1mm pixel size tomographical scan of freeze-dried kidney sample 2 at 59.5 keV photon energy is displayed in Figure 7.8a, which is accompanied with a line scan (Figure 7.8b) of the linear attenuation coefficient across the image through a position just lower than the centre of the sample that intersects the highly attenuating region. Initial features to note about Figure 7.8a are the high attenuating areas around the edge and within the sample. The former is attributed to the polystyrene container (\( \mu =19.6m^1 \))
Figure 7.8 Tomographical scan at 59.5 keV photon energy of freeze-dried porcine kidney sample 2 (a), and a line scan (b) across the image through the high attenuating area in the centre of the sample. The pixel size is 1mm×1mm.
Figure 7.9 Tomographical scan at 59.5 keV photon energy of freeze-dried porcine kidney sample 2 (a), and a line scan (b) across the image through the high attenuating area in the centre of the sample. The pixel size is 2mm×2mm.
[HUB82, WEA86] at 60 keV photon energy), which would not be easily identifiable if the kidney specimen were in a fresh state as seen in Figure 7.5. The area with high linear attenuation coefficient within the sample is due to the presence of sinus fat. This will be discussed in detail later. It may be seen from the image and line scan that the freeze-dried kidney sample is heterogeneous indeed. However, the modulation of $\mu$ across the sample is not solely due to the sample itself, but noise within the image which can be seen significant by observation of the area around the sample. This is probably due to the accumulation of insufficient counts for each ray-sum which was improved in the remaining tomographical scans of samples. Figure 7.8 may be compared to Figure 7.9 which is a scan of kidney sample 2 at the same energy (59.5 keV), but data accumulated employing 2mm source and detector collimation and reconstructed upon a 2mm x 2mm pixel scale. Note that because of the large collimation diameter employed, a high number of ray-sum counts may be accumulated and the image noise is less than that observed in Figure 7.8a [ALV79] (compare points external to the sample between tomographs). However, the spatial resolution of Figure 7.9a is inferior to that in Figure 7.8a, this being the most obvious when observing the pixels describing the polystyrene container. This is most apparent when comparing line scans (however, the object in the images are slightly oriented to one another), the container walls being sharper in Figure 7.8b, whilst the sample data in Figure 7.9b being smoother due to averaging of $\mu$ over larger volumes.

Figure 7.10a illustrates a tomographical scan (2mm x 2mm pixel size) of kidney sample 2, taken by the transmission of photons at 17.8 keV energy. Note that this scan is slightly orientated to that shown in Figure 7.9a. As expected the linear attenuation coefficients of the specimen are higher than those in the previous tomographs (compare the line scans, tomograph grey scales and the data presented in Table 7.3). This should make delineation of areas of similar density and composition easier [KOU82a]. This will be discussed in detail shortly. Again the area of the specimen containing the fat deposition is highlighted as a high photon attenuation region, as also is the polystyrene container. A large variation of the linear attenuation coefficient for the cortex and medulla is seen.

Tomographical scans at 59.5 keV photon energy using 1mm and 2mm source and detector collimation (1mm x 1mm and 2mm x 2mm reconstructed image pixel size respectively) and 17.8 keV photon using 2mm collimation were obtained for the two
Figure 7.10 Tomographical scan at 17.8 keV photon energy of freeze-dried porcine kidney sample 2 (a), and a line scan (b) across the image, through the high attenuating area in the centre of the sample. The pixel size is 2mm×2mm.
Figure 7.11 Example tomographical images of a) liver 1 (59.5 keV) and b) lung 1 (17.8 keV) with pixel dimensions of 2mmx2mm.
samples each of porcine liver and lung. The data corresponding to these scans is found in Table 7.3. Note that 17.8 keV photon energy tomographs with 1mm collimation could not be obtained due to the extremely low count rate for ray-sums.

Example tomographs of liver and lung samples may be found in Figure 7.11. Shown are images of liver sample 1 and lung sample 1, both with 2mm×2mm pixel dimensions, the former however being scanned at 59.5 keV photon energy whilst the latter at 17.8 keV. Comparison of the grey scales between the images reveals little difference of linear attenuation coefficient of the specimens even though they are taken at different energies. This may be ascribed to differences in density, that of lung being substantially lower than liver due to the presence of air. Features to note about the tomograph of liver (Figure 7.11a) is that the polystyrene container is easily discernible from the freeze-dried tissue and that a number of low photon attenuating areas exist. Those two areas in the volume of the sample are due to the hepatic blood supply whilst that near the container wall where the specimen did not fill the polystyrene vessel. Also, the fluctuation of the linear attenuation coefficient of the main liver tissue is smaller in comparison to the other tissues, a feature substantiated by the data in Table 7.3.

The polystyrene container is also easily identifiable in Figure 7.11b. The higher attenuating portion in the centre of the lung sample is due to the presence of a large bronchus, this attenuation occurring from its walls. Other areas of greater linear attenuation coefficient than from the surrounding material may be seen, these probably being from smaller bronchial formations.

The data presented in Table 7.3 gives an initial indication of the physical density fluctuations and elemental homogeneity of the studied specimens. Generally, the kidney and lung exhibit the greatest standard deviations upon mean values of linear attenuation coefficient, whilst liver the smallest. Large variations of μ for the kidney may be attributed to the cortex and medulla not being identifiable in images whilst for lung the presence of bronchial formations and alveoli. Mean linear attenuation coefficients agree well between samples of the same organ as also when using different collimation for their imaging. However greater standard deviations were observed for 1mm×1mm pixel sizes for the reasons described earlier. Values of μ for liver are higher than those for kidney and lung, the most probable reason being the larger hepatic tissue density. This will be covered in detail in the following sections.
Table 7.3 Tomographical scan parameters and linear attenuation coefficients for images obtained of lyophilised porcine kidney, liver and lung samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Energy (keV)</th>
<th>Pixel array</th>
<th>Region of interest</th>
<th>Co-ordinates and size</th>
<th>Mean linear attenuation coefficient m(^{-1}) ((\mu\pm\sigma_{n-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney sample 1</td>
<td>59.5</td>
<td>37×37</td>
<td>cortex/medulla</td>
<td>(15,12)-(23,16) n=45</td>
<td>3.93±0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sinus fat</td>
<td>(29,17)-(30,19) n=6</td>
<td>9.08±1.68</td>
</tr>
<tr>
<td>Kidney sample 2</td>
<td>59.5</td>
<td>59×59</td>
<td>cortex/medulla</td>
<td>(19,10)-(35,25) n=276</td>
<td>4.09±3.69</td>
</tr>
<tr>
<td></td>
<td>59.5</td>
<td>31×31</td>
<td>cortex/medulla</td>
<td>(10,6)-(19,11) n=60</td>
<td>4.04±1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sinus fat</td>
<td>(13-16)-(16,17) n=8</td>
<td>10.1±2.7</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>31×31</td>
<td>cortex/medulla</td>
<td>(9,6)-(18,10) n=50</td>
<td>25.1±3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sinus fat</td>
<td>(12,12)-(14,14) n=9</td>
<td>41.0±4.4</td>
</tr>
</tbody>
</table>

cont.
<table>
<thead>
<tr>
<th></th>
<th>59.5</th>
<th>61x61</th>
<th>1</th>
<th>Main hepatic tissue</th>
<th>(19,21)-(44,34)</th>
<th>7.08±1.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver sample 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=364</td>
<td></td>
</tr>
<tr>
<td>Liver sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(22,15)-(39,32)</td>
<td>7.60±2.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=324</td>
<td></td>
</tr>
<tr>
<td>Liver sample 1</td>
<td>17.8</td>
<td>31x31</td>
<td>2</td>
<td>Main hepatic tissue</td>
<td>(10,10)-(22,17)</td>
<td>6.82±0.60</td>
</tr>
<tr>
<td>Liver sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=104</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(11,10)-(20,17)</td>
<td>7.10±0.59</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>n=80</td>
<td></td>
</tr>
<tr>
<td>Lung sample 1</td>
<td>59.5</td>
<td>61x61</td>
<td>1</td>
<td>Main lung tissue</td>
<td>(23,17)-(39,30)</td>
<td>1.81±1.93</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=238</td>
<td></td>
</tr>
<tr>
<td>Lung sample 1</td>
<td>59.5</td>
<td>31x31</td>
<td>2</td>
<td>Main lung tissue</td>
<td>(11,11)-(20,15)</td>
<td>0.96±0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(12,9)-(21,16)</td>
<td>2.08±0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=80</td>
<td></td>
</tr>
<tr>
<td>Lung sample 1</td>
<td>17.8</td>
<td>31x31</td>
<td>2</td>
<td>Main lung tissue</td>
<td>(11,11)-(20,15)</td>
<td>7.21±2.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(12,9)-(21,16)</td>
<td>12.27±4.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n=80</td>
<td></td>
</tr>
</tbody>
</table>
7.4.3 Comparison of data from fresh and freeze-dried specimen tomographs

An immediate difference that may be identified between tomographs of samples scanned under fresh and freeze-dried conditions is that linear attenuation coefficients for kidney (cortex/medulla) are greater in the former state. This is obviously accredited to the volume of water present within the specimen (this accounts for roughly 83.3% of the wet weight). Also, the mean linear attenuation coefficient standard deviations for the freeze-dried samples are greater (between 15% and 30% for 2mm×2mm scans at 59.5 keV) than those for the fresh kidney samples (roughly 8% for 2mm×2mm scans at 59.5 keV). The main reason for this may be attributed to differences in density and elemental composition between the two states of sample. A portion of the standard deviation will be due to image noise, this however not being considered at this point. In a wet state, the renal cortex and medulla have different but similar densities [ICR75]. This is due to the tissues possessing different physiological functions, the medulla containing more water and hence its density being slightly lower than cortex. Chapter 4 and 5 have highlighted that differences in kidney composition are found depending upon the composite tissue studied. However, it is thought that these differences (seen as large μ standard deviations) are more apparent in tomographs of freeze-dried samples than fresh samples due to the far lower density of the specimens found in the latter. That is, it is easier to discern differences of linear attenuation coefficient due to small quantities of elements in a matrix as the photon attenuation from that matrix is decreased [KOU81]. Also, the difference in density between the cortex and medulla in the freeze-dried state is probably greater.

A comparison of linear attenuation coefficients of fat and soft tissue in fresh and freeze-dried states highlights that larger differences are found for the latter sample preparation procedure. This is due to the substantially lower water content of adipose than soft tissue [WHI91], and hence the elemental composition and density of fat is unchanged to a lesser extent when freeze-dried. Therefore, the linear attenuation coefficient of adipose tissue is altered to a small degree when lyophilised, and differences between surrounding soft tissue is seen to be great. This was observed in the tomographs of the kidney sample (Figures 7.8, 7.9 and 7.10), the area of fat displayed as a higher photon attenuating region. This has implications when identifying areas of adipose tissue in soft tissue, fat being more difficult to discern when samples are in a fresh state.
7.5 Heterogeneity of scanned biological samples

Mean linear attenuation coefficients and standard deviations for specific regions of interest in each of the biological sample tomographs are presented in Table 7.3. The standard deviations consist of contributions from image noise and elemental and density fluctuations of the scanned specimens. It is the aim of this section to investigate if a measure of elemental heterogeneity may be derived from the regions of interest from biological sample tomographical scans.

