Kinetic-Spatial Filters for PET Dynamic Studies in Oncology

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Abstract

A novel kinetic filtering technique has been developed, which enhances the visualisation of tumours imaged using $^{18}$FFluorothymidine Positron Emission Tomography. $^{18}$FFluorothymidine is a cell proliferation marker which undergoes a predominantly hepatic metabolism, and therefore shows a high level of accumulation in the liver, as well as in rapidly proliferating cancerous tissue.

A linear classification algorithm to isolate cancerous tissue against such healthy organs was thus developed and validated using 28 images of patients with locally advanced or metastatic breast cancer. A reduction in signal from the liver and heart of over 80% is observed following application of the filter, whilst the majority of signal from both primary tumours and metastases is retained. The algorithm can be applied to images reconstructed using either analytic or iterative methods, and a scan acquisition time of 60 minutes is thought to be sufficient to obtain the necessary kinetic data.

The technique could have direct relevance in clinical oncology, as well as being applicable to a variety of tracers, and to the removal of other organs, including the bladder.
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Chapter 1

Introduction

Cancer is one of the most significant causes of death worldwide, accounting for 13% of all deaths in 2005 [1]. There are around 200 different types of the disease [2], but the most common are cancer of the lung, breast, bowel and prostate, which together accounted for over 50% of all newly diagnosed cases in 2005, excluding non-melanoma skin cancer [3]. Whilst there is no single treatment, there are a range of options, including local treatments such as surgery and radiotherapy, and whole-body treatments such as chemotherapy and hormone therapy [2]. The treatment chosen depends on the tissue in which the cancer originated, how far it has spread anatomically, and how aggressively it is growing, as well as the overall health of the patient [4]. Amongst the variety of diagnostic tools that can be used to obtain the information required to select the most appropriate treatment and monitor its progress, a significant role is played by Positron Emission Tomography (PET).

PET is a non-invasive molecular imaging technique, whose special feature is its ability to follow a functional process in real-time in vivo. The technique can be useful in the early diagnosis of disease, since organs tend to fail first in their biological function, and only later can the resulting structural changes be identified using an anatomic imaging modality, such as X-Ray Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) [5].

PET has been in use in clinical practice for around 15 years [6], with over 90% of its present clinical usage in the field of oncology [7]. The technique has a role in imaging a variety of cancers [6], and can be useful in the diagnosis and grading of both primary tumours and metastases, differentiating between tumour recurrence and tissue necrosis, and in the selection and monitoring of treatment plans [8]. At present, radiological studies of tumour response to treatment rely on an assessment of the change in tumour volume [9], but PET scanning could be applied to instead assess changes in the biological
activity of a tumour. This may provide an early indication of whether a particular treatment approach is likely to be beneficial, since biochemical changes may be visible on a PET scan weeks or months before there is an identifiable change in tumour volume [10].

Full details of the principles behind PET imaging are given in Chapter 2, but essentially it involves injecting the patient with a radiotracer, designed to target a particular physiological process, and then scanning them to obtain an image of the spatial distribution of this tracer, from which the process can be quantified. There are a variety of PET tracers available, but the most widely used, and the only one currently approved by the Food and Drug Administration for routine use in cancer imaging, is $^{18}$F-fluorodeoxyglucose (FDG) [8].

FDG is a glucose analogue that, like glucose, is taken into cells by glucose transporters, where it undergoes phosphorylation, catalysed by the enzyme hexokinase [11]. However, unlike glucose, FDG is resistant to further metabolism and, since it does not significantly diffuse out through the cell membrane, it becomes metabolically trapped inside the cell [12]. The accumulation of phosphorylated FDG inside cells can therefore provide an indication of their rate of glucose uptake and hexokinase activity. As a result, PET images of the distribution of FDG within the body can be used to provide quantitative estimates of the rate of glucose utilisation in various tissues. The tracer therefore has applications in the field of oncology, because most cancers show an over-expression of glucose transporters, and therefore have an accelerated glucose metabolism [13].

However, FDG is not a tumour-specific tracer, and false-positive results are common, for example at sites of inflammation or infection [14]. This creates difficulties in oncology, especially following treatment, as the risk of false-positive results may be elevated for up to eight weeks following surgery, and for as much as six months following radiotherapy [6]. False-negative results also present a problem, and can be caused by slowly growing or highly differentiated tumours [14] which have a low metabolic rate. For these reasons, alternative, more tumour-specific tracers are being actively sought.

One such emerging tracer is $^{18}$F-fluorothymidine (FLT), a thymidine analogue, whose accumulation in cells provides an indication of their proliferation rate [15]. Increased proliferation is a more tumour-specific marker than increased glucose utilisation [16], and recent research, a review of which is presented in Chapter 3, suggests that FLT may therefore have applications in the field of oncology.

However, FLT accumulates in the liver as well as in proliferating tissues, since it undergoes a predominantly hepatic metabolism [17]. The resulting high intensity obscures signal from proliferating tissues in and around this
organ, with the consequence that tumours in the stomach, bowel, kidneys and pancreas, as well as the liver itself, can be difficult to identify. Since the liver is the second most likely site for metastases [4], and cancers of the bowel, stomach and pancreas are fairly common [2], for FLT to have a role in clinical oncology, it is important that tumours in these tissues can be detected.

The present project was aimed at solving this clinical problem, using a strategy based on the observation that the kinetics of FLT differ between, say, liver and tumour tissue. The idea was therefore to exploit these differing kinetics to filter out healthy organs showing high signal intensity, thus allowing the detection of tumours in and around them. As well as potentially being applicable to other PET tracers which undergo simple hepatic metabolism, the technique developed could be used to filter out organs other than the liver, such as the heart, vertebra and bladder, which also show high signal intensity, both for FLT and other PET tracers.
Chapter 2
Positron Emission Tomography

The basic procedure for a PET scan involves injecting the patient with a tracer labelled with a positron-emitting nucleus, and then scanning them. A positron emitted inside the body can travel only a short distance through tissue, losing kinetic energy by Coulomb scattering from atomic electrons as it does so, until it is almost at rest. Then, when this low energy positron interacts with an atomic electron, the particles can annihilate to produce two gamma ray photons that are detectable outside the body. In order to conserve energy and momentum, the photons must be emitted in opposite directions, and each with an energy of 511 keV. Since the elements of the PET detector form closed rings around the patient, the two photons are detected simultaneously in opposite detector elements. This process, known as coincidence detection, allows for spatial localisation of the tracer within the body, and therefore the production of an image showing its distribution.

2.1 Radiotracers

Positron-emitting nuclei are unstable because they possess a high number of protons, and they stabilise by the decay of a proton into a neutron, positron and electron neutrino. The nuclei most commonly used in PET imaging are carbon-11, nitrogen-13, oxygen-15 and fluorine-18 [18], and some relevant properties of these nuclei are shown in Table 2.1.

The nuclei are short-lived, and must therefore be artificially produced using a cyclotron, which is a machine that uses strong electric and magnetic fields to accelerate a beam of charged particles to a high energy. This beam then collides with a stationary target material, inducing nuclear reactions which result in the production of positron-emitting nuclei. For example, bombarding $^{18}$O with protons, such that the nucleus gains a proton and
loses a neutron, results in the production of $^{18}$F [15].

<table>
<thead>
<tr>
<th>Positron-Emitting Nucleus</th>
<th>Half-Life (mins)</th>
<th>Mean Free Path (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}$C</td>
<td>20</td>
<td>0.3</td>
</tr>
<tr>
<td>$^{13}$N</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>$^{15}$O</td>
<td>2.8</td>
<td>1.5</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>110</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 2.1: Properties of the most commonly used PET radionuclei [15]; the half-life is the time taken for half the radioactive nuclei in a sample to undergo radioactive decay, and the mean free path is the average distance that the positron travels through tissue before annihilation.

As shown in Table 2.1, positrons are not annihilated instantaneously upon emission inside the body, but first travel a short distance through the tissue. This finite mean free path, which is dependent on the energy with which the positron is emitted from the nucleus, means that the photons detected are actually emitted a short distance away from the location of the tracer molecule within the body. In general there is also some residual momentum when the electron and positron annihilate, which means that the photons are not emitted exactly $180^\circ$ apart. These two sources of error define the absolute physical limit to the resolution of PET imaging which, in clinical scanners, is negligible compared with the much larger intrinsic resolution of the detector [15].

Prior to PET imaging, a tracer molecule, selected to target a particular physiological process, is radiolabelled with a positron-emitting nucleus in a reaction that is generally performed in a computer-controlled synthesis system. The automated system allows for rapid labelling, which is important because of the short half-lives of the nuclei involved, and is shielded with lead to protect workers from radiation exposure.