Theoretical mass attenuation coefficients of the studied samples may be calculated with a knowledge of their elemental composition [BRO80]. These compositions are presented in Table 7.4, data covering the tissues liver (liver 2 in chapter 4), lung and bronchi (see chapter 5). Kidney will be considered in the latter stages of this section. Elemental concentration data is derived by Rutherford backscattering analysis (RBS) for atomic numbers 6-20, whilst proton induced X-ray emission (PIXE) analysis 17 upwards and hence there is an overlap of data. A full description of theoretical and experimental aspects of these techniques may be found in chapters 2, 3, 4 and 5. It may be noted that no data is presented for hydrogen in the biological samples. This is because this element could not be studied by either of the above mentioned analytical techniques [KHA81a]. The hydrogen contents of the freeze-dried specimens were estimated by the initial assumption that liver, lung and bronchi contain 10% H wet weight [WHI91,ICR75]. Given the dry/wet weight sample ratios for liver (0.278), lung (0.18) and bronchi (0.22) from chapter 4 and chapter 5, it was possible to calculate the approximate dry weight hydrogen content in each specimen, these being 6.9%, 4.6% and 5.8% respectively. The effect of the introduction of this element into the matrix composition upon sample mass attenuation coefficient is investigated and results shown in Table 7.5.

Concentrating upon Table 7.4, although there is a large variation of trace element content between tissues, differences are relatively small when comparing major element compositions (mostly denoted by *). This may be an initial indicator that the differences in linear attenuation coefficient between specimens is a product of density dissimilarity. Values contained within Table 7.4 are mean concentrations which are accompanied with their standard deviations. As discussed in chapter 4 and chapter 5, these standard deviations will be dependent upon the mass employed to derive them, these being 1.28g and 92±12|ig for liver and lung respectively. The large difference in mass is due
to the variation in analysis approach between the two samples. A number of features may be noted from Table 7.4, namely the presentation of two concentrations for K and P and S compositions given as one summed value. The element edges for P and S in the RBS spectra could not be resolved and hence only their approximate summed composition derived. As for potassium, the composition from this element could be calculated using either techniques, however, the value from PIXE analysis is probably the most accurate. The above sample compositions were used to calculate the mass attenuation coefficients by the mixture rule [KOU78] at energies 59.5 keV and 17.8 keV and results presented in Table 7.5.

Table 7.4 Major and trace element compositions (ppm dry weight) of liver and lung derived by PIXE and RBS analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>Liver (n=13)</th>
<th>Lung (n=59)</th>
<th>Bronchi (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.584±0.013*</td>
<td>0.583±0.016*</td>
<td>0.585±0.006*</td>
</tr>
<tr>
<td>N</td>
<td>0.122±0.010*</td>
<td>0.136±0.014*</td>
<td>0.148±0.006*</td>
</tr>
<tr>
<td>O</td>
<td>0.225±0.009*</td>
<td>0.177±0.012*</td>
<td>0.186±0.004*</td>
</tr>
<tr>
<td>Na</td>
<td>0.011±0.007*</td>
<td>0.025±0.013*</td>
<td>0.012±0.006*</td>
</tr>
<tr>
<td>P/S</td>
<td>0.039±0.008*</td>
<td>0.035±0.013*</td>
<td>0.028±0.005*</td>
</tr>
<tr>
<td>Cl</td>
<td>0.002±0.001</td>
<td>0.007±0.003</td>
<td>0.008±0.001</td>
</tr>
<tr>
<td>K</td>
<td>0.016±0.003*</td>
<td>0.037±0.012*</td>
<td>0.033±0.002*</td>
</tr>
<tr>
<td>Ca</td>
<td>(1.83±0.15) 10^-5</td>
<td>(3.7±1.1) 10^-4</td>
<td>(6.64±1.34) 10^-4</td>
</tr>
<tr>
<td>Mn</td>
<td>(9.9±1.4) 10^-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>(6.84±0.22) 10^-6</td>
<td>(2.0±0.6) 10^-4</td>
<td>(5.63±0.70) 10^-5</td>
</tr>
<tr>
<td>Cu</td>
<td>(1.96±0.14) 10^-5</td>
<td>(4.3±1.2) 10^-6</td>
<td>(6.6±4.4) 10^-6</td>
</tr>
<tr>
<td>Zn</td>
<td>(2.24±0.05) 10^-6</td>
<td>(8.0±1.6) 10^-6</td>
<td>(5.20±0.24) 10^-5</td>
</tr>
<tr>
<td>Br</td>
<td>(7.0±1.9) 10^-6</td>
<td>(2.0±0.6) 10^-5</td>
<td>(1.76±0.48) 10^-5</td>
</tr>
<tr>
<td>Rb</td>
<td>(1.74±0.34) 10^-6</td>
<td>(1.6±0.4) 10^-5</td>
<td>(3.7±5.9) 10^-6</td>
</tr>
</tbody>
</table>

Values derived by PIXE apart from * by RBS analysis
A number of mass attenuation coefficients are given for each tissue, these representing values derived from the matrix composition (C, N, O, Na, P/S, Cl and K) presented in Table 7.4, the adjusted matrix composition with an estimated hydrogen content, and the matrix and hydrogen composition with trace element contributions. Values for liver and lung are also included which are derived from the data presented by White et al [WHI91]. Initial observations about the data contained within Table 7.5 are that mass attenuation coefficients differ more greatly at the lower photon energy and also calculated theoretical values do not correlate with literature derived coefficients as closely as one would expect. The former feature is expected [KOU81] whilst the latter is not, however matrix data that has been adjusted for an estimated hydrogen content compares

Table 7.5 Theoretical mass attenuation coefficients for liver, lung and bronchi at 17.8 keV and 59.5 keV and their ratios compared to experimentally derived values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Theoretical μ/ρ (m²/kg)</th>
<th>Experiment ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17.8 keV</td>
<td>59.5 keV</td>
</tr>
<tr>
<td>Liver</td>
<td>Matrix</td>
<td>1.263 10⁻¹</td>
<td>1.940 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>1.203 10⁻¹</td>
<td>2.031 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H+TE</td>
<td>1.243 10⁻¹</td>
<td>2.044 10⁻²</td>
</tr>
<tr>
<td></td>
<td>Literature</td>
<td>1.135 10⁻¹</td>
<td>2.027 10⁻²</td>
</tr>
<tr>
<td>Lung tissue</td>
<td>Matrix</td>
<td>1.600 10⁻¹</td>
<td>2.028 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>1.540 10⁻¹</td>
<td>2.084 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H+TE</td>
<td>1.559 10⁻¹</td>
<td>2.090 10⁻²</td>
</tr>
<tr>
<td></td>
<td>Literature</td>
<td>1.197 10⁻¹</td>
<td>2.025 10⁻²</td>
</tr>
<tr>
<td>Bronchi</td>
<td>Matrix</td>
<td>1.482 10⁻¹</td>
<td>1.998 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>1.419 10⁻¹</td>
<td>2.072 10⁻²</td>
</tr>
<tr>
<td></td>
<td>H+TE</td>
<td>1.436 10⁻¹</td>
<td>2.077 10⁻²</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>CH₅</td>
<td>5.384 10⁻²</td>
<td>1.871 10⁻²</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H - Mass attenuation coefficients calculated with estimated hydrogen concentration.
H+TE - Mass attenuation coefficients calculated with estimated hydrogen concentration and trace element content.
Table 7.6 Comparison of the matrix composition derived by RBS analysis with estimated H content and literature concentrations in liver.

<table>
<thead>
<tr>
<th>Element</th>
<th>Composition (proportion by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBS derived</td>
</tr>
<tr>
<td>H</td>
<td>0.069</td>
</tr>
<tr>
<td>C</td>
<td>0.544</td>
</tr>
<tr>
<td>N</td>
<td>0.114</td>
</tr>
<tr>
<td>O</td>
<td>0.209</td>
</tr>
<tr>
<td>Na</td>
<td>0.010</td>
</tr>
<tr>
<td>P/S</td>
<td>0.036</td>
</tr>
<tr>
<td>Cl</td>
<td>0.002</td>
</tr>
<tr>
<td>K</td>
<td>0.015</td>
</tr>
</tbody>
</table>

more favourably with that presented by White et al [WHI91] (see Table 7.6). We may see the greater dissimilarities occur for the higher Z matrix elements. There are a number of reasons for this, the most significant being the relatively small number of counts accumulated in their edges in RBS spectra. Also, for P and S, their backscattered proton signals were not resolvable and hence an estimate of their summed compositions could only be made. This involved the assumption that all counts in this edge belonged to one element only (P in this case), and hence the proton backscattering cross-section for this element employed in matrix composition calculations. The mass attenuation coefficients for P were used in photon attenuation calculations. Variations in major element compositions between RBS derived values and literature derived values may also occur because of the differences in mammalian species studied i.e. pig and man respectively, however these differences are thought to be small [BOW66].

It can be seen that significant differences in mass attenuation occur when taking into account the hydrogen content of biological specimens and also their trace element contents. Hence when requiring to calculate accurate values of these coefficients, contributions from H and trace elements must be taken into account.
The density of the scanned biological samples dictate largely the linear attenuation coefficients measured and are one source of \( \mu \) variation in tomographs. Mean density values may be calculated using the mass attenuation coefficients presented in Table 7.5 (2mmx2mm pixel size for each sample) and the linear attenuation coefficients presented in Table 7.3. These densities are given in Table 7.7, values being shown for a number of matrices at 17.8 keV and 59.5 keV in liver, lung, bronchi and polystyrene. Bronchial and polystyrene densities are based upon measured linear attenuation coefficients at 17.8 keV and 59.5 keV of 28.75±5.09m\(^{-1}\) and 5.10±0.85m\(^{-1}\) \( (n=14) \) and 35.18±3.81m\(^{-1}\) and 11.20±1.34m\(^{-1}\) \( (n=15) \) respectively.

The density of lung tissue is by far the smallest of the studied tissues, and explains why the linear attenuation coefficient of this organ is low in comparison to liver and bronchi given their relatively similar elemental compositions. The lung also exhibits the greatest fluctuation of \( \rho \), this partly being ascribed to bronchial formations within the tissue, these are illustrated in the tomographical scans. Image noise also affects the degree of fluctuation. The variation of \( \rho \) within the liver is relatively small implying the relatively constant physiological form of this tissue. Densities are presented for bronchi and

<table>
<thead>
<tr>
<th>Sample</th>
<th>Matrix description</th>
<th>Density (kg/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17.8 keV</td>
</tr>
<tr>
<td>Liver</td>
<td>H+TE</td>
<td>307,322</td>
</tr>
<tr>
<td></td>
<td>Literature</td>
<td>337,353</td>
</tr>
<tr>
<td>Lung</td>
<td>H+TE</td>
<td>46,79</td>
</tr>
<tr>
<td></td>
<td>Literature</td>
<td>60,103</td>
</tr>
<tr>
<td>Bronchi</td>
<td>H+TE</td>
<td>200</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>CH(_3)</td>
<td>653</td>
</tr>
</tbody>
</table>

Table 7.7 Densities of various specimens calculated using mass and linear attenuation coefficients from Table 7.3 and Table 7.5.
Figure 7.12 Tomograph of the data acquired from the division of the 17.8 keV scan by 59.5 keV scan for liver sample 1 (a), and a plot of the ratios across the image through the centre of the object (b).
polystyrene, an accepted value for the latter being 1050kg/m$^3$ [WEA86]. The figure presented in Table 7.7 is somewhat smaller than that given above, the reason being the averaging of $\mu$ over the surrounding air, polystyrene container and lung specimen. It is thought that $\rho$ for bronchi is underestimated due to the same effect.

Densities presented in Table 7.7 are mean values and $\rho$ is expected to vary throughout the biological specimens, the degree being dependent upon the origin of the tissue extraction. Without a knowledge of this variation, it is impossible to estimate the fluctuation of the linear attenuation coefficient which is solely accountable to major and trace element contents. As it is difficult, if not impossible to evaluate the variance of $\rho$ throughout the samples, another method of relaxing the density dependence in tomographs is required. This is achieved by the division of data from 17.8 keV scans by that from 59.5 keV scans, the tomographs obviously being obtained under identical circumstances.

One such scan is illustrated in Figure 7.12a, which has been obtained from the division of the 17.8 keV tomograph of liver sample 1 by its 59.5 keV counterpart. Note now that the grey scale is no longer in units m$^{-1}$, but a ratio which covers values from under 2 to greater than 6. The image has been enhanced to a minor extent, values of 20m$^{-1}$ in the 17.8 keV scan set to zero in order to avoid spurious signals in the background surrounding the sample and to highlight the features seen in Figure 7.11. The data describing the liver tissue is unchanged from its raw values. A feature to note about Figure 7.12a is that the polystyrene container is no longer seen as a high photon attenuating region around the sample. Rather, it takes a ratio (3.17±0.40) that is smaller than that of the liver tissue which has an average value of 5.66 (see Table 7.5). The experimental ratio for polystyrene is slightly higher than the theoretical value, the probable reason being the averaging effect which was discussed earlier.

Table 7.5 contains the tomograph ratio data, two values presented for each tissue type represent sample 1 and sample 2 respectively. These mean values with their standard deviations are taken over the regions of interest listed in Table 7.3. The ratios may be compared to theoretical values which are derived using the differing matrix compositions discussed already. It may be seen that for liver, the theoretical ratios derived from the literature [WHI91] and derived by RBS with adjustment for hydrogen content (H), compare best to experimental ratios. The variation of the ratio across the tomograph (Figure 7.12a) may be seen in the line scan (Figure 7.12b) across the image, the standard
Figure 7.13 Density relaxed tomographs of lung sample 1 with a) no image enhancement and b) image enhancement.
deviations included in Table 7.5 giving a quantitative measure of this fluctuation although this does not only represent elemental variances but comprises partly of noise.

In an attempt to estimate the degree of linear attenuation fluctuation in images which may be attributed to photon counting statistics (main contribution to image noise), equation 6.5 was employed in a slightly modified form [Gil84] which is given in equation 7.1 and assumes $m = \frac{n\pi}{4}$ rather than $n\pi/2$ (see section 6.4 for definitions).

$$\Delta \mu = k \left( \frac{4\pi}{dcD} \right)^{\frac{1}{2}}$$

(Eq. 7.1)

The factor D is the dimension of the reconstructed object, whereas k a factor which is dependent upon the reconstruction algorithm employed ($k = 0.289$). However, investigation by Folkard [Fol83] showed the above value of k to overestimate pixel noise and $k = 0.16$ recommended instead.

The estimated $\mu$ variations in tomographs for liver and lung samples are presented in Table 7.8 and these are compared to total standard deviations from the regions of interest listed in Table 7.3. Note that these are estimated variations due to equation 7.1 assuming constant counts for each ray-sum, the tomography carried out in this work employing constant time criteria. However, as a consequence of low photon attenuation in samples due to their low Z composition and densities, relative standard deviations associated with ray-sum counts did not alter significantly.

It may be seen from Table 7.8 that estimated and measured percentage $\mu$ deviations for liver correlate relatively closely, thus implying that noise is a major contributor to linear attenuation fluctuations in these tomographs. This noise is transferred to the density relaxed tomographs, ratio standard deviations being 10.4% and 11.1% for liver sample 1 and liver sample 2 respectively. It is difficult to predict the consequences of image division upon noise, however this is probably reduced due to averaging effects.

The predicted relative noise contributions to the lung tomographs is significantly less than those for the liver images. Hence, it is expected a large proportion of the total image noise is due to density and composition heterogeneities, the former being the greatest source of variation. This was tested by obtaining density relaxed tomographs of
Table 7.8 Predicted and total measured linear attenuation coefficient variations in the 2mm×2mm pixel size tomographs of the liver and lung samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Energy (keV)</th>
<th>Ray-sum counts</th>
<th>( \Delta \mu )</th>
<th>( \Delta \mu / \mu ) (%)</th>
<th>Experiment SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver 1</td>
<td>17.8</td>
<td>400</td>
<td>3.253 ( \times 10^3 )</td>
<td>6.7</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>59.5</td>
<td>11000</td>
<td>6.203 ( \times 10^3 )</td>
<td>7.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Liver 2</td>
<td>17.8</td>
<td>500</td>
<td>2.909 ( \times 10^3 )</td>
<td>5.7</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>59.5</td>
<td>11000</td>
<td>6.203 ( \times 10^3 )</td>
<td>6.8</td>
<td>8.3</td>
</tr>
<tr>
<td>Lung 1</td>
<td>17.8</td>
<td>1500</td>
<td>1.680 ( \times 10^5 )</td>
<td>18.2</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td>59.5</td>
<td>14000</td>
<td>5.498 ( \times 10^4 )</td>
<td>44.9</td>
<td>63.5</td>
</tr>
<tr>
<td>Lung 2</td>
<td>17.8</td>
<td>900</td>
<td>2.168 ( \times 10^3 )</td>
<td>13.9</td>
<td>39.9</td>
</tr>
<tr>
<td></td>
<td>59.5</td>
<td>13000</td>
<td>5.706 ( \times 10^4 )</td>
<td>21.5</td>
<td>44.7</td>
</tr>
</tbody>
</table>

the lung samples, an example being shown in Figure 7.13a, this not being exposed to any form of image enhancement as was performed in Figure 7.12. Again, the polystyrene container is seen as a lower photon attenuating regions, however the bronchial formations present within Figure 7.11b are no longer seen, this re-iterating the conclusion that differences between its linear attenuation coefficient and that of the surrounding lung tissue are due to the higher density of the former. This is highlighted by comparison of the experimental and theoretical ratios for the two tissues given in Table 7.5.

Unfortunately the linear attenuation coefficient of the lung tissue does not vary considerably from the background image noise, and hence it was impossible to enhance Figure 7.13a with the results obtained for liver in Figure 7.12. Rather a misleading density relaxed tomograph is obtained (Figure 7.13b), which implies the bronchi have largely differing composition to the lung tissue which is known to be untrue (see Tables 7.4 and 7.5). This was obtained by setting \( \mu \) values of less than 20m\(^{-1}\) to zero in the 17.8 keV scan whilst \( \mu \) values of less than 3m\(^{-1}\) to unity in the 59.5 keV scan. However, this image serves to highlight these structures as well as showing the lower ratio of the photon attenuations in polystyrene rather than bronchi.
Although Table 7.4 shows elemental variations in liver (observe standard deviations), and differences in concentrations to occur between lung and bronchi which should be exhibited in density relaxed tomographs (compare theoretical ratio values in Table 7.5), it has been demonstrated that differences will generally not be observed due to fluctuations in the images from noise. In normal tomographs, density variations will also be a source of fluctuations to linear attenuation coefficients. However, it is not yet

![Diagram showing extraction sites of samples](image)

**Figure 7.14** Schematic representing the extraction sites of the samples which were tomographically scanned and utilised in sub-sample selection for analysis by RBS and PIXE.

It is not yet clear if different component tissues of freeze-dried kidney samples may be delineated due to the large difference in trace and minor element contents as was identified in chapter 4 and chapter 5. This will be discussed next.

As presented earlier, the tomographs obtained of the lyophilised porcine kidney samples were employed in the selection of sub-samples for trace, minor and major element analysis. The positions from where the two circular samples were extracted from the kidney slice is illustrated in Figure 7.14. Sample 2 is that illustrated in the tomographs in Figure 7.8a, 7.9a and 7.10a, and the positions of the 11 sub-samples shown which were

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each approximately 125mm³ in volume, this equating to a mass of roughly 0.03g. Sub-samples 1-7 were extracted along a line which is directly across Figure 7.9a, and intersects the fat deposits near the left hand side polyethylene container wall (sub-sample 1) and in the centre (sub-samples 3 and 4). The remaining sub-samples (8-11) are extracted in a direction orthogonal to this stretching from near the adipose in the centre (sub-sample 8) to near the lower outer portion of the sample (sub-sample 11). The approximate anatomical composition of each sub-sample is listed in Table 7.9, these being recorded prior to preparation for PIXE and RBS analysis (see section 4.4 for preparation procedures).

Table 7.9 Approximate anatomical descriptions of the sub-samples from kidney specimen 2 which were chosen with the aid of tomography for analysis by PIXE and RBS.

<table>
<thead>
<tr>
<th>Sub-sample</th>
<th>Anatomical description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Medulla and sinus fat (from inner renal pelvis).</td>
</tr>
<tr>
<td>2</td>
<td>Medulla, cortex and sinus fat.</td>
</tr>
<tr>
<td>3</td>
<td>Medulla and sinus fat.</td>
</tr>
<tr>
<td>4</td>
<td>Medulla and large portion of sinus fat.</td>
</tr>
<tr>
<td>5</td>
<td>Cortex and medulla with small portion of sinus fat.</td>
</tr>
<tr>
<td>6</td>
<td>Very small portion of fat with roughly 50% cortex, 50% medulla.</td>
</tr>
<tr>
<td>7</td>
<td>Composition as sample 6.</td>
</tr>
<tr>
<td>8</td>
<td>Medulla with small portion of sinus fat.</td>
</tr>
<tr>
<td>9</td>
<td>100% medulla.</td>
</tr>
<tr>
<td>10</td>
<td>75% cortex, 25% medulla (juxtamedullary region).</td>
</tr>
<tr>
<td>11</td>
<td>100% cortex (subscapular)</td>
</tr>
</tbody>
</table>
Initially it was investigated if differences in mass attenuation coefficient could be detected when comparing cortex and medulla for freeze-dried specimens. As can be seen from Table 7.9, sub-samples are essentially a mixture of composite kidney tissues.