The way in which the molecule is labelled is important, and isotopic labelling, such as the replacement of $^{12}$C by $^{11}$C, is preferable because the resulting tracer has identical behaviour to the unlabelled molecule. This is often possible when labelling with $^{11}$C, $^{13}$N or $^{15}$O as many endogenous molecules contain carbon, nitrogen or oxygen, but it is generally not possible when using $^{18}$F as few biological molecules contain fluorine. However, labelling with $^{18}$F is attractive because its longer half-life compared to the other nuclei means that synthesis of the tracer does not necessarily have to occur on-site at the PET imaging centre. $^{18}$F is used as a pseudo-isotopic substitute for hydrogen in labelling a variety of PET tracer molecules, because such an exchange generally has only a small effect on the behaviour of the molecule in vivo, which can be easily accounted for.
2.2 Image Acquisition

A PET scanner consists of a series of coaxial rings around the patient, with each ring containing a number of detector elements. In the most commonly used detection systems, these elements are made up of an array of scintillating crystals, such as bismuth germinate oxide or cerium-doped lutetium oxyorthosilicate [19], that are optically coupled to location-sensitive photomultiplier tubes [15]. When a gamma ray photon interacts with the scintillating crystal, electrons in the lattice are excited from the valence band up into the conduction band. These electrons return to the valence band at impurities in the crystal and, in doing so, dissipate energy in the form of light. This is converted into a weak electronic pulse, which is then amplified into a measurable signal in the photomultiplier tubes.

The physical properties of the chosen crystal are important, and ideally it should have a high stopping power for 511 keV photons, produce a short and intense light pulse and be capable of providing accurate energy measurements [19]. The detection system should also have a short dead time, which is the time required for a single event to be processed, and during which the detector cannot record any new events that occur [15].

To describe how data are acquired, a single ring of the detector is considered in isolation. Each element in the ring is connected in a coincidence circuit with every other element, such that an event is registered if photons are detected simultaneously in two elements. For the detection of two photons to be considered simultaneous, it must occur within a short coincidence window which, in modern clinical scanners, is between 10 and 12 nanoseconds [15].

![Figure 2.1: Stages of image acquisition; (A) shows an annihilation event and the corresponding line of response, (B) the grouping of parallel lines of response to form projections, and (C) the construction of a sinogram.](image)

Registration of an event determines a path across the detector, known as the line of response, along which the two photons must have been emitted,
as shown in Figure 2.1 A. For every possible orientation of the ring, parallel lines of response are grouped together as illustrated in Figure 2.1 B to form projections, and the number of events recorded along each of the lines of response in a single projection forms one row of a data matrix called a sinogram. Each row of this matrix contains data from a different projection around the patient, such that a complete sinogram contains all the information recorded from a single ring, as shown in Figure 2.1 C.

In reality, a PET detector has many rings, and data may be acquired in either 2-D or 3-D mode. In 2-D mode, lead or tungsten septa are inserted between the rings, as shown in Figure 2.2 A, and data are obtained in planes, with each plane containing data from only a single ring and its close neighbours. These 2-D planes may be combined to produce a 3-D image if required. In 3-D mode, the septa are removed, as shown in Figure 2.2 B, allowing all possible lines of response between the rings, which results in a significantly higher sensitivity because many more photons are detected. This increased sensitivity can potentially be exploited to reduce either the amount of tracer required or the image acquisition time [19].

![Figure 2.2](image.png)

Figure 2.2: Modes of image acquisition [20]; (A) shows the detector in 2-D mode with septa between the rings, and (B) shows the detector in 3-D mode with no septa present.

### 2.3 Photon Attenuation in Tissue

At energies around 511 keV, the dominant interaction of photons with tissue is by Compton scattering from outer-shell electrons, which results in both a loss of energy and deflection from the original path. Data must therefore be corrected for errors occurring due to this attenuation, as well as other effects, before an image can be reconstructed.

The probability that a photon undergoes no interactions as it travels through tissue along a line $l$ is known as its survival probability, and is given
by the expression

$$P = \exp\left(-\int_{l}^{\mu(x)dx}\right)$$

in which $\mu(x)$ is the linear attenuation coefficient of the tissue [21]. The survival probabilities of the pair of photons produced as shown in Figure 2.1 A are independent, and so the combined probability that neither photon interacts is given by

$$P_C = \exp\left(-\int_{a}^{b} \mu(x)dx\right).$$

The attenuation factor, $(1 - P_C)$, is therefore independent of the position of the annihilation event along the line $l$, and can be calculated for every line of response. The resulting values can then be used to correct the PET image for attenuation. Traditionally, this attenuation map is obtained from a transmission scan which is acquired using an external 511 keV germanium rod source while the patient is in the scanner, but before injection of the radiotracer. However, the use of a combined PET/CT machine provides an alternative, as the transmission data can instead be obtained from the CT scan.

Not all attenuated photons are deflected out of the field of view and, if such scattered photons are subsequently detected, an incorrect line of response may be registered. However, since energy loss is correlated with the angle of scatter, the registration of such photons may be supressed by only considering those with an energy above a certain threshold value [15]. This is an important consideration when deciding on the mode of image acquisition, since scattered photons present a far greater problem when using the 3-D mode because of its higher sensitivity. For imaging the brain, shielding can be positioned around the head to reduce scatter, but for imaging the thorax this is not possible, and the 3-D mode of imaging is generally not used.

### 2.4 Image Reconstruction

The aim of image reconstruction is to obtain a quantitative map of the spatial distribution of a radiotracer within the body. The simplest method is Filtered Back Projection (FBP) and, in this brief description of the process, a single slice through an object, shown in Figure 2.3 A, is considered for simplicity. First, the projection at each angle is extracted from the sinogram as an intensity profile, illustrated in Figure 2.3 B. Then, since the angle at which each projection was acquired is known, the intensities are simply re-projected across the image matrix, as shown in Figure 2.3 C, from which it is clear that
this technique is indeed successful in retrieving the original image, assuming sufficient projections are used.

![Figure 2.3](image.png)

Figure 2.3: Image reconstruction by Simple Back Projection; (A) shows a slice through the object, (B) illustrates the intensity profile from a single projection, and (C) shows the image obtained following reconstruction.

The FBP algorithm is routinely used in the reconstruction of PET images, but its widespread use can be attributed mainly to historical popularity, as there are several limitations to the technique. These include a loss of fine detail, noise amplification, and a so-called star artefact, visible in Figure 2.3 C, whose magnitude depends on the number of projections used. Whilst some improvement can be made by applying Fourier filters, even then the resulting image is not free from artefacts generated by the reconstruction process and, since computational demand is no longer a limiting factor, the use of sophisticated iterative approaches is becoming more common.

One such example is the Maximum Likelihood Expectation Maximisation (MLEM) algorithm, which aims to find the image most likely to result in the observed projections, given some modelling of the data, noise and detection procedure. The algorithm begins with an estimate of the image, often that obtained using FBP, which it then modifies, based on a comparison of the observed projections with those obtained from the image estimate [22]. In theory, this procedure is repeated until convergence, but in practice it can be very slow, and the number of iterations is often specified, although enough must be allowed for the algorithm to reach a sufficient level of convergence. One way to accelerate the process is to use Ordered Subsets Expectation Maximisation (OSEM), in which the projections are divided up into a number of subsets, each of which is iterated separately [23]. However, as the number of subsets is increased, the results become more biased and less quantitative, and the number chosen therefore depends on the particular application, involving a trade-off between reconstruction speed and image quality.
2.5 Image Analysis

PET images may be acquired in static or dynamic modes, discussed below in further detail, or in gated mode, in which the image acquisition is synchronised with a physiological function, such as the cardiac cycle [19].

In static mode, images of several planes through the body provide visual information showing differences in the accumulation of radiotracer in various tissues. Whilst such visual analysis can be a useful diagnostic tool, its validity is dependent on the experience of the observer [11]. Semi-quantitative objective measures may also be obtained from static images, such as the commonly used standardised uptake value (SUV). This measure normalises the decay-corrected activity in a given tissue \( A \) to the injected dose \( Q_{inj} \) and body surface area of the patient \( BSA \), and is given by the expression

\[
SUV(m^2/ml) = \frac{A(kBq/ml) \times BSA(m^2)}{Q_{inj}(kBq)}.
\]

In dynamic mode, a time-series of images are acquired, from which time-activity curves (TACs) can be generated for various tissues, which show the tracer kinetics. The behaviour of the tracer can then be described using a compartmental model, illustrated in Figure 2.4, from which pharmacokinetic parameters can be derived. An estimate of the rate of radiotracer delivery, known as the input function, is often required, and may be obtained, for example, by arterial blood sampling.

![Figure 2.4: A two-compartment model; \( C_P \) represents the concentration of tracer present in the blood plasma, \( K_1 \) and \( k_2 \) describe its exchange between plasma and tissue, and \( k_3 \) and \( k_4 \) its exchange between compartments within the tissue.](image)

The behaviour of a tracer is dependent on both the input function, and intrinsic response of the tissue, which is known as the Impulse Response Function (IRF). A tracer modelled as illustrated in Figure 2.4 can therefore be described by

\[
C_T(t) = C_P(t) \otimes IRF(t) = C_P(t) \otimes (\phi_1 e^{-\theta_1 t} + \phi_2 e^{-\theta_2 t})
\]
where $C_T(t)$ is the concentration of tracer in the tissue at time $t$, $\phi$ and $\theta$ are linear combinations of the $k$s, and $\otimes$ represents the mathematical operation of convolution. It is therefore clear that the general equation for the behaviour of a tracer described by an $n$-compartment model can be written as

$$C_T(t) = C_P(t) \otimes \left( \sum_{i=1}^{n} \phi_i e^{-\theta_i t} \right).$$

Given an appropriate choice of compartmental model, algorithms exist to calculate the values of the rate constants ($\phi_i$ and $\theta_i$) that best fit the observed data. It is important to note at this point that compartmental models represent a simplification of complex biological systems, and so the individual compartments and their corresponding rate constants may not necessarily represent any real divisions present in the tissue. The rate constants can, however, be used to calculate parameters that describe important physiological processes, such as the rate of cell proliferation in the case of FLT-PET, which is discussed in further detail in Section 3.2.