Table 7.10 Dry weight elemental compositions of various portions of porcine kidney derived from PIXE and RBS analysis and taken from the literature.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cortex (n=13)</th>
<th>Medulla (n=8)</th>
<th>Whole (n=17)</th>
<th>Literature [WHI91]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0.052</td>
<td>0.052</td>
<td>0.052</td>
<td>0.062</td>
</tr>
<tr>
<td>C</td>
<td>0.593±0.012*</td>
<td>0.562±0.022*</td>
<td>0.584±0.020*</td>
<td>0.574</td>
</tr>
<tr>
<td>N</td>
<td>0.106±0.009*</td>
<td>0.127±0.010*</td>
<td>0.110±0.012*</td>
<td>0.130</td>
</tr>
<tr>
<td>O</td>
<td>0.146±0.010*</td>
<td>0.156±0.009*</td>
<td>0.149±0.011*</td>
<td>0.190</td>
</tr>
<tr>
<td>Na</td>
<td>0.018±0.009*</td>
<td>0.026±0.009*</td>
<td>0.021±0.011*</td>
<td>0.009</td>
</tr>
<tr>
<td>P/S</td>
<td>0.033±0.006*</td>
<td>0.027±0.008*</td>
<td>0.032±0.006*</td>
<td>0.017</td>
</tr>
<tr>
<td>Cl</td>
<td>0.014±0.004*</td>
<td>0.009±0.011*</td>
<td>0.012±0.006*</td>
<td>0.009</td>
</tr>
<tr>
<td>K</td>
<td>0.038±0.008*</td>
<td>0.039±0.007*</td>
<td>0.039±0.007*</td>
<td>0.009</td>
</tr>
<tr>
<td>Ca</td>
<td>(4.56±1.74)×10^4</td>
<td>(2.38±0.73)×10^4</td>
<td>(3.89±1.91)×10^4</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>(3.2±0.4)×10^4</td>
<td>(1.1±1.0)×10^4</td>
<td>(2.8±1.0)×10^6</td>
<td>-</td>
</tr>
<tr>
<td>Fe</td>
<td>(1.69±0.53)×10^4</td>
<td>(1.47±0.38)×10^4</td>
<td>(1.70±0.46)×10^4</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>(3.1±0.6)×10^4</td>
<td>(1.2±0.6)×10^5</td>
<td>(2.7±0.9)×10^5</td>
<td>-</td>
</tr>
<tr>
<td>Zn</td>
<td>(9.9±1.8)×10^4</td>
<td>(6.1±0.5)×10^5</td>
<td>(8.9±2.4)×10^5</td>
<td>-</td>
</tr>
<tr>
<td>Br</td>
<td>(1.1±0.2)×10^4</td>
<td>(1.8±0.8)×10^4</td>
<td>(1.3±0.4)×10^5</td>
<td>-</td>
</tr>
<tr>
<td>Rb</td>
<td>(2.3±0.5)×10^5</td>
<td>(2.1±1.9)×10^5</td>
<td>(2.6±0.8)×10^5</td>
<td>-</td>
</tr>
</tbody>
</table>

Values derived by PIXE apart from * by RBS analysis.
and hence this data could not be employed in this investigation. However, RBS and PIXE data from chapter 5 could be utilised due to the nature of the samples studied and elemental compositions are available for cortex and medulla individually. This data is presented in Table 7.10, notice that values for whole kidney are also included. These were derived following the criteria stated by Kratochvil and Taylor [KRA81] that a ratio of sampling positions in cortex and medulla is equal to the mass proportions of these tissues i.e. 0.7 [ICR75] (see section 5.6.3). Again, the tissues were assumed 10% hydrogen wet weight and thus given the specimen dry/wet weight ratio of 0.2 (see section 5.2), the dry sample H content was estimated. It should be noted that the proportion of water attributed to the wet weight of the sample is an average value, and it is expected that the medulla contains more water than the cortex [WHI91].

Table 7.10 highlights that differences in elemental concentration between cortex and medulla occur even for the major elements. This implies that these tissues may be differentiated in tomographs due to their differing linear attenuation coefficients. However, note that elemental concentrations in whole kidney do not correlate as well as those for liver (see Table 7.6) when compared to literature values. The reasons for dissimilarities have been discussed earlier, although an added source may originate from the anatomical heterogeneous nature of the kidney specimens and in particular surface roughness of samples [EDG80] (see section 5.4). When converted to wet weight, matrix element concentrations compare more favourably to literature values. Again, one should be aware that errors in derived compositions may arise from the cortex and medulla possessing different water contents, an average value only being applied to this conversion. Differences in compositions between renal cortex and medulla are less when samples are in a fresh state, and hence these tissues should be more difficult to differentiate in tomographs in this state.

Data from Table 7.10 and Table 7.11 were employed to calculate total mass attenuation coefficients at energies 17.8 keV and 59.5 keV (Table 7.12). As previous, this was done by the mixture rule [KOU78], utilising element mass attenuation coefficients from Storm and Israel [STO70] which were interpolated to obtain the energy of interest. Two compositions were considered for tissues in a particular state, one concentrating solely upon the main matrix elements (H) whilst the other, including trace element levels (H+TE) in calculations. Variations are seen between the two matrices considered, their
Table 7.11 Wet weight elemental compositions of various portions of porcine kidney derived from RBS and PIXE analysis and taken from the literature.

<table>
<thead>
<tr>
<th>Element</th>
<th>Tissue</th>
<th>Literature [WHI91]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortex (n=13)</td>
<td>Medulla (n=8)</td>
</tr>
<tr>
<td>H</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>C</td>
<td>0.119±0.002*</td>
<td>0.112±0.004*</td>
</tr>
<tr>
<td>N</td>
<td>0.021±0.002*</td>
<td>0.025±0.002*</td>
</tr>
<tr>
<td>O</td>
<td>0.740±0.051*</td>
<td>0.742±0.043*</td>
</tr>
<tr>
<td>Na</td>
<td>0.004±0.002*</td>
<td>0.005±0.002*</td>
</tr>
<tr>
<td>P/S</td>
<td>0.007±0.001*</td>
<td>0.005±0.001*</td>
</tr>
<tr>
<td>Cl</td>
<td>0.003±0.001*</td>
<td>0.002±0.002*</td>
</tr>
<tr>
<td>K</td>
<td>0.008±0.002*</td>
<td>0.008±0.001*</td>
</tr>
<tr>
<td>Ca</td>
<td>(9.12±3.48) $10^{-5}$</td>
<td>(4.76±0.15) $10^{-5}$</td>
</tr>
<tr>
<td>Mn</td>
<td>(6.4±0.8) $10^{-7}$</td>
<td>(2.2±2.0) $10^{-7}$</td>
</tr>
<tr>
<td>Fe</td>
<td>(3.38±1.06) $10^{-5}$</td>
<td>(2.94±0.76) $10^{-5}$</td>
</tr>
<tr>
<td>Cu</td>
<td>(6.2±1.2) $10^{-6}$</td>
<td>(2.4±1.2) $10^{-6}$</td>
</tr>
<tr>
<td>Zn</td>
<td>(2.0±0.4) $10^{-5}$</td>
<td>(1.2±0.1) $10^{-5}$</td>
</tr>
<tr>
<td>Br</td>
<td>(2.2±0.4) $10^{-5}$</td>
<td>(3.6±1.6) $10^{-6}$</td>
</tr>
<tr>
<td>Rb</td>
<td>(4.6±1.0) $10^{-6}$</td>
<td>(4.2±3.8) $10^{-6}$</td>
</tr>
</tbody>
</table>

degree being a function of photon energy and tissue state. Largest differences are seen at the lower energy with samples in a dry condition. This also applies when comparing mass attenuation coefficients between cortex and medulla.

Concentrating upon fresh weight kidney, very little difference for $\mu/p$ coefficients exists between cortex and medulla at 59.5 keV photon energy. Greater differences occur at 17.8 keV, the option of tomographically scanning samples at this
energy however not being available (see section 7.4.1). Assuming densities of cortex and medulla to be 1.049g/cm$^3$ and 1.044g/cm$^3$ respectively [ICR75], linear attenuation coefficients at 59.5 keV are calculated to be 21.8m$^{-1}$ and 21.6m$^{-1}$ (using $\mu/p$ data for H+TE matrix in Table 7.12). These compare well to data presented in Table 7.1 and the literature values from Rao and Gregg [ROA75] and ICRU publication 46 [ICR92]. Linear attenuation coefficients between cortex and medulla differ only to a small degree (about 1%) and given the estimated noise to be 8.5% (from equation 7.1) in the fresh kidney sample scans, it is no great surprise that the two tissues are not discernible by tomography. This is the same conclusion drawn by Curtis et al [CUR80].

Mass attenuation coefficients for cortex and medulla in freeze-dried conditions differ to a larger extent than those for fresh specimens, values at 17.8 keV energy producing the most pronounced effect. This coupled with the expected differences in density of the two tissues may enhance the variations in linear attenuation coefficient further.

Table 7.12 Calculated mass attenuation coefficients at 17.8 keV and 59.5 keV of renal cortex and medulla, elemental data being extracted from Table 7.10 and Table 7.11.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sample state</th>
<th>Matrix</th>
<th>Calculated $\mu/p$ (m$^2$/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>fresh</td>
<td>H</td>
<td>1.186 10$^{-1}$</td>
</tr>
<tr>
<td>Cortex</td>
<td>fresh</td>
<td>H+TE</td>
<td>1.190 10$^{-1}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>fresh</td>
<td>H</td>
<td>1.168 10$^{-1}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>fresh</td>
<td>H+TE</td>
<td>1.171 10$^{-1}$</td>
</tr>
<tr>
<td>Cortex</td>
<td>dry</td>
<td>H</td>
<td>1.620 10$^{-1}$</td>
</tr>
<tr>
<td>Cortex</td>
<td>dry</td>
<td>H+TE</td>
<td>1.641 10$^{-1}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>dry</td>
<td>H</td>
<td>1.574 10$^{-1}$</td>
</tr>
<tr>
<td>Medulla</td>
<td>dry</td>
<td>H+TE</td>
<td>1.589 10$^{-1}$</td>
</tr>
</tbody>
</table>

H-Mass attenuation coefficients calculated with estimated hydrogen content
H+TE-Mass attenuation coefficients calculated with estimated hydrogen content and trace element contributions.
Table 7.13 Calculated and measured photon attenuation coefficients of the eleven sub-samples extracted from freeze-dried porcine kidney.