Alternatively, data-driven methods such as Spectral Analysis can be used, which do not require the selection of a particular compartmental model. Spectral Analysis involves the selection of a basis set

$$\psi_i = C_P \otimes e^{-\beta_i t}$$

such that the general equation describing the behaviour of the tracer can be written as

$$C_T = \sum_i \alpha_i \psi_i$$

and the problem is therefore reduced to that of determining the values of the coefficients $\alpha_i$ that provide the best fit to the observed data.
A critical factor in the development and progression of cancer is a loss of regulation of the cell cycle [16] which, under normal physiological conditions, ensures that an appropriate balance is maintained between proliferating and non-proliferating cells in a given tissue. At present, the evaluation of cell proliferation \textit{in vivo} generally involves the analysis of tissue samples by, for example, counting the percentage of cells undergoing mitoses during a microscopic analysis [24], or the immunohistochemical analysis of proteins such as Ki-67 [25]. This protein is present during all active phases of the cell cycle, but absent in non-proliferating cells, and the fraction of Ki-67-positive cells, known as the Ki-67 labelling index (Ki-67 LI), therefore provides a measure of the proliferation rate. This has been shown to be of prognostic value in several cancers, including breast cancer [26, 27].

However, taking tissue samples from a patient is an invasive procedure, and the results are prone to sampling errors due to, for example, intra-tumour heterogeneity or differences in pathology between primary tumours and metastases [11]. An alternative, non-invasive, and more easily repeatable method is therefore desirable, such as PET imaging with a tracer aimed at targeting cell proliferation. Since the incorporation of extra-cellular thymidine into DNA is considered a gold-standard measure for this process [11], there has been significant research into the development of thymidine analogues for such a use.

### 3.1 Thymidine Analogues in PET

In mammalian cells, the majority of thymidine is obtained \textit{via} the endogenous pathway, which involves the methylation of deoxyuridine monophosphate to produce thymidine monophosphate (TMP) [11], which is then phosphory-
lated twice before being incorporated into DNA [9]. Alternatively, TMP can be obtained directly via the salvage pathway, in which extra-cellular thymidine is transported into the cell by facilitated transport, and then phosphorylated by the enzyme thymidine kinase (TK) [11]. TK exists in two forms; cytoplasmic TK1, which is selectively upregulated just before and during the DNA replication phase of the cell cycle [11], and mitochondrial TK2, whose expression does not vary according to the cell cycle [8]. Before entering the DNA synthesis pathway, thymidine brought into the cell may instead be degraded by the enzyme thymidine phosphorylase (TP) [9].

In 1972, thymidine isotopically labelled with $^{11}$C, as shown in Figure 3.1 A, was proposed as a PET tracer for cell proliferation [28] and, since then, its potential has been investigated in humans with positive results [29, 30]. However, because of its short half-life, and rapid in vivo degradation into labelled metabolites, it is not practical for routine clinical use since, even with very careful kinetic modelling, accurate quantification is difficult to achieve [31].

![Figure 3.1: Thymidine analogues used in PET; (A) shows $^{11}$Cthymidine, and (B) shows $^{18}$Ffluorothymidine [8].](image)

An alternative is FLT, shown in Figure 3.1 B, a thymidine analogue originally developed as an anti-viral treatment for HIV [11]. Whilst its clinical use in that setting was limited due to toxicity at therapeutic doses [32], it is potentially valuable as a PET tracer when labelled with $^{18}$F, and at tracer doses it is non-toxic [9], and produces a radiation dose within the accepted limits [33]. It also has the advantages of having a longer half-life than $^{11}$Cthymidine and being resistant to degradation in vivo, as it is not a substrate for TP [9].

FLT follows the same salvage pathway as thymidine but, since it is not significantly incorporated into DNA, it becomes trapped within cells [11].
Unlike thymidine, it is a substrate for only the cell-cycle dependent TK1, and its accumulation in cells is therefore proportional to the activity of this enzyme, which in turn is tightly regulated to correspond with cell proliferation [9]. The retention of FLT in cells therefore provides an indication of their proliferation rate.

### 3.2 Kinetic Modelling of FLT

For clinical applications, FLT retention is generally quantified using the SUV, whose calculation does not require either the arterial input function or particular modelling expertise. However, there is ongoing investigation into the potential of using kinetic modelling to obtain fully quantitative measures which may provide better correlation with the rate of cell proliferation.

The behaviour of FLT and its radiolabelled metabolite, FLT-glucuronide, can be described by the compartmental model illustrated in Figure 3.2. The rate constant $k_4$ is zero because the efflux of phosphorylated FLT (FLT-P) from tissue is negligible over the timescale of PET image acquisition.

The retention of FLT in tissue can be quantified using a modification of the graphical analysis technique first described in 1987 by Herholz and Patlak [34]. At the later stages of a PET scan, the retention of FLT is dominated by its irreversible uptake from plasma to tissue, and the associated rate constant, $K_i$, can be calculated, using linear regression, from the curve with equation

$$\frac{C_T(t)}{C_P(t)} = K_i \int_0^t \frac{C_P(t)d\tau}{C_P(t)} + constant.$$
In the standard Patlak method, it is assumed that there is a single radiotracer and, for use with FLT, the method must therefore be modified to account for the presence of the radiolabelled metabolite FLT-glucuronide, which is assumed to have no irreversible uptake.

### 3.3 Correlation with Immunohistochemistry

A number of recent studies have compared the retention of FLT, quantified using the SUV, with Ki-67 immunohistochemistry, and the results are summarised in Table 3.1. The correlation is described by the Pearson product moment correlation coefficient ($-1 \leq r \leq +1$), which provides a measure of the linear relationship between variables, with $r > 0$ representing positive correlation and $r < 0$ negative correlation.

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Number of Patients</th>
<th>Correlation ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>12</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.01</td>
</tr>
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<td>0.84</td>
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<td>9</td>
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<td></td>
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<td>0.60</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.84</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>21</td>
<td>0.84</td>
</tr>
<tr>
<td>Oesophageal</td>
<td>10</td>
<td>-0.74</td>
</tr>
</tbody>
</table>

Table 3.1: Correlation ($r$) between the SUV and Ki-67 LI for breast cancer [17, 35], glioma [36, 37], lung cancer [38, 39, 40, 41], lymphoma [42], and oesophageal cancer [43].

In interpreting these results, it is important to note that the way in which the SUV is determined varies between studies. For example, the time after which it is calculated varies, as does the activity chosen to represent the tissue, with some using the highest intensity pixel in the region, and others the mean intensity of all pixels in the region above some threshold value. The lack of correlation observed in the second breast cancer study, and the negative correlation observed for oesophageal cancer could therefore be attributed to the use of sub-optimal methods for calculating the SUV.

Further studies of the correlation of FLT retention with pathology-based assessment should be performed, both to validate these existing results, and to extend the investigation across the whole range of cancer types. It will
also be important to determine the best method for evaluating the SUV, both to ensure that future studies are comparable, and to give this measure of proliferation a clinical role in determining tumour aggressiveness.

Studies by Kenny et al. [17] and Vesselle et al. [41] have investigated the potential of quantifying the retention of FLT using the modified Patlak method described in Section 3.2. Correlations of $r = 0.92$ and $r = 0.94$ were observed in breast [17] and lung [41] cancer respectively, compared with correlations of $r = 0.79$ and $r = 0.84$ obtained using the SUV analysis. These results demonstrate that significantly improved correlation may be obtained with the use of kinetic modelling although, due to its added complexity, this may be applicable only in a research setting, and not routine clinical practice.

3.4 Clinical Applications

The role of FLT-PET in detection and staging has been investigated in various cancers [9], and it appears that, whilst it may provide greater specificity for malignancy than FDG-PET [40, 42], the lower uptake of FLT compared with that of FDG results in a lower overall sensitivity [36, 40]. It is therefore unlikely to replace FDG-PET as a tool for diagnosis and staging, although the two could be used together to provide additional specificity.

The roles of FLT-PET are more likely to involve the prediction and monitoring of tumour response to therapy, as well as determination of tumour aggressiveness, and the corresponding patient prognosis, for which it is expected to be more suitable than FDG.

Present radiological studies of tumour response to treatment rely on an assessment of the change in tumour volume, generally using the RECIST criteria [44], which are given in Table 3.2. When using these criteria, the response of the tumour is assessed by measuring the change in length of the maximum tumour diameter, and assuming that this provides an accurate guide to the overall change in size [11].