<table>
<thead>
<tr>
<th>Sub-sample</th>
<th>Theoretical $\mu/\rho$ (m$^{2}$/kg)</th>
<th>Measured $\mu$ (m$^{-1}$)</th>
<th>Mean density $\rho$ (kg/m$^3$)</th>
<th>Attenuation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.8 keV</td>
<td>59.5 keV</td>
<td>17.8 keV</td>
<td>59.5 keV</td>
</tr>
<tr>
<td>1</td>
<td>$1.803 \times 10^{-1}$</td>
<td>$2.163 \times 10^{-2}$</td>
<td>$29.02 \pm 6.11$</td>
<td>$5.02 \pm 3.29$</td>
</tr>
<tr>
<td>2</td>
<td>$1.507 \times 10^{-1}$</td>
<td>$2.084 \times 10^{-2}$</td>
<td>$23.52 \pm 5.01$</td>
<td>$4.63 \pm 2.20$</td>
</tr>
<tr>
<td>3</td>
<td>$1.517 \times 10^{-1}$</td>
<td>$2.087 \times 10^{-2}$</td>
<td>$31.92 \pm 8.55$</td>
<td>$7.13 \pm 3.70$</td>
</tr>
<tr>
<td>4</td>
<td>$1.392 \times 10^{-1}$</td>
<td>$2.053 \times 10^{-2}$</td>
<td>$32.74 \pm 7.26$</td>
<td>$4.69 \pm 2.56$</td>
</tr>
<tr>
<td>5</td>
<td>$1.479 \times 10^{-1}$</td>
<td>$2.078 \times 10^{-2}$</td>
<td>$25.89 \pm 5.29$</td>
<td>$3.92 \pm 1.46$</td>
</tr>
<tr>
<td>6</td>
<td>$1.451 \times 10^{-1}$</td>
<td>$2.071 \times 10^{-2}$</td>
<td>$26.77 \pm 3.89$</td>
<td>$4.34 \pm 0.92$</td>
</tr>
<tr>
<td>7</td>
<td>$1.521 \times 10^{-1}$</td>
<td>$2.089 \times 10^{-2}$</td>
<td>$25.60 \pm 3.48$</td>
<td>$4.83 \pm 1.12$</td>
</tr>
<tr>
<td>8</td>
<td>$1.523 \times 10^{-1}$</td>
<td>$2.090 \times 10^{-2}$</td>
<td>$22.77 \pm 3.41$</td>
<td>$2.77 \pm 1.20$</td>
</tr>
<tr>
<td>9</td>
<td>$1.424 \times 10^{-1}$</td>
<td>$2.064 \times 10^{-2}$</td>
<td>$27.65 \pm 2.63$</td>
<td>$4.09 \pm 1.15$</td>
</tr>
<tr>
<td>10</td>
<td>$1.360 \times 10^{-1}$</td>
<td>$2.048 \times 10^{-2}$</td>
<td>$28.29 \pm 2.99$</td>
<td>$3.53 \pm 1.25$</td>
</tr>
<tr>
<td>11</td>
<td>$1.411 \times 10^{-1}$</td>
<td>$2.062 \times 10^{-2}$</td>
<td>$26.90 \pm 1.51$</td>
<td>$4.50 \pm 1.16$</td>
</tr>
</tbody>
</table>
The sub-samples extracted from freeze-dried porcine kidney sample 2 (see Table 7.9 for their anatomical description) were used to calculate theoretical photon attenuation coefficients from elemental composition data derived by RBS and PIXE analysis and were compared to values obtained by transmission tomography. This comparison may be seen in Table 7.13. Theoretical mass attenuation coefficients are presented for each sub-sample at 17.8 keV and 59.5 keV whilst experimentally derived linear attenuation coefficients at these photon energies are extracted from the indicated sites (Figure 7.14) from 2mm×2mm pixel dimension tomographs. Standard deviations accompany mean μ coefficients, the largest relative values occurring at sampling sites 1 to 4 inclusive, this being attributed to fat deposits in these volumes. Linear attenuation coefficients at some of these sub-sample extraction points are also elevated due to their adipose content. Mean tissue densities were calculated from linear and mass attenuation coefficient data values of between 180-200 kg/m³ the most commonly observed for cortex /medulla. Sub-samples 9 and 11 are essentially pure medulla and cortex respectively and hence it may be expected that a difference in density be seen. However ρ values are identical, density differences probably being too subtle for this measurement approach. Densities for sub-samples 3 and 4 are higher than other sites due to the large proportion of fat in their anatomical composition.

Ratios of attenuation coefficients (17.8 keV over 59.5 keV) were obtained from both experimentally and theoretically derived data, the former to relax the density dependence upon the linear attenuation coefficients, whilst the latter for comparison purposes. Theoretically derived ratios show little variation between sampling sites even for those with large fat compositions. Values for sub-samples 9 and 11 (medulla and cortex), are similar and hence this implies that cortex and medulla are not discernible due to their differences in elemental composition. The linear attenuation coefficients of sub-samples 9 and 11 are also similar thus implying that density variation contributions for each tissue type are negligible. Experimental attenuation coefficient ratios are on the whole lower than theoretically derived ratios probably for the reasons stated earlier for the values found in Table 7.5. These ratios also exhibit higher variation, this being accredited to tomograph noise, fluctuation values of approximately 9% and 23% (from equation 7.1) upon values of μ in 17.8 keV and 59.5 keV photon transmission scans are found.
7.6 Conclusions

The ability of photon transmission tomography to provide a measure of biological specimen heterogeneity and to differentiate between constituent tissues and identify regions of interest has been tested. Samples were scanned in fresh and dry states and their tomographs compared. This comparison implicates that greatest differences in linear attenuation coefficient between constituent tissues are seen when samples are in a dry state. Scans of freeze-dried porcine kidney, liver and lung easily identified deposits of fat in the former and the presence of blood vessels and bronchi in the latter two organs. This may aid in the selection of sub-samples for subsequent analysis as these may be chosen to exclude tissues which do not contribute to the trace element study. However, it has been found that tissues are mostly discernible from each other because of differences occurring in their physical density. Those biological specimens with similar densities and differing elemental composition are difficult to differentiate between as in the case of renal cortex and medulla, the main reason being the fluctuation of $\mu$ in the image which may be attributed to the relatively small number of counts accumulated for each ray-sum. Image noise from the source may be reduced by the employment of an intense polychromatic energy X-ray generator, the accurate determination of linear attenuation coefficients however being difficult.

The fluctuation of $\mu$ in tomographs originates from image noise and density and elemental composition variations of the scanned samples. The density dependence of $\mu$ in scans was relaxed by the division of tomographs obtained at two discrete photon energies. The remaining fluctuation of the attenuation ratio in the resultant image is then only attributable to photon counting statistics and elemental fluctuations in the sample, however only a small proportion is correlated with the latter in these tomographs. For photon transmission tomography to provide a reliable measure of sample elemental homogeneity, the number of image counts must be considerably increased. However, as the technique stands, it provides a viable method to initially screen samples so that areas of interest may be selected for consequent study, and with reduced image noise, tissues of similar but different elemental and physical composition may be differentiated.
Chapter 8
Conclusions and Suggestions for Further Work

The trace element levels in various biological organs may be used to indicate their health status or exposure to man-made pollutants. However, due to these specimens being composed in an elementally heterogeneous manner, it is often difficult to extract sub-samples from them such that their concentrations are representative of the tissue as a whole. This makes the diagnosis of disease and the identification of occupational and pollution exposure difficult unless the change in elemental concentration due to these factors is greater than the intra-organ/tissue variation. Hence it is desirable to quantify elemental distributions in specimens so that questions concerning the size, number and extraction site of sub-samples may be addressed. These issues are considered only with low frequency in the literature or where they are answered, conclusions are often contradictory.

Proton induced X-ray emission (PIXE) analysis and photon transmission tomography were employed in the study of the distributions of elements in various biological specimens. Samples analysed by PIXE were irradiated in two different modes, namely, in the form of cylindrical pellets manufactured from homogenised material or thick specimen sections. In the construction of pellets, a minimum mass of material with associated volume is required which limits the spatial resolution on which elemental variations may be derived. As a high degree of spatial resolution is a merit of PIXE over competing element analysis techniques, specimens were prepared in sections so that they could be irradiated directly thus utilising the capabilities of proton induced X-ray emission. Results from both approaches were compared.

In the analysis of pellets, sub-samples were initially extracted from defined positions within porcine liver, kidney and heart which were freeze-dried, homogenised and then manufactured into targets. These targets were irradiated with a 2 MeV proton beam in vacuum and concentrations in each determined by the external standard comparator method although an attempt to derive levels by the single element comparator method and absolute analysis was made. Both of the comparator approaches derived concentrations which were close to accepted values, however the absolute approach yielded
concentrations which differed by roughly a factor of 8. This was mainly attributed to uncertainties in the detector solid angle and efficiency and in the charge collection process. It is necessary to investigate and remedy these sources of error in absolute PIXE analysis such that this approach may be accurately applied to the quantitative element determination in samples thus alleviating the reliance upon standard reference materials which themselves may introduce uncertainties into the analytical procedure and in any case increase the time and cost involved in the analysis process.

The areas sampled within the three porcine hearts were each of the atria and ventricles and the aortic, mitral and pulmonary valves. When comparing element concentrations between valve and heart muscle, significant differences were found to occur for Cl, K, Ca, Fe, Cu and Zn. Calcium however was the only element in valve to exceed levels in muscle. The fact that elemental levels between these tissues are found to differ is not surprising, however dissimilar concentrations for K and Fe between ventricle and atrium sub-samples is an unexpected and interesting feature. It is thought that differences in these element levels occur as a result of atria and ventricles composing of varying portions of heart muscle, that being greater in the latter whilst the former comprises of a larger percentage of covering tissue that requires less blood for function.

Sampling areas in liver were the left lobe, right lobe central and right lobe peripheral. Concentrations of the elements Fe, Cu and Zn were found to differ when comparing the left lobe to either of the right lobe sites. Also, Zn was identified to vary within the right lobe itself. These findings were unexpected due to the constant physical and physiological function of this organ. Disagreements in Fe levels throughout the liver may be a consequence of the varying blood content between sampling sites.

The kidney is composed of two discrete tissues namely the cortex and medulla. It was important in the sampling studies to quantify trace element levels in each composite tissue. The elements Cu, Zn, Mn and Cd were found to preferentially collect within the renal cortex whilst K, Ca and Fe were evenly distributed throughout the organ. It is thought that Cu, Zn and Cd deposit within the cortex due to their transport from the liver by the protein metallothionein. Concentration gradients for Cd and Zn are noted in the literature in cortex, this however could not be verified due to the limits in spatial resolution as a result of the extraction of sub-samples for pelletisation.