<table>
<thead>
<tr>
<th>Complete response</th>
<th>Complete resolution of all the target lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial response</td>
<td>Decrease of ≥ 30% of the longest diameter</td>
</tr>
<tr>
<td>Stable disease</td>
<td>Neither partial response nor progressive disease</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>More than a 20% increase in size</td>
</tr>
</tbody>
</table>

Table 3.2: The RECIST criteria used in radiological assessment of tumour response to treatment, in which the change in size is determined by comparing measurements of the maximum tumour diameter [11].
A small number of studies have investigated the potential application of FLT-PET in predicting and monitoring tumour response to therapy in breast cancer [45, 46] and lymphoma [47], with positive findings.

In a study of 13 patients with locally advanced or metastatic breast cancer, Kenny et al. [45] performed FLT-PET scans both before and one week after starting chemotherapy treatment. The changes in FLT retention, quantified using both the SUV and $K_i$ from a Patlak analysis, were compared with the clinical response determined using the RECIST criteria. The tumour size was measured using either electronic callipers or a CT scan both before and 60 days after starting the treatment. It was found that patients with either a complete or partial clinical response at day 60 also showed a reduction in both the SUV and $K_i$ after one week. Figure 3.3 shows the FLT-PET scans of both a clinical responder and non-responder.

Figure 3.3: FLT-PET images obtained by Kenny et al. for breast cancer patients; (A) and (B) show pre and post-treatment images of a patient whose cancer responded to chemotherapy treatment, and (C) and (D) images of a patient whose cancer did not respond [45].

In a similar study of 14 patients with newly diagnosed primary or metastatic breast cancer, Pio et al. [46] performed FLT-PET scans both before and two weeks after the first cycle of either cytotoxic or hormone-only treatment. Good positive correlation ($r = 0.74$) was found between the change
in FLT retention, quantified using the SUV, and the change in tumour size determined from a CT scan taken around three months after starting the treatment.

Finally, in a study of 22 patients with high-grade non-Hodgkin’s lymphoma, Hermann et al. [47] performed FLT-PET scans both before and one week after starting chemotherapy treatment. It was found that the reduction in FLT retention, quantified using the SUV, correlated well with the clinical response determined, using the RECIST criteria, from a CT scan taken three months later.

These results are encouraging, and suggest that a reduction in FLT retention as soon as one week after starting therapy can provide an indication of whether the treatment is likely to be successful. This will be of particular use when treating patients with cytostatic agents, for which a change in tumour volume will not necessarily occur following successful treatment [9], as they cause cell-cycle arrest, rather than cell death. It seems likely that this will be the main area of application of FLT-PET, and further studies should be performed to validate these results across all cancers.
Chapter 4

Image Segmentation

The ability to accurately segment an image into its constituent tissue types is essential, both to aid in the visualisation of structures, and to define the regions to be analysed in a quantitative study. A method that is widely used in both clinical practice and research is for a clinician to manually draw regions of interest (ROIs) onto the image but, as well as being time-consuming, the success of this method is dependent on the experience of the clinician. In some cases, it may be possible to transform the data to fit a standard anatomic atlas, but this is unlikely to be suitable for use in oncology due to problems caused by the presence of pathology in the image. Alternatively, the PET image can be coregistered with a corresponding anatomic image, whose segmentation results may then be used as a guide. However, the process of image registration between modalities is not trivial, and this method does not remove the need for image segmentation.

The automatic, or semi-automatic, segmentation of PET images is challenging, and conventional techniques that rely only on the spatial information can produce inaccurate results due to difficulties in differentiating between adjacent tissues with different functions. The segmentation may be improved by the use of techniques, such as those described in this Chapter, that exploit the fact that tissues with different functions are likely to have differing kinetic properties.

4.1 Cluster Analysis

The aim of a cluster analysis is to partition a large number of objects into a finite number of groups, or clusters, according to certain criteria, such that objects within a cluster are similar, but objects in different clusters are dissimilar. There are a number of clustering algorithms available but,
in order to demonstrate the application of the technique to dynamic PET imaging, the commonly used example of k-means clustering is described [48], with an illustration of the process shown in Figure 4.1.

It is assumed that the image contains a finite number of tissue types, each with a unique kinetic behaviour, and that it can therefore be divided into a finite number of clusters. Following initialisation of the cluster centres, each pixel is allocated to the cluster for which the distance between its TAC and that of the cluster centre is minimised, according to the chosen metric. The centre point of each cluster is then re-calculated as the average TAC of the pixels it contains. This whole process is repeated until the chosen convergence criterion is met, for example that the pixel assignments to clusters no longer change following further iteration.

Figure 4.1: The k-means clustering algorithm; (A) shows the initialisation of cluster centres, (B) the initial groupings of pixels into clusters, (C) the re-calculation of cluster centres, and (D) the final segmented image obtained after several iterations [49].

The value of performing cluster analysis on PET data is clear, and current research is therefore concentrated on refining the algorithms to produce the most accurate possible segmentation in the shortest possible time, such as the two-stage cluster analysis proposed by Guo et al., in which the introduction of a pre-clustering step was found to significantly reduce the time required to perform the segmentation of brain data [50]. Another area of recent research is into techniques that utilise both the temporal and spatial characteristics of the data, such as the study performed in 2006 by Kim et al., in which cluster analysis was followed by a region-growing approach [51]. The resulting hybrid technique was found to produce ROIs with better separation from the background than standard clustering algorithms, for both simulated phantom data and clinical brain data.

It should, however, be noted that clustering algorithms are often very sensitive to both the number of clusters chosen, and the initial selection of their centres. As a result, it may be necessary to run an algorithm several times,
with different initial conditions, in order to obtain a satisfactory solution. In 2007, Dueck and Frey developed a new method, known as affinity propagation, in which all pixels are simultaneously considered as potential cluster centres [52]. Although this technique has yet to be applied to PET data, for a variety of other applications it has been found to produce a solution much more rapidly, and with a lower error than other clustering methods.

### 4.2 Principal Component Analysis

Principal Component Analysis (PCA) uses simple matrix algebra to transform an image to a new orthonormal coordinate system, whose basis vectors point in the directions associated with variances of the data. Such a coordinate transformation is achieved by finding the variance-covariance matrix of the image data, and calculating its eigenvalues and the corresponding eigenvectors, which then form the basis of the new coordinate system [53]. The eigenvector associated with the largest variance is known as the first principal component, that associated with the next largest variance as the second principal component, and so on.

![Figure 4.2: Projections along the first three principal components; (A) before noise-normalisation, and (B) after, in which the epileptic focus is clearly visible as the brightest region in the image of the second principal component [54].](image)

PCA can be applied to reduce the dimensionality of image data, based on
the assumption that directions associated with the largest variances contain interesting data, whilst those associated with the smallest variances contain mostly noise. However, this is not necessarily the case for PET data, due to the inherently poor signal-to-noise ratio of the modality. It can also be used to aid in visualisation, since different tissues can be emphasised by projecting the image along the direction of a particular eigenvector. However, a study performed in 1994 by Pedersen et al. found that the initial results obtained using this approach were unsatisfactory, as shown in Figure 4.2 A. When PCA was instead applied to noise-normalised data, the results were significantly improved, as shown in Figure 4.2 B, and the technique was successful in enhancing the visualisation of structures of interest in the brain, heart and abdomen [54].

4.3 Independent Component Analysis

Independent Component Analysis (ICA) relies upon the assumption that an image contains a finite number of tissue types, each of which has a unique kinetic behaviour that is distinguishable from that of every other tissue type. The TAC of an individual pixel can therefore be made up from a linear combination of the TACs of these tissue types. The image can be transformed, using matrix algebra, such that the independence of these components is maximised, and this transformation then used to segment the image, such that each resulting region contains pixels belonging to the same tissue type [53].

ICA has been successfully applied to separate the cardiac components, thus allowing the left ventricular input function to be derived, and compared with that obtained using arterial blood sampling [55]. This method could potentially provide an alternative, and less invasive, way to obtain the input function required for kinetic modelling.

4.4 Applications in Oncology

Although the majority of studies to date have been performed in the brain and heart, there are potential applications in the field of oncology. For example, image segmentation could be employed to allow the clear visualisation of tumours with respect to the surrounding tissues, which would result in a more accurate quantification of their behaviour. This could then prove useful in monitoring therapy response, determining patient prognosis, or in radiation therapy planning. At present, CT images are generally used to delineate tumours prior to radiotherapy, but it is thought that the use of PET
may be beneficial, for example it may allow the dose delivered to be boosted
to the most metabolically active regions of the tumour, in order to have
the maximum effect [56]. However, image segmentation poses a much more
challenging problem if the whole body is to be considered, as is necessary in
oncological studies.
Chapter 5

FLT-PET Image Dataset

The dataset used for this project contained dynamic FLT-PET images, and the corresponding blood data, from 15 patients with breast cancer, who were attending the Hammersmith and Charing Cross Hospitals in London. The patients were scanned between 2003 and 2005, and the results of two previous analyses of the data have been published by Kenny et al. [17, 45], and were discussed in Sections 3.3 and 3.4.