The analysis of lyophilised kidney sections enabled concentration gradients to
be studied as a higher degree of spatial resolution was achievable. Higher levels of Zn and Cu were found in subscapular cortex whilst concentrations decreased to lower values in juxtamedullary portions and remained relatively constant across the renal pyramid. Concentrations of K, Fe, Br and Rb were essentially unchanged across the kidney sections although Ca did exhibit preferential deposition within the cortex. This is contrary to findings presented in the literature and results from the analysis of pelletised samples. When comparing elemental concentrations derived from pelletised samples and sections, there was on the whole, good agreement. There is a potential for dissimilarities to occur because of surface roughness effects and proton irradiation and X-ray take-off angles may be less defined when analysing specimen sections. Stylised models were developed in order to investigate the magnitude of their effects upon PIXE analysis of biological sample slices.

A single groove model was adopted to estimate the effect of surface roughness upon X-ray yield. The model parameters were varied i.e. matrix composition and density, groove dimensions, X-ray take-off angle, proton energy and trace element of interest and rough to smooth X-ray yield ratios calculated which were used as a measure of the groove effect. It was found that the influence of sample surface roughness upon X-ray yield decreases as the photon energy increases (or as Z number increases). The incident proton energy was varied and the effect of the groove upon PIXE analysis identified to increase, albeit to a small extent, as proton energy decreased. Positive correlations were found between the groove depth and X-ray yield ratio and the matrix density and X-ray yield ratio. An interesting feature to note about the magnitude of the effect of sample surface roughness is that this decreases as the X-ray take-off angle increases, and when the detector is positioned perpendicular to the sample surface plane, the effect totally disappears. Hence, this implies that when analysing a sample with irregular surface by PIXE, an X-ray take-off angle as close to 90° should be adopted.

The effect of sample surface tilt upon PIXE analysis was investigated by employing two worst case scenarios. That is, specimen tilt toward and away from the detector were considered. Two angles i.e. 5° and 10° were chosen for investigation and the effects of these inclinations upon X-ray yield for differing elements was derived. As expected, the magnitude of the effect upon X-ray yield was greater for 10° than 5°. However both angles exhibited a similar trend where the tilt to flat surface photon ratio...
was largely different from 1 for low Z elements, and this approached unity as Z increased, the effects for roughly calcium upwards being small to negligible. The effect of sample surface tilt upon PIXE analysis is independent of specimen matrix density.

Although the magnitude of the effects of sample surface roughness and tilt upon PIXE analysis are dictated by physical parameters e.g. matrix composition and density, groove depth and X-ray take-off angle in the former whilst sample surface tilt in the latter, it may be generalised that they only become significant for elements emitting lower energy photons. That is, for the lower Z elements (roughly Z<20) when considering K-shell transitions and medium atomic number elements (roughly Z<50) for the L-shell X-rays. The stylised groove model may be superseded by the generation of a randomly rough surface. This would represent more closely the rough biological surfaces analysed in PIXE. However, it was observed from SEM photographs that the specimen volume contains ducts and vessels which are associated with the physiological nature of the tissue, and some account may be taken of this in an improved model. These features would allow general rules to be formulated so that quantitative estimates of the effect of surface roughness upon X-ray yield in PIXE may be evaluated.

Thick specimen sections of porcine liver and lung were also analysed in the above mentioned manner. Plots of elemental concentrations across the freeze-dried liver sections revealed fluctuations which were attributable to analytical variances and heterogeneity of the sample itself. There were no identifiable concentration trends, which is expected due to the constant physiological nature of this organ, apart from where a sampling point was positioned near an hepatic vein wall. The most significant change in concentration at this position was observed for calcium, the level of which was more than double that of the main liver tissue. Analysis of a section of hepatic vein wall revealed similar concentrations.

Ten specimen sections in all were subtracted from a pair of lungs so that the elemental levels in each of the lobes and bronchi could be quantified. A study of the concentrations of elements across one section (extracted from the bottom of the right lung) revealed the absence of any trends and the modulation of levels due to analytical and heterogeneity factors. Differences between mean specimen section concentrations however were identified. Calcium was found to significantly differ between lungs, the reason being unknown, whilst Zn preferentially collected in areas correlated with high aerosol
deposition. The elements Cu and Rb remained relatively constant throughout both lungs. Analysis of the bronchi walls showed this tissue to contain high calcium levels.

In the PIXE analysis of biological specimens in the form of pellets or lyophilised sections, it is imperative to limit beam current to inhibit sample damage. A current density of roughly $1 \times 10^{-15} \text{ A m}^{-2}$ was employed in the analysis of a porcine liver sample in the form of a pellet, and elemental losses were evaluated at differing proton doses. It was found that at this current density, there was negligible effect upon derived concentrations. However, at a higher value of roughly $8 \times 10^{-14} \text{ A m}^{-2}$, elemental losses occurred and in particular for Br. For lyophilised biological sections and higher proton energies this effect will be more severe. Hence, this highlights the need for current density limitation when analysing biological samples and the quantification of safe limits for various proton irradiation scenarios so that no elemental losses occur.

The trace element analysis of biological specimens by the extraction of sub-samples for subsequent pelletisation and the direct proton irradiation of thick specimen sections has highlighted the heterogeneous nature of this type of sample. Also, differences in elemental concentration may be derived depending upon which portion of an organ or tissue is selected for analysis. Hence, it is necessary when comparing concentrations between individuals or in the diagnosis of disease or exposure to man-made pollutants to employ clearly defined sampling positions. For example, misleading conclusions may be drawn when comparing element levels in liver between individuals that have been derived from samples extracted from different areas within each of the organs e.g. left lobe and right lobe, and hence one specific sampling site must be employed throughout. Also, the objectives of the study must be clearly defined. If interest is centred upon the accumulation of Cd or Zn in the kidney as a result of smoking, then it is apparent that sub-samples should be extracted from the cortex as opposed to the medulla as the former tissue is the most sensitive in indicating these element levels. Similarly, in the study of airborne pollution by the analysis of lung specimens, sub-samples should be extracted from the apex as this is the main deposition site for aerosols. However, for a defined sampling position or tissue, it is still possible to derive a widely varying range of concentrations due to heterogeneity and a minimum mass should be extracted to reduce elemental variations to a given level. This mass is derived from sampling factor theory and is calculated by the replicate analysis of a material using constant weight sub-samples.
The relationship between total elemental variance and that attributed to the analytical procedure is considered.

Sampling factors were calculated for porcine liver, kidney, heart and lung from the data derived by PIXE analysis of homogenised sub-samples manufactured into pellets and thick specimen sections. These sampling factors are presented for 1% \( (K_1) \) and 5% \( (K_5) \) elemental variations, and those derived from the analysis of pelletised targets are typically of the order of grams or tenths of grams (dry-weight) respectively. Each element in a particular tissue has an associated sampling factor, and it is usually observed that their values are smaller as concentrations increase. Converted to a wet weight basis, the sampling factor range for a variety of elements in each organ translates to tens and hundreds of grams for the 1% elemental variation level. This finding has a number of implications one of which is that the upper sampling factor range limit approaches the total organ weight thus inferring that in particular cases, representative elemental levels may only be derived with analysis of material from the whole organ. Hence, this demonstrates the impracticability of employing 1% elemental variation criteria to sampling factor studies as the derived masses are invariably too large to be put into practical use and a higher level of elemental variance settled for, e.g. 5% may be acceptable in some cases. The sample factor \( K_5 \) derives masses that are 25 times smaller than \( K_1 \) values and hence are more applicable to sampling studies. An interesting feature to note when comparing sampling factors between different organs was that no tissue is clearly more elementally heterogeneous than another. This was unexpected given the anatomical composition of the various organs e.g. compare kidney to liver.

Sampling factors were calculated from data derived by the PIXE analysis of thick biological sections and these compared to those discussed above. A large difference was noted (roughly a factor of a thousand) which arises from the analysis of samples by the employment of small masses (\( \mu \text{g} \)) which are dictated by the proton beam surface scanning area. It is thought that the sampling factor theory breaks down at this order of mass, however, this could not be reliably linked to the incorrect assumption that concentration distributions are normal in form and rather, positively skewed. It is recommended that an investigation be completed upon the magnitude of mass at which sampling factors become invalid, and the reasons identified for this break-down.

Photon transmission tomography was investigated for the ability of the technique
to provide a measure of elemental heterogeneity of biological samples. This may be possible due to tomography providing an image of the distribution of the linear attenuation coefficient throughout a selected slice of an object which is a function of both physical density and elemental composition. Tomographically scanning specimens prior to sub-sample selection may aid in the identification of regions of interest and may be particularly useful when limited to a small number of sample aliquots which may either be analysed by a technique such as PIXE or stored as in the case of specimen banks.

The tomographical scanning rig and image reconstruction algorithm were initially tested for their performance, and an excellent correlation between theoretically and experimentally derived linear attenuation coefficients was obtained. Samples of soft (kidney) and adipose tissue were imaged in a fresh state, and these compared to tomographs obtained from freeze-dried specimens (kidney, liver and lung). This comparison showed that composite tissues e.g. cortex and medulla in kidney, should be more easily discernible when samples are in a dry state which is due to differences in their elemental composition and density. Also, features such as fat deposits and blood vessels are easy to identify in freeze-dried sample tomographs, these however being more difficult to locate when specimens are imaged in a fresh state. The ability to differentiate these features prior to sub-sample extraction may aid in the reduction of potential contamination as less manipulation of these specimens may be required.

An attempt to quantify the contribution to image linear attenuation coefficient fluctuation from elemental variances was made. This involved an estimation of $\mu$ fluctuation from image noise which is a product of a finite number of counts being collected in each ray-sum. Also, the density dependence of $\mu$ in tomographs was relaxed by the division of two images collected under similar conditions but at different energies. It was found that most of the fluctuation in tomographs is attributed to image noise, although the significance of this contribution was reduced in lung due to the presence of bronchial formations. Therefore, the modulation in $\mu$ which was predicted to occur from elemental variations is found to be smaller than contributions from noise and density and hence tissues such as renal cortex and medulla cannot be differentiated in images. For this to be so, total image counts need to be increased thus reducing tomograph noise.

A method to increase image counts would be to replace the discrete energy radioactive source with an X-ray tube. Although the employment of a polychromatic X-
ray source affects the determination of sample linear attenuation difficult as photons are attenuated to differing extents which is dependent upon their energy, this should aid in delineating between areas of similar but different elemental composition and density. If human specimens are analysed, particular regions in organs may be enhanced due to the exposure of environmental pollutants and personal habits. For example, the accumulation of Cd in renal cortex is accelerated due to smoking, and thus this tissue may be differentiable from medulla due to the relatively high concentration of this medium atomic number element in an essentially low Z matrix. This may also be detectable by imaging samples using a discrete energy radioactive source.