5.1 Patient Details

Patients aged between 18 and 80 with histologically proven American Joint Committee on Cancer stage II-IV breast cancer [57] that were to be treated with combination 5-fluorouracil, epirubicin and cyclophosphamide chemotherapy were eligible. The patients were receiving no active treatment at the time of the study, and had been treatment free for at least three weeks for effective cytotoxic chemotherapy or hormonal therapy, and at least four weeks for radiotherapy. Between the 15 patients, there were a total of 21 discrete lesions, including 13 primary tumours and 8 metastases, with a range of proliferation rates, as detailed in Table 5.1.

Three FLT-PET scans were scheduled for each patient, including two reproducibility scans prior to treatment, and one further scan approximately one week after treatment commenced. Of the 15 patients studied, 9 successfully completed two pre-treatment scans, and 14 completed both a pre-treatment and post-treatment scan, providing a total of 38 FLT-PET images. Tumour biopsies were taken from the patients prior to treatment, and the proliferation rates were assessed using the Ki-67 LI, with suitable histology available from 12 of the 15 patients.
Table 5.1: Patient details; all primary tumours are in the breast, and axillary, mammary and pretracheal nodes refer to the lymph system. Numbers in brackets show into how many regions each primary tumour was divided for analysis, although in patient 4, where the primary tumour was very large, the whole tumour and its rim were sampled in addition.

5.2 PET Imaging

Each patient was intravenously administered with a tracer dose (between 151 and 380 MBq, median 338 MBq) of FLT, which was synthesised by Hammer-smith Imanet using the method described by Cleij et al. [58]. Patients were then scanned for 95 minutes using an ECAT962/HR+ PET scanner, which allows the simultaneous acquisition of data to form 63 trans-axial planes. The data were then binned into 31 discrete time frames of varying duration, including ten frames of 30 seconds, five of 60 seconds, five of 120 seconds, five of 180 seconds and six of 600 seconds.

The images were reconstructed using the FBP technique described in Section 2.4 and, from the summed image data, ROIs were defined manually by the same observer using the Analyze image analysis software\(^1\). All planes showing an identifiable tumour uptake were delineated, as well as areas of the heart, liver, vertebra, lung, and normal breast. Large tumours were divided into several regions for analysis, as detailed in Table 5.1, and areas of obvious

\(^1\)Analyze is a Biomedical Imaging Resource from the Mayo Foundation
necrosis were excluded.

As explained in Section 2.5, to allow for a complete analysis of the images, including the use of kinetic modelling, the activity of the injected radiotracer in the blood plasma, known as the parent plasma input function, is required. Arterial blood sampling was therefore performed continuously for the first ten minutes of scanning, and discrete arterial samples were also taken at baseline, and after 2.5, 5, 10, 20, 30, 45, 60, 75 and 90 minutes. The total blood radioactivity was then determined from both the continuous and discrete samples by gamma counting.

However, to obtain the parent plasma input function, two corrections had to be applied to this measured radioactivity, and the required information was obtained from the discrete samples. The first correction accounts for the fact that only tracer that is free in the blood plasma is of relevance, and the second for the metabolism of FLT. The blood was centrifuged to separate the plasma, whose radioactivity was then determined by gamma counting, and the fraction of FLT, as opposed to its radiolabelled metabolite, was determined using reversed-phase high performance liquid chromatography with radiochemical detection.

5.3 Initialisation

As mentioned in Section 2.4, one of the problems associated with the FBP method of reconstruction is the presence of artefacts in the final images, which degrade their quality. An image reconstructed using FBP is shown in Figure 5.1 A, in which streaks from the regions of high intensity can be seen across the image.

![Figure 5.1: A single slice, at the level of the liver, shown reconstructed using (A) FBP, and (B) OSEM.](image)

Since the determination of accurate TACs for the various tissues was
essential to this project, it was decided that images reconstructed using an iterative method should instead be used. The OSEM algorithm, described in Section 2.4, was applied, using 360 iterations and six subsets, as these parameters provided an appropriate reconstruction speed, whilst maintaining the quantitative nature of the data. The same image reconstructed using OSEM is shown in Figure 5.1B, in which streak artefacts are not visible.

Following the successful reconstruction of all 38 images using the OSEM algorithm, a statistics file was obtained from each dynamic image using Analyze. This file contains information about every ROI in the image, including the mean and standard deviation activity at every slice, and every time frame. From these statistics files, a TAC was generated for every ROI in every image using Clickfit$^2$.

### 5.4 Preliminary Study

Before commencing work on the possible filtering techniques, a preliminary study of the images was performed. Slices from an image at the level of the liver and heart are shown in Figure 5.2 and, as expected, high activity is observed in the heart, liver and vertebra, as well as in the tumour, whereas the lung and normal breast tissue show relatively low activity.

![Figure 5.2: Image at the level of (A) the liver, and (B) the heart, showing the distribution of FLT within the body.](image)

All tumours show both uptake and retention of FLT, although heterogeneity was observed, both within a single tumour and between different tumours in the same patient. This clearly illustrates the fact that a tissue biopsy may not be the ideal way to assess proliferation, since such samples are not necessarily representative.

$^2$Clickfit is an in-house MATLAB-based toolbox
The TACs were also examined, and typical curves for tissues showing high activity are illustrated in Figure 5.3, from which it is clear that the kinetic behaviour of FLT does vary between tissues. For example, no significant retention occurs in the heart, but there is rapid uptake as blood containing the tracer passes through, whereas both uptake and retention are observed in the liver, vertebra and tumour. However, since FLT is metabolised in the liver, its retention decreases with time in this organ, whereas in the tumour and vertebra, both of which proliferate rapidly, such reduction in retention does not occur.

It appears that the behaviour of FLT in all tissues becomes stable around 15 minutes after injection, and no new kinetic information is therefore gained after this time. As a result, studies aiming to exploit the tracer kinetics could be significantly shorter, which would be beneficial if any such technique were to be applied clinically. For the purposes of quantification, it would, however, be important to allow sufficient time to achieve the linear behaviour required for the calculation of $K_i$.

Figure 5.3: TACs from patient 14 for tissues showing high activity, normalised by the injected dose, but not corrected for radioactive decay. This patient moved during the final time frame, and the corresponding data points are therefore artificially low.
Chapter 6
Filtering

This first approach involved the development of several kinetic filters, which aimed to remove the signal from selected healthy organs, whilst retaining that from tumours. The healthy organs selected included the liver, heart and vertebra, since these show high signal intensities on FLT-PET scans, as described in Section 5.4, and could therefore potentially conceal tumours.

The filters were designed following similar principals to the PCA and ICA techniques described in Sections 4.2 and 4.3, in that they involve transforming the coordinate system of the image, in order to highlight new features. In this case, the transformation was chosen such that an improved contrast-to-noise ratio (CNR) between tumour tissue and the selected healthy organ might be achieved.

6.1 Methods

The methods employed involved projecting the dynamic image along a particular direction, either orthogonal to the TAC of the organ to be removed, or along the direction of maximum variance between either the TACs or IRFs of the tumour and this organ. The potential of combining such filters with an additional denoising technique was also investigated. The filters were designed and optimised using only a subset of the 38 available images, including pre-treatment scans from patients 4, 6, 11, 12 and 14, which contained tumours with a range of Ki-67 LI, as detailed in Table 5.1.

6.1.1 Orthogonal Projection

Each of the five images was projected along the direction orthogonal to the TAC of the liver, heart and vertebra. This technique aimed to remove signal
CHAPTER 6. FILTERING

from the selected organ as illustrated in Figure 6.1.

Figure 6.1: Illustration showing projections orthogonal to the direction of the liver. The green arrow represents a liver TAC, whose intensity is reduced to zero upon projection. The black arrow represents a tumour TAC, whose reduction in intensity is dependent on its similarity to the liver TAC.

6.1.2 Maximum Variance

For each of the five images, a PCA approach was used to determine the direction of maximum variance between the TACs of the liver and tumour ($\sigma_t^2$), and the image was then projected along this direction. In effect, this means that a weighting was assigned to each time frame, such that the summed image data amplified differences between the two tissues. The image was then also projected along the direction of maximum variance between the IRFs of the liver and tumour ($\sigma_i^2$), for which a bar chart of the weightings is displayed in Figure 6.2 A.

Figure 6.2: Bar charts showing the weightings assigned to each time frame for projection along the direction of (A) $\sigma_t^2$, (B) 10,000 $\sigma_i^2$, (C) 0.1 $\sigma_i^2$.
The potential to further amplify the observed differences was investigated, by using a non-linear function to scale the weightings assigned to each time frame, as illustrated in Figures 6.2 B and C.

6.1.3 Wavelet Denoising

Wavelets are mathematical functions which can be used to decompose a signal into different frequency components [59], in much the same way as Fourier analysis can be used to reduce a signal to a series of sines and cosines. With wavelets, however, the spatial information is retained, and one application is to the denoising of signals without smoothing out discontinuities [59].

When a signal is decomposed using wavelet analysis, the resulting coefficients may be divided into the low frequency components representing the main features of the signal, and high frequency components representing fine details and, potentially, noise. The signal can therefore be denoised by setting these high frequency components to zero, and the application of this technique to a two-dimensional image is illustrated in Figure 6.3.

![Figure 6.3: An illustration of two-dimensional wavelet space, in which the coefficients have been divided into low and high frequency components in each direction. The image is denoised by setting all components to zero, except for those in the bottom left-hand quadrant.](image)

This denoising technique was applied, using a utility from the PiWave software\(^1\), to the images obtained following the orthogonal projection method. It was also incorporated into the maximum variance filtering method, such

\(^1\)PiWave is an in-house Matlab-based software for parametric imaging
that the filter was effectively applied only to the lowest frequency wavelet components, with all others set to zero.

6.2 Results

The performance of each filter was assessed both qualitatively, by examination of the resulting images, and quantitatively, by determination of their CNRs. For the five images, the contrast and noise were calculated after the application of each filter, according to the following expressions

\[
\text{contrast} = \frac{\mu_t}{\mu_o} \quad \text{and} \quad \text{noise} = \frac{\sigma_t}{\mu_t}
\]

in which \(\mu_t\) and \(\sigma_t\) are the mean and standard deviation activities in the tumour, and \(\mu_o\) the mean activity in the healthy organ under consideration. These activities were determined from the summed image data, by averaging over all slices containing the relevant tissue. The values obtained were then compared with those from the original images, and the tabulated results are expressed as the ratio of the CNR from the filtered image to that from the original image. A value greater than one therefore means that the filter produced improvement, either by increasing the contrast or reducing the noise.

The results obtained following application of the orthogonal projection method are displayed in Table 6.1, and demonstrate significant improvement in the CNR between heart and tumour tissue across all images. Improved CNR between liver and tumour tissue was, however, observed only for images showing rapidly proliferating tumours, and the variation in CNR between vertebra and tumour tissue was inconsistent across the five images.

<table>
<thead>
<tr>
<th>Patient (Ki-67 LI)</th>
<th>Filtered</th>
<th>Filtered and Denoised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
</tr>
<tr>
<td>4 (45.22%)</td>
<td>110</td>
<td>7.50</td>
</tr>
<tr>
<td>6 (33.57%)</td>
<td>215</td>
<td>9.73</td>
</tr>
<tr>
<td>11 (3.53%)</td>
<td>134</td>
<td>0.48</td>
</tr>
<tr>
<td>12 (8.85%)</td>
<td>50.2</td>
<td>0.93</td>
</tr>
<tr>
<td>14 (29.7%)</td>
<td>244</td>
<td>19.2</td>
</tr>
</tbody>
</table>

Table 6.1: Quantitative results obtained following projection of the five images orthogonal to the TACs of the heart, liver and vertebra, both before and after wavelet denoising.
The subsequent application of wavelet denoising produced further improvement of all results, although a reduction in CNR between vertebra and tumour tissue was still observed in some cases.

The results obtained following application of the maximum variance methods are displayed in Table 6.2, and demonstrate that, whilst projection along the direction of $\sigma^2_t$ resulted in a reduced CNR for almost every image, projection along the direction of $\sigma^2_i$ gave slightly improved results.

<table>
<thead>
<tr>
<th>Patient (Ki-67 LI)</th>
<th>TAC</th>
<th>IRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (45.22%)</td>
<td>0.92</td>
<td>1.17</td>
</tr>
<tr>
<td>6 (33.57%)</td>
<td>0.91</td>
<td>1.13</td>
</tr>
<tr>
<td>11 (3.53%)</td>
<td>1.01</td>
<td>1.10</td>
</tr>
<tr>
<td>12 (8.85%)</td>
<td>0.98</td>
<td>1.11</td>
</tr>
<tr>
<td>14 (29.7%)</td>
<td>0.93</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Table 6.2: Quantitative results obtained following projection of the five images along the direction of maximum variance between the TACs and IRFs of the liver and tumour. The results obtained following additional non-linear scaling and wavelet denoising are also shown.

Non-linear scaling by a function of $10,000 \sigma^2_i$ produced further improvement, whilst scaling by $0.1\sigma^2_i$ resulted in a reduction in CNR for every image. The combined effect of wavelet denoising and projection along $\sigma^2_i$ produced the most significant improvement, with a CNR increase of around a factor of three observed for each of the five images.

6.3 Discussion

The orthogonal projection method was found to be capable of removing signal from the heart, liver and vertebra, although only projection orthogonal to the TAC of the heart resulted in a significant retention of tumour signal that was consistently observed across the five images. These differences in behaviour can be explained by a consideration of the TACs of the various tissues, examples of which were displayed in Figure 5.3. Whereas the TAC of the heart differs significantly from that of the tumour at all times, those of the liver and vertebra are more similar, and projection orthogonal to these TACs therefore results in the simultaneous removal of a proportion of the tumour signal, as illustrated in Figure 6.1. Since the signal in both vertebra and tumour tissue is caused by rapid proliferation, the observed similarity between their TACs was anticipated, and the potential to remove vertebra
signal using a filtering approach was not considered further. It was observed that only signal from the more rapidly proliferating tumours was significantly retained following projection of the image orthogonal to the TAC of the liver. This could be due to the higher degree of irreversible retention exhibited by such tumours, which means that they display particularly high signal intensity in the later time frames, during which signal in the liver is at a minimum.

Since the detection of tumours located in or near the liver is so important, and the orthogonal projection method achieved only partial success, the removal of this organ by filtering techniques was further investigated. The maximum variance method was found to produce improved CNRs when the image was projected along the direction of maximum variance between the IRFs, but not when projected along the direction of maximum variance between the TACs. This could be because convolution of the IRF with the plasma input function, to create the TAC, smooths out some of the variation between the intrinsic responses of these two tissues, and thus greater variation may be observed by considering the IRFs than the TACs. Some further improvement was obtained by scaling the weightings applied to each time frame using a non-linear function that further amplified the later time frames, during which the liver signal intensity was lowest. The combined effect of wavelet denoising and projection along the direction of maximum variance between the IRFs produced the most significant improvement. However, this could be attributed mainly to the denoising procedure, and even the observed factor of three improvement in the CNRs did not produce significant visual difference in the images.

In conclusion, it appears that filtering techniques of this kind may only be successfully used to remove the signal from organs whose TAC differs significantly from that of the tumour. For other tissues, whose TAC is more similar, the technique exhibits only partial success and, although slight improvements were made, they were not sufficient to be of clinical use.
Chapter 7

Classification

Following the partial success of the filtering technique described in Chapter 6, it was decided to next approach the problem as that of a linear classification. This approach shares several features of the cluster analysis technique described in Section 4.1, in that its overall aim is to partition a large number of objects into a finite number of groups, according to certain criteria. A classification algorithm, however, assigns each object to one of several groups, or classes, with predefined properties, unlike a cluster analysis, in which only the total number of groups is defined.

For this project, each image pixel was to be classified according to the tissue type it was most likely to represent, based on a comparison of its time profile with those of the predefined classes. Images of only pixels likely to show tumour tissue could therefore be produced, by setting the intensities of all other pixels to zero.

7.1 Methods

The image data were first used to generate average TACs for each of the major tissue types present, including the heart, lungs, liver, tumour, normal breast and vertebra. An additional background curve was also defined, to fit pixels located within the field of view, but outside the body of the patient. These then provided the set of predefined classes, according to whose properties the images were to be classified.

Although it was intended that data from all the available images should be used, only 28 proved to be suitable. The scans from patients 2 and 10 were rejected because the liver was not present in the field of view, along with others displaying artefacts such as contamination from the injection site in the field of view, or image degradation caused by patient motion.
7.1.1 Generation of Classes

In order to obtain truly representative classes, the TACs from every image were first normalised, according to the dose with which the patient had been injected. The mean and standard deviation activity were then calculated for every tissue type, using all 28 curves, at each of the 31 mid-frame times. The additional background curve was created with a mean of 0, and standard deviation of 0.005 at every mid-frame time, and the resulting curves for the highest activity tissue types are shown in Figure 7.1.

For scans which had several TACs for a given tissue type, such as in larger tumours which were divided into regions, the mean of the TACs for that tissue type was used in generating the relevant class. This was done to avoid the introduction of unnecessary bias towards a particular patient.

Only primary breast tumours, and not their metastases, were considered in the generation of classes since, with only five metastases of different types between the patients, there were insufficient data to determine whether they should be included with the primary tumours, or allocated a separate class.
7.1.2 Classification Algorithm

The image to be classified was first normalised to account for the dose with which the patient had been injected, and then each pixel in turn was compared against the seven predefined classes. The time profile of an individual pixel was, however, too noisy to allow the accurate determination of the tissue type it represented and, in performing comparisons, the mean time profile obtained from a pixel and its six nearest neighbours was therefore considered, which was far less noisy, as illustrated in Figure 7.2.

![Figure 7.2: Time profiles of an individual pixel from the heart region, the mean obtained from this pixel and its six nearest neighbours, and the TAC obtained by sampling the whole heart, taken from an image of patient 14.](image)

The likelihood for a pixel to represent each of the possible tissue types was assessed using a distance measure known as the Mahalonobis distance \([60]\), \(D_M\). This is given by the expression

\[
D_M = \sqrt{\sum_{t=1}^{31} \left( \frac{p_t - \mu_t}{\sigma_t} \right)^2}
\]

in which \(p\) represents the activity of the pixel under consideration, and \(\mu\) and \(\sigma\) the mean and standard deviation activities respectively of the class it is being compared against.