The photon transmission tomography performed in this work has illustrated that various composite biological tissues are differentiable in samples. This is mainly due to density differences rather than elemental. However, with the reduction of image noise, tissues of similar but different composition and density may become discernible thus making photon transmission tomography a viable technique for sub-sample selection for subsequent trace element analysis or storage as in the case of biological and environmental specimen banks.
Appendix 1

Beam Alignment and Focusing

Following the 2 MeV proton beam being steered into line 5, the initial stage of the beam alignment procedure is to bring one of the object apertures into the beam path. This is achieved by firstly removing the aperture plate from the ion beam by the micrometer screw-gauge which controls the position of the plate. Now the beam spot seen on the quartz view port will be roughly 5 mm in diameter, if properly adjusted by the technicians. Now screw in the micrometer gauge until the correct aperture setting is selected. A 1.0 mm diameter aperture was employed for all ion beam analysis in this work. The beam spot seen in the quartz view port should now be circular in shape and roughly in the centre of it. A beam spot shape other than circular implies improper adjustment of the object aperture. Lower the first quartz view port and check that the beam may be seen in the second. If this is so, then the beam may be let into the target chamber but only if the pressure of this chamber has reached $2 \times 10^5$ torr or below.

If the above mentioned pressure has been achieved, open the mechanical gate valve and observe the charge integration digital readout or current monitor. Any reading on either of these implies that the beam is incident at some point upon the sample plate holder. If no beam current is recorded then select greater sensitivity for these displays. A full-scale deflection of either 10 nA or 100 nA is employed whilst using the 1.0 mm object aperture. If however a smaller aperture is employed, greater sensitivity will be required. If still no current is recorded, the ion beam is not traversing into the target chamber. This is resolved by the application of voltage upon the beam steering plates which is performed ‘blindly’ i.e. it is not necessary to simultaneously view into the target chamber and adjust the 2 kV stable power supplies, as observation of the beam current monitor is sufficient.

Looking through the microscope configuration under high magnification, move the sample plate holder with the stepper motor controller until the glass plate is visible. It may be necessary to move the goniometer either in or out to achieve a focused image of this. Having completed this, adjust the voltages upon the lower deflection plates so that the beam now falls into the centre of the field of view. The reason for this will become
apparent shortly. Also, as we have adjusted the voltage upon the beam deflection plates which lies below the object aperture, this requires to be re-examined so that the beam spot is still of a correct form. With these adjustments made, it is now possible to focus the ion beam using the quadrupole magnet configuration.

With the magnet power supplies turned off, switch the controls into series mode (this is achieved by adjustment of the switch on the power supplies and on top of the magnets). Now the current supplied to each magnet may be controlled individually. Set the current to zero for each magnet, and then increase the current supplying each of the magnets in turn whilst viewing the beam down the microscope. One should observe the beam focusing in a straight line as the current increases which then becomes de-focused as the current increases further. Note here that no more than 0.6 amperes should be applied to each magnet. Magnets 1 and 3 focus the beam in a horizontal line whilst magnets 2 and 4 in a vertical line. If whilst focusing, the beam moves from the centre of the field of view and converges elsewhere then the beam is not co-linear with that magnet and the position of the magnets are required to be adjusted. This adjustment is made by a set of jacks which control vertical and horizontal motion of the quadrupole magnet bed. Horizontal motion brings magnets 2 and 4 into a co-linear position with the beam, whilst vertical movements, magnets 1 and 3. The jacks closest to the magnet at fault are used for this adjustment. When the beam is co-linear with all magnets, focusing may begin.

Zero the current controls, switch off the magnet power supplies and return the two switches to normal mode. Now magnets 1 and 4 and magnets 2 and 3 are controlled as pairs. Current adjustment potentiometers 1 and 2 are used to control these respectively and employed in focusing the beam into a circular spot. Now the flapping units may be invoked and the scanning area chosen. The form of this scanning area may be observed on the oscilloscope positioned above these units. Ion beam analysis may now be carried out upon samples.
Appendix 2
Sample Output from Batty

Sample output from Batty which is part of PIXAN, the PIXE analysis suite of programs developed by the Australian Atomic Energy Commission, Lucas Heights [CLA86,CLA87b]. The multichannel analyser data used is that from the 2 MeV proton induced X-ray emission analysis of Bowen’s kale standard. Shown is a 5th order polynomial background fit accompanied with fitted coefficients and chi-square and root mean square values for assessment of goodness of fit. Corrections for matrix self absorption, silicon escape peaks and sum peaks are made. Element Kα and Lα peak areas collected over 10.54μC are shown accompanied with errors, chi-square fits of gaussians to characteristic peaks and minimum detectable limits.
INPUT DATA

ENERGY (KEV) = 0.04725 * I + -1.25202

INITIAL VALUES FOR SIGMA AND MU

SIGMA 2.97020D-02 + 3.79460D-03*ENERGY
CENTROID 1.08262D-02 + 9.97429D-01*ENERGY

FILTER THICKNESS (MM) = 0.3500

THE CALCULATION WILL BE REPEATED IF NEGATIVE ELEMENTS ARE FOUND

THE FITTING OPTIONS AVAILABLE ARE
1 VARY ONLY PEAK HTS
2 VARY PEAK HTS AND SCALE BACKGROUND
3 VARY PEAK HTS AND BACKGROUND POLYNOMIAL
4 VARY PEAK HTS AND TAIL PARAMETERS
5 VARY PEAK HTS, TAILS AND SCALE BACKGROUND
6 VARY PEAK HTS, TAILS AND BACKGROUND POLYNOMIAL

THE OPTION CHOSEN IS IFITX = 3

SPECTRA ANALYSED FROM 3.000 TO 20.000 KEV

MATRIX CONSTRUCTION FOR SAMPLE
C CONC. 0.4435 N CONC. 0.0424 O CONC. 0.3930 H CONC. 0.0562
CA CONC. 0.0407 K CONC. 0.0242

STRIPPING SILICON ESCAPE CONTRIBUTION

*** POLY AS FITTED
1.98891D+00 -9.26811D-01 -1.08383D-01 -1.16417D-02 2.17966D-03
-2.30139D-06

BACKGROUND FITTING NORD ENORT :- 5 10.000

******* BLOCK 1 *******
PIXE SPECTRUM PEAK AREAS

THE ELEMENTS BEING SUMMED ARE CA K TI Fe CO

227
AFTER 7 ITERATIONS \( \text{CHISQR} = 5.70737 \times 10^{00} \)
\( \text{ROOT MEAN SQUARE} = 4.44357 \times 10^{-02} \)

FINAL BACKGROUND PARAMETERS
\( 1.41035 \times 10^{00} -8.87993 \times 10^{-01} 7.97884 \times 10^{-02} 2.36637 \times 10^{-02} -2.27596 \times 10^{-04} -5.60057 \times 10^{-04} \)

NEGATIVE OR SMALL ELEMENTS *************
DELETING ELEMENTS AS ZR S NB MO Y CO

AFTER 5 ITERATIONS \( \text{CHISQR} = 5.61426 \times 10^{00} \)
\( \text{ROOT MEAN SQUARE} = 4.44727 \times 10^{-02} \)

FINAL BACKGROUND PARAMETERS
\( 1.44791 \times 10^{00} -9.24278 \times 10^{-01} 4.99780 \times 10^{-02} 2.12072 \times 10^{-02} 6.87992 \times 10^{-04} -4.48373 \times 10^{-04} \)

NEGATIVE OR SMALL ELEMENTS *************
DELETING ELEMENTS P

AFTER 5 ITERATIONS \( \text{CHISQR} = 5.59651 \times 10^{00} \)
\( \text{ROOT MEAN SQUARE} = 4.44548 \times 10^{-02} \)

FINAL BACKGROUND PARAMETERS
\( 1.44737 \times 10^{00} -9.23741 \times 10^{-01} 5.03598 \times 10^{-02} 2.12187 \times 10^{-02} 6.71102 \times 10^{-04} -4.50169 \times 10^{-04} \)

\( \text{SIGMA} = 3.61128 \times 10^{-02} + 4.68570 \times 10^{-03} \times \text{ENERGY} \)
\( \text{CENTROID} = 6.42933 \times 10^{-02} + 9.94568 \times 10^{-01} \times \text{ENERGY} \)

********** BLOCK 1 **********
PIXE SPECTRUM PEAK AREAS

CHARGE COLLECTED IS (\( \mu \text{C} \))
10.5430

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Appendix 3

Flow Chart of the Groove Model which Estimates the Effect of Sample Surface Roughness upon PIXE Analysis.

Aspects relating to the groove model illustrated in the flow chart shown overleaf may be found in chapter 5. The groove model estimates the effects of sample surface roughness upon the X-ray yield in proton induced X-ray emission analysis, and the flow chart represents the methodology employed in the evaluation. Essentially the flow chart contains a loop which is repeated NIRR times, NIRR representing the Number of proton Irradiation points employed. When the loop is executed, a number of decisions are made. These are as follows. Initially it is decided where the proton irradiation point is with respect to it being either on or in the vicinity of the groove i.e. S1, S2 or S3. The resultant outcome dictates the modes that produced X-rays may leave the specimen and the magnitude of attenuation that they are subject to. Once this has been decided, $\theta_{ro}$ is tested against $\alpha$ as only certain effects may occur depending upon which angle is greater. Following this, rough surface X-ray yields are calculated for each effect e.g. S3i indicates that the proton beam is incident upon surface 3 and we are calculating the X-ray yield from effect i. Numerical integration is carried out between upper energy limit $E_{ul}$ and lower energy limit $E_{ll}$. These are shown for every effect and often given as the proton energy at a certain depth within the sample e.g. $E_{ll}$=$E(R'\tan\theta_{ro})$ in the case of S3i. Once the effect of the groove upon X-ray yield at a certain x-position has been evaluated, given NIRR has not been reached, the proton irradiation point is incremented by INTVW and the whole process is repeated until ultimately an average groove effect may be calculated.
How many irradiation points are needed? 

Set initial x-value irradiation point and initialise rough surface integral, i.e. \( x = 0 \), \( INT = 0 \).

Calculate smooth surface yield \( SINT \).

For \( 1 \leq J \leq NIRR \) do:

1. Set initial x-value irradiation point and initialise rough surface integral, i.e. \( x = 0 \), \( INT = 0 \).
2. Calculate smooth surface yield \( SINT \).
3. Set boundary limits with \( INT \) yield.

Input parameters - D, E, \( \theta \), \( \beta \).

Material composition - D, E, \( \theta \), \( \beta \).

X-ray source - X-ray energy, \( Z \) number.

Loop to calculate the X-ray yield for each of the irradiation points.

END.
Effect iv

Effect v

END

AVRT INTIN

Calculate effective roughness integral

NO

YES

END

CD

\[ x = x + \text{INTVW} \]

Effect of groove = \[ \frac{\text{AVINT}}{\text{SINT}} \]

\[ \text{AVINT} = \frac{\text{INT}}{\text{NIRR}} \]

\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]

\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]

\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]

\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]

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\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]

\[ \sum (R_{\max} \tan \theta - \frac{1}{2}D \tan \theta) \tan \alpha \]
How does $\theta_{TO}$ relate to $\alpha$?