It was determined that if the smallest distance was to either the tumour or vertebra class, and the four largest were to the heart, lungs, normal breast
and background classes, in any order, then the pixel was likely to represent tumour tissue. To obtain the filtered image, the intensities of all pixels which did not satisfy these conditions were set to zero, thus producing an image of only those pixels likely to represent tumour tissue.

7.2 Results

The classification algorithm was applied to all 28 images, and five were then selected for further analysis, from patients 1, 3, 4, 6, and 14. The ability of the filter to remove signal from the liver, heart and vertebra, whilst retaining that from tumours, was assessed quantitatively, by calculation of the SUV at 60 minutes for these four tissue types, using the expression given in Section 2.5. The tabulated results are expressed as the percentage reduction in SUV between the original and filtered images for each tissue type. Studies were also performed to investigate the possibilities of using shorter scan times, and applying the technique to images reconstructed using FBP.

7.2.1 Filter Validation

In order to validate the potential application of the classification algorithm to new scans, whose data were not used to generate the predefined classes, a technique known as leave-one-out cross-validation was used. This means that, for each image to be classified, the predefined classes were generated using data from the remaining 27 images.

A slice at the level of the liver, both before and after filtering, is shown in Figure 7.3. This result is representative of the findings observed across all 28 images, and clearly illustrates the almost complete removal of liver signal.

![Figure 7.3: A slice at the level of the liver both before (left) and after (right) application of the classification algorithm. This particular image is taken from patient 14, but it is representative of the results observed in all 28 images.](image)

Figure 7.3: A slice at the level of the liver both before (left) and after (right) application of the classification algorithm. This particular image is taken from patient 14, but it is representative of the results observed in all 28 images.
For each of the 13 patients, a slice at the level of the primary breast tumour, both before and after filtering, is shown in Figure 7.4. These images illustrate the almost complete removal of signal from both the heart and liver, as well as significant reduction in that from the lung and normal breast tissue. Whilst the majority of signal from the primary tumours is retained, some reduction is observed, particularly in the larger, or more heterogeneous, tumours. The vertebra does not appear to show either consistent removal or retention across the images.
Figure 7.4: Slices at the level of the primary breast tumour both before (left) and after (right) application of the classification algorithm, with tumours indicated by the white arrows.

For the relevant four patients, a slice at the level of each metastatic tumour, both before and after filtering, is shown in Figure 7.5. As for the primary tumours, the majority of signal from the metastases is retained, except in the case of the rib metastasis of patient 4, whose signal is almost entirely removed by the filtering process.
Quantitative results describing the efficiency of this filtering technique are presented in Table 7.1, which fully support the statements made so far, that signal from the heart and liver is almost entirely removed following filtering, whilst the majority of tumour signal is retained, and the vertebra exhibits inconsistent behaviour.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Liver</th>
<th>Heart</th>
<th>Vertebra</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.48</td>
<td>84.38</td>
<td>13.74</td>
<td>2.36</td>
</tr>
<tr>
<td>3</td>
<td>86.07</td>
<td>89.38</td>
<td>44.07</td>
<td>16.56</td>
</tr>
<tr>
<td>4</td>
<td>91.37</td>
<td>88.82</td>
<td>45.23</td>
<td>28.15</td>
</tr>
<tr>
<td>6</td>
<td>92.51</td>
<td>90.87</td>
<td>10.16</td>
<td>8.47</td>
</tr>
<tr>
<td>14</td>
<td>97.14</td>
<td>87.59</td>
<td>23.17</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Table 7.1: Percentage reductions in SUV between the original and filtered images, calculated for the tissue types which show high signal intensities in FLT-PET images.

### 7.2.2 Scan Acquisition Time

The potential application of this technique to scans obtained using a shorter acquisition time was investigated, by performing the comparisons between pixel time profiles and predefined classes using only a sample of the available kinetic information. Three studies were performed, which made use of only
data obtained during the first 60, 30, and 15 minutes after injection. Both
the images displayed in Figure 7.6, and quantitative results presented in
Tables 7.2–7.4, clearly illustrate the almost complete removal of both liver
and heart signal for all three studies. However, as the amount of data used
in the classification was reduced, so was the observed retention in tumour
signal.

Figure 7.6: Slices at the level of the liver and tumour after filtering, with only data
obtained during the first (A) 60, (B) 30, and (C) 15 minutes after injection used
to perform the classification. These particular images are taken from patient 14,
but are representative of the results observed across the five images.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Liver</th>
<th>Heart</th>
<th>Vertebra</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.38</td>
<td>86.36</td>
<td>22.39</td>
<td>4.02</td>
</tr>
<tr>
<td>3</td>
<td>86.40</td>
<td>90.78</td>
<td>47.69</td>
<td>18.95</td>
</tr>
<tr>
<td>4</td>
<td>91.54</td>
<td>90.57</td>
<td>62.01</td>
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<td>6</td>
<td>92.63</td>
<td>92.41</td>
<td>15.64</td>
<td>8.89</td>
</tr>
<tr>
<td>14</td>
<td>97.23</td>
<td>88.77</td>
<td>32.35</td>
<td>1.51</td>
</tr>
</tbody>
</table>

Table 7.2: Percentage reductions in SUV between the original and filtered images,
when using only data obtained during the first 60 minutes after injection to perform
the classification.
CHAPTER 7. CLASSIFICATION

<table>
<thead>
<tr>
<th>Patient</th>
<th>Liver</th>
<th>Heart</th>
<th>Vertebra</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.09</td>
<td>89.49</td>
<td>27.99</td>
<td>13.96</td>
</tr>
<tr>
<td>3</td>
<td>88.26</td>
<td>92.45</td>
<td>49.95</td>
<td>25.35</td>
</tr>
<tr>
<td>4</td>
<td>92.56</td>
<td>91.52</td>
<td>68.29</td>
<td>32.23</td>
</tr>
<tr>
<td>6</td>
<td>93.44</td>
<td>94.67</td>
<td>20.09</td>
<td>10.48</td>
</tr>
<tr>
<td>14</td>
<td>97.63</td>
<td>90.53</td>
<td>40.42</td>
<td>6.48</td>
</tr>
</tbody>
</table>

Table 7.3: Percentage reductions in SUV between the original and filtered images, when using only data obtained during the first 30 minutes after injection to perform the classification.

<table>
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<th>Liver</th>
<th>Heart</th>
<th>Vertebra</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.90</td>
<td>95.63</td>
<td>36.81</td>
<td>39.03</td>
</tr>
<tr>
<td>3</td>
<td>92.63</td>
<td>96.94</td>
<td>63.58</td>
<td>50.22</td>
</tr>
<tr>
<td>4</td>
<td>95.74</td>
<td>96.28</td>
<td>71.49</td>
<td>43.53</td>
</tr>
<tr>
<td>6</td>
<td>96.26</td>
<td>98.45</td>
<td>31.39</td>
<td>27.81</td>
</tr>
<tr>
<td>14</td>
<td>98.75</td>
<td>95.50</td>
<td>51.23</td>
<td>30.28</td>
</tr>
</tbody>
</table>

Table 7.4: Percentage reductions in SUV between the original and filtered images, when using only data obtained during the first 15 minutes after injection to perform the classification.

An additional study was then performed, in which the classification algorithm made use of only data obtained between 15 and 60 minutes after injection. Both the images displayed in Figure 7.7, and quantitative results presented in Table 7.5, illustrate that, although retention of the tumour signal was maintained, the removal of signal from the liver or heart was not achieved.

Figure 7.7: Slices at the level of the liver and tumour after filtering, with only data obtained between 15 and 60 minutes after injection used to perform the classification. This particular image is taken from patient 14, but is representative of the results observed across the five images.
Patient Liver Heart Vertebra Tumour
1   11.46  53.04   0.77  3.77
3   1.00   73.74  1.05  18.30
4   14.56  86.35  9.44  10.07
6   14.15  49.49  2.48  0.97
14  11.55  66.22  1.84  1.39

Table 7.5: Percentage reductions in SUV between the original and filtered images, when using only data obtained between 15 and 60 minutes after injection to perform the classification.

7.2.3 FBP Reconstruction

Finally, the potential application of the technique to images reconstructed using the FBP method described in Section 2.4 was investigated, since this reconstruction method is widely used, particularly for quantitative studies. The predefined classes were generated as described in Section 7.1.1, using data obtained from images reconstructed using FBP, and the classification algorithm described in Section 7.1.2 was then applied to each of the five images, with a representative result shown in Figure 7.8.

![Figure 7.8: Slices at the level of the liver and tumour both before (left) and after (right) application of the classification algorithm to an image reconstructed using FBP. This particular image is taken from patient 14, but it is representative of the results observed in all five images.](image)

This result clearly illustrates the almost complete removal of signal from the liver and heart, as well as the lungs and normal breast tissue, whilst the
signal from tumour tissue was retained. The vertebra, once again, displayed inconsistent behaviour across the five images.

7.3 Discussion

The classification technique was found to be consistently successful in removing signal from the liver, heart, lungs and normal breast, whilst retaining that from tumour tissue. Inconsistent results were, however, obtained for the vertebra, whose TAC appears to be too similar to that of the tumour for reliable separation of the two tissues to be achieved. As a result, signal from the vertebra, as well as that from other bones such as the sternum and ribs, cannot be removed using the current method, which may therefore not be directly applicable to cases in which the presence of cancerous tissue in or near the bones is likely.

Although the majority of tumour signal was retained, some reductions were observed, particularly in the larger, or more heterogeneous tumours. This effect is most noticeable in the images of patient 4, where signal from one region of the primary tumour was almost completely retained, as shown in Figure 7.4, whilst that from other regions was removed, as shown in Figure 7.5. It was suggested that the areas of signal removal might correspond to regions of non-proliferating tissue, which were therefore not recognised by the classification algorithm as belonging to a tumour. The rate constant describing the irreversible uptake of FLT from plasma to tissue, $K_i$, which has been found to correlate well with tumour proliferation [17, 41], was therefore calculated for the tumours present in patients 1, 3, 4, 6 and 14. The modified Patlak method described in Section 3.2 was applied, using the Clickfit software, and the results are presented in Table 7.6.

<table>
<thead>
<tr>
<th>Patient</th>
<th>$K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>3</td>
<td>$2.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>$4^+$</td>
<td>$9.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>$4^-$</td>
<td>$1.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>6</td>
<td>$5.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>14</td>
<td>$5.3 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 7.6: $K_i$ values for five tumours; for patient 4, $+$ indicates a region in which signal was retained, and $-$ a region in which signal was removed.

For patient 4, the $K_i$ value of the tumour region in which signal removal was observed is an order of magnitude higher than those of all other re-
regions quantified. Whilst this does not support the suggestion that areas
of signal removal might correspond to regions of non-proliferating tissue, it
does demonstrate a clear difference between this region, and those for which
the majority of signal is retained. It is hypothesised that this region of the
tumour actually proliferates much more rapidly than any other tumour in
the dataset, with the result that its TAC does not produce a good fit when
compared against the predefined tumour class. An important aspect of the
further work on this project will therefore involve the acquisition of additional
data, in order to ensure that the predefined tumour class is representative of
tumours covering the full range of proliferation rates.

It appears that a scan of 60 minutes should be sufficient to acquire the
kinetic information necessary for accurate classification, since the results ob-
tained using only data from the first 60 minutes after injection were com-
parable with those obtained using all the available data. The use of only
data acquired during the first 15 or 30 minutes, however, produced images
in which the tumour signal was not sufficiently retained. Since the TACs of
the various tissues differ most during the early time frames, as illustrated in
Figure 7.1, the use of only data acquired between 15 and 60 minutes after
injection also produced unsatisfactory results, with no significant removal of
signal from the liver or heart observed.

It was found that this technique can be applied to images reconstructed
using either an analytic or iterative method, although there is a concern that
the streaks from regions of high intensity that are often observed in FBP
images could potentially conceal tumours located in or near such tissues.
Further work must therefore be performed in order to determine whether the
technique could provide useful information in such cases.

In conclusion, this technique could have direct relevance in clinical on-
cology, where the images produced could be used in conjunction with the
originals, to provide additional diagnostic information. It could also be ap-
plied to other tracers which undergo a simple hepatic metabolism, as well
as to the removal of other organs, such as the bladder, which displays high
signal intensity for any tracer that is excreted in the urine.
Chapter 8

Conclusions and Further Work

PET has an important role in clinical oncology, with applications in both diagnosis and staging, as well as the selection and monitoring of treatment plans. Although by far the most widely used tracer is the glucose analogue, FDG, its lack of tumour-specificity means that alternatives are being actively sought. One such emerging tracer, with potential applications in the field of oncology, is the cell proliferation marker, FLT. However, as well as accumulating in rapidly proliferating cancerous tissue, this tracer also shows high signal intensities in tissues such as the heart, liver and vertebra, which could potentially obscure signal from tumours located in or near these organs. Since the liver is the second most common site for metastases, this is of particular concern, as for FLT to have a significant role in clinical oncology, it is vital that tumours located around this organ can be identified.

The aim of this project was therefore to develop a technique which would exploit the differing kinetics of the various tissues in order to remove signal from healthy organs, whilst retaining that from tumours. The first method tested involved projecting the image along a direction chosen to improve the CNR between the tumour and healthy organ under consideration. This method achieved only partial success, and an alternative involving linear classification was therefore investigated, which produced much more satisfactory results.

The classification technique has so far been validated across 28 images of patients with locally advanced or metastatic breast cancer, and found to consistently remove signal from the heart, liver, lungs and normal breast. Signal from tumours, however, was found to be retained, whilst inconsistent behaviour was observed in the vertebra. The method has potential clinical applications, as the filtered images could be used in conjunction with the originals, in order to provide additional diagnostic information.

There is, however, further work to be done before the technique can be
applied in clinical practice, including the acquisition of additional data to further develop the predefined classes. This is particularly important for the tumour class, since it is thought that this does not currently represent tumours spanning the full range of proliferation rates, as well as containing no data from metastatic tumours. Additional classes could also be generated, which represent combinations of the various tissues, in order to improve the definition of tissue boundaries. This would be particularly relevant for tumours located within, say, the liver, for which the boundaries would be best represented by a combination of the liver and tumour classes. Finally, data describing other organs which show high signal intensity, such as the kidneys or bladder, could be included.

The technique should be further validated by the application to new datasets, including patients with tumours located within organs such as the liver, and the potential of removing signal from the bones using information from a coregistered CT scan could be investigated.
Bibliography


[13] A. M. Groves, T. Win, S. Ben Haim et al., 2007, Non-\[^{18}\text{F}]\text{FDG PET in clinical oncology}, Lancet Oncology, 8, 822


[26] T. Beck, E. E. Weller, W. Weikel et al., 1995, *Usefulness of immunohistochemical staining for p53 in the prognosis of breast carcinomas: correlations with established prognosis parameters and with the proliferation marker, MIB-1*, Gynecologic Oncology, **57**, 96


[29] A. F. Shields, D. A. Mankoff, J. M. Link et al., 1998, *\[^{11}C\]-thymidine and FDG to measure therapy response*, Journal of Nuclear Medicine, **39**, 1757


[33] H. Vesselle, J. Grierson, L. M. Peterson et al., 2003, 18F-Fluorothymidine Radiation Dosimetry in Human PET Imaging Studies, Journal of Nuclear Medicine, 44, 1482

[34] K. Herholz and C. S. Patlak, 1987, The influence of tissue heterogeneity on results of fitting nonlinear model equations to regional tracer uptake curves: with an application to compartmental models used in positron emission tomography, Journal of Cerebral Blood Flow and Metabolism, 7, 214


[36] W. Chen, T. Cloughesy, N. Kamdar et al., 2005, Imaging Proliferation in Brain Tumors with 18F-FLT PET: Comparison with 18F-FDG, Journal of Nuclear Medicine, 46, 945


[38] A. K. Buck, G. Halter, H. Schirrmeister et al., 2003, Imaging proliferation in lung tumors with PET: 18F-FLT versus 18F-FDG, Journal of Nuclear Medicine, 44, 1426


[40] C. S. Yap, J. Czernin, M. C. Fishbein et al., 2006, Evaluation of thoracic tumors with 18F-fluorothymidine and 18F-fluorodeoxyglucose-positron emission tomography, Chest, 129, 393


[42] A. K. Buck, M. Bommer, S. Stilgenbauer et al., 2006, Molecular Imaging of Proliferation in Malignant Lymphoma, Cancer Research, 66, 11055
[43] H. L. van Westreenen, D. C. P. Cobben, P. L. Jager et al., 2005, Comparison of $^{18}$F-FLT PET and $^{18}$F-FDG PET in Esophageal Cancer, Journal of Nuclear Medicine, 46, 400


[46] B. S. Pio, C. K. Park, R. Pietras et al., 2006, Usefulness of 3'-$[^{18}F]$fluoro-3'-deoxythymidine with positron emission tomography in predicting breast cancer response to therapy, Molecular Imaging and Biology, 8, 36


[50] H. B. Guo, R. Renaut, K. W. Chen et al., 2003, Clustering huge data sets for parametric PET imaging, Biosystems, 71, 81


[52] B. J. Frey and D. Dueck, 2007, Clustering by passing messages between data points, Science, 315, 972

[54] F. Pedersen, M. Bergstrom, E. Bengtsson et al., 1994, Principal component analysis of dynamic positron emission tomography images, European Journal of Nuclear Medicine, 21, 1285

[55] J. S. Lee, D. S. Lee, J. Y. Ahn et al., 2001, Blind separation of cardiac components and extraction of input function from (H2O)-O-15 dynamic myocardial PET using independent component analysis, Journal of Nuclear Medicine, 42, 938

[56] E. C. Ford, P. E. Kinahan, L. Hanlon et al., 2006, Tumor delineation using PET in head and neck cancers: Threshold contouring and lesion volumes, Medical Physics, 33, 4280

[57] S. E. Singletary, C. Allred, P. Ashley et al., 2002, Revision of the American Joint Committee on Cancer staging system for breast cancer, Journal of Clinical Oncology, 20, 3628


[60] P. C. Mahalanobis, 1936, On the generalized distance in statistics, Proceedings of the National Academy of Science, India, 12, 49
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