$\theta_{TO} \leq \alpha$

Effect i

$H_{LL} = H_0$
$H_{UL} = 0$
$\text{INT} = \text{INT} + S1i$

$\theta_{TO} > \alpha$

Effect i

$H_{LL} = \beta R / \alpha (\tan \theta_{TO} - \tan \alpha)$
$H_{UL} = 0$
$\text{INT} = \text{INT} + S1i$

Effect ii

$H_{LL} = H_0$
$H_{UL} = \beta R / \alpha (\tan \theta_{TO} - \tan \alpha)$
$\text{INT} = \text{INT} + S1ii$

F
Effect i

If \( R + \frac{D}{\tan \alpha} \geq -2D \tan \alpha \)

then \( x_{UL} = \frac{-2D}{\tan \alpha} \) else

\[ x_{UL} = \frac{R}{(\tan \alpha + \tan \theta_{TO})} + \frac{D}{\tan \alpha} \]

If \( x \leq x_{UL} \) then

\[ R_{LL} = E(R' = x(\tan \alpha + \tan \theta_{TO}) \quad D(x(\tan \theta_{TO}) + 1)) \]

\[ R_{UL} = 0 \]

\[ \text{INT} = \text{INT} + S_{2i} \]

Effect ii

If \( R + \frac{D}{\tan \alpha} \geq -2D \tan \alpha \)

then \( x_{UL} = \frac{-2D}{\tan \alpha} \) else

\[ x_{UL} = \frac{R}{(\tan \alpha + \tan \theta_{TO})} + \frac{D}{\tan \alpha} \]

If \( x \leq x_{UL} \) then

\[ R_{LL} = E(R' = x(\tan \theta_{TO} + \tan \alpha) \quad D(\tan \theta_{TO} + 1)) \]

\[ R_{UL} = 0 \]

\[ \text{INT} = \text{INT} + S_{2i} \]
Effect iii

\[ R_{LL} = \begin{cases} R_0 & \text{if } R + 2D \geq 2D, \\
\frac{R + 2D}{\tan \alpha} & \text{else}
\end{cases} \]

\[ x_{UL} = \begin{cases} 2D \frac{1}{\tan \alpha} & \text{if } x \leq x_{UL} \text{ then} \\
\frac{R + 2D}{\tan \alpha + \tan \theta_{TO}} & \text{else}
\end{cases} \]

\[ \tan \alpha \]

\[ \text{INT} = \text{INT} + S_{2iv} \]

Effect iv

\[ E_{LL} = E_0 \]

\[ R_{UL} = \begin{cases} R' = x(\tan \theta_{TO} + \tan \alpha) \cdot D(\tan \theta_{TO} + 1) & \text{tan } \alpha \leq 2D \\
\tan \alpha & \text{else}
\end{cases} \]

\[ \text{INT} = \text{INT} + S_{2iv} \]
Appendix 4
Sampling Factors

The calculation of representative elemental concentrations in biological materials is difficult as these tissues are heterogeneously composed and thus there will be an associated uncertainty attached to derived values which is dependent upon the mass of the extracted sub-sample. It is apparent that as the sub-sample mass \( m \) is increased, the uncertainty in the concentrations decreases as the effects of heterogeneity are averaged out. It is of great interest to be able to derive a mass such that the variation upon elemental concentrations is limited to a given level of precision. This mass is predicted by the sampling factor \( K_1 \), and for 1% variation level, it is given by equation A4.1,

\[ K_1 = \frac{10^4 m}{\bar{c}_Z^2} \left( \sigma_T^2 - \sigma_A^2 \right) \]  

(Eq A4.1)

where \( m \) represents the sub-sample mass, \( \bar{c}_Z \) is the average concentration of element \( Z \) over all sub-samples, and \( \sigma_T^2 \) and \( \sigma_A^2 \) are the variances accredited to concentrations between sub-samples (including experimental errors) and the experimental procedure respectively. The value of \( \sigma_T^2 \) is given by equation A4.2,

\[ \sigma_T^2 = \frac{1}{n-1} \sum_{i=1}^{n} (c_i^Z - \bar{c}_Z)^2 \]  

(Eq A4.2)

where \( n \) is the number of sub-samples and \( c_i^Z \) the concentrations of element \( Z \) in each of the sub-samples. In order to derive the sampling factor, \( n \) sub-samples of replicate mass \( m \) are extracted and analysed (Figure A4.1). The factor \( \bar{c}_Z \) is the average concentration in that sub-sample in terms of proportion by weight. It must be noted here that each element has its own specific sampling factor in a particular material. Therefore, with \( \sigma_T^2 \) remaining constant, differing sub-sample masses \( m \) of material may be extracted and the same sampling factor will be derived (\( \sigma_A^2 \) is essentially reliant upon the collected counts in a photopeak and the certified concentrations of a standard in PIXE analysis and hence can be set to a constant value). This is because as the extracted sub-sample mass \( m \) becomes
Figure A4.1 Illustration of the procedure for calculating the sampling factor for a biological material by the extraction of sub-samples of replicate mass $m$.

Smaller, $\sigma_2^2$ becomes larger because larger differences in concentration will be encountered due to heterogeneity, and as $m$ becomes larger, $\sigma_1^2$ becomes smaller due to heterogeneity effects being averaged out.

The procedure for deriving sampling factors is easily understood when all of each sub-sample is analysed to ascertain the concentrations $c_i$. However, in some instances, not all of the sub-sample is analysed by the analytical technique (e.g. PIXE) or a portion is reserved for analysis by another procedure. When using thick target PIXE analysis, although the proton beam is allowed to scan over target surfaces, only analysis of a finite depth into the samples takes place and hence analysis of the total sub-sample mass $m$ is not possible. In this case a portion of the original sub-sample is analysed and the same concentrations $c_i$ of the original whole sub-samples are obtained. This is satisfied by the homogenisation of each sub-sample (Figure A4.2) so that a smaller portion of the original sub-sample may be analysed and the concentration $c_i$ derived by this method is the same as that of the original sub-sample (remember the concentration $c_2$ is
in terms of proportion by weight). Homogenised sub-samples are made into pelletised targets to ease the analysis procedure for PIXE.

Analysis of the homogenised sub-samples is complicated in that the material is never truly homogeneous. Therefore, a minimum mass of the homogenate has to be analysed so that the derived concentration is representative of the original sub-sample. Therefore, as long as the mass analysed by the proton beam is greater than this minimum mass, the derived concentration will be representative of the original sub-sample.

Analysis of the homogenised sub-samples that have been manufactured into pellets provide concentrations that are the same as the original sub-sample concentrations.
and hence these concentrations are directly used in equation A4.1. The original replicate sub-sample mass \( m \) must be employed in these calculations for a number of reasons. Firstly, the sub-sample mass \( m \) will dictate the concentrations \( c_z \) derived. Secondly, as discussed earlier, the sub-sample mass \( m \) will dictate the variation of concentrations found between sub-samples, the smaller the value of \( m \) the larger is the variation of concentration values obtained and vice versa. As the concentrations derived from analysis of the pellets are the same as those of the original sub-samples, the variance of concentrations \( \sigma^2_z \) between the original sub-samples is the same as the variance of concentrations between pelletised targets. Therefore, the variance of the concentrations between pellets is attributed to the replicate sub-sample mass \( m \).

It may be argued that the mass probed by the proton beam \( m_1 \) be employed in calculations of the sampling factor for the whole organ. However, if this were the case, a number of sampling factors \( K \) may be derived for the same element in a matrix, which is contrary to sampling factor concepts. This is because the same concentrations \( c_z \) for each pellet are derived irrespective of the mass probed by the proton beam (as long as this is greater than the minimum mass required to be representative of the homogenised material), and thus \( \sigma^2_z \) and \( c_z \) are constant in equation A4.1. With \( \sigma^2_z \) remaining constant, the sampling factor changes proportionally with respect to \( m_1 \).

Sampling factors may also be derived in terms of X-ray yield \( Y_z \) rather than concentration \( c_z \). The X-ray yield \( Y_z \) is dependent upon the concentration of element Z (\( c_z \)) and the number of protons incident upon the sample (see section 2.3.3), and has no dependence upon the mass \( m_1 \) sampled by the proton beam (as long as a minimum mass has been sampled as indicated earlier). This is evident when we think of the physical processes taking place, the probability of X-ray production being greater when there are more atoms of element Z present within the sample and when there are more protons incident to ionise these atoms. From this, it is apparent why X-ray yield is independent of mass \( m_1 \) sampled by the proton beam on a sample as we may have a focused beam covering a small area and a beam scanned over a larger area and as long as the number of incident protons are identical, the yield of X-rays are the same. Hence, if we are analysing pellets manufactured from homogenised sub-samples, the X-ray yield \( Y_z \) will be the same for analysis of the pellets and whole sub-samples if the same proton charge is collected for each. Then \( \sigma^2_z \) expressed in terms of X-ray yield is the same for the pellets.
and whole sub-samples, and the sub-sample mass $m$ is employed in the sampling factor equation.
References


[ALB89] Albury, D M, Selected applications of proton induced X-ray emission to trace element analysis, Ph.D., University of Surrey, (1989).


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[DAV89] Davies, G, Emission tomography in the determination of the spatial distribution of neutron induced radionuclides, Ph.D., University of Surrey, 1989.


[FOL83] Folkard, M Development and applications of a gamma-ray tomographic scanner, Ph.D., University of Surrey, 1983.


[HAR85] Harrison, H, Final year undergraduate project, Department of Physics, University of Surrey, 1985.


[HER80] Herman, G T, Image reconstruction from projections, the fundamentals of computerized tomography, Academic, San Francisco, (1980).


[ING76] Ingamells, C O, Derivation of the sampling constant equation, Talanta, 23(1976)263-264.


Iyengar, G V, Concentrations of 15 trace elements in some selected adult human tissues and body fluids of clinical interest from several countries: results from a pilot study for the establishment of reference values, Institute of Medicine, Juelich Nuclear Research Centre, Juelich, (1985).


**[Lacosta, G]**, Final year undergraduate project, Department of Physics, University of Surrey, 1990.


Sanders, J M, Detection and measurement in emission and transmission tomography and applications, Ph.D., University of Surrey, 1982.


[STO70] Storm, E, Israel, H I, Photon cross sections from 1keV to 100MeV for elements Z=1 to Z=100, Nucl. Data Tabl., A7(1970)565-681.


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Publications and Presentations of Work


Beach, A C, Spyrou, N M, Determination of the elemental homogeneity of selected biological organs by 2MeV proton induced X-ray emission analysis, Presented at Meth. Applic. Radioanal. Chem. (MARC) - III, Kailua-Kona, Hawaii, 10-16 April, 1994, Under consideration for publication J. Radioanal. Nucl. Chem..