Developing Methods for Quantitative PET: Application to Multimodal Human and Rat Brain Imaging

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Molecular and Medical Pharmacology

by

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2014
ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy in Molecular and Medical Pharmacology
University of California, Los Angeles, 2014
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Positron Emission Tomography (PET) is a functional medical imaging tool that enables the visualization of radio-labeled biologically active molecules (tracer) distributed inside a living body. PET is also combined with other modalities such as CT and MRI with either software or hardware methods to gain synergy. However numerous technical and biological issues remain to be addressed to improve the utility of PET in multimodal imaging for both clinical and preclinical applications. Patient movement during the PET/CT dynamic scan is one of the major problems in clinical study and automated MRI template-based volume of interest (VOI) analysis is one of the key issues in preclinical brain studies for PET.
PET/CT is an imaging system that combines PET and CT, in which CT not only provides structure information but also aids attenuation correction for PET. This multimodal medical imaging has become prevalence in clinical diagnosis. However, head movements occurring during PET/CT dynamic scans can create large artifacts in CT-based attenuation corrected PET due to mismatches between CT and PET images. We have thus developed an automated movement correction (MC) procedure for PET/CT dynamic brain scans. MC method was first validated in a Hoffman phantom study and further evaluated with patient FDDNP (a tracer that binds β-amyloid and τ-protein depositions in tissue) and FDG (a glucose analogue) scans. Results showed that the use of MC for dynamic PET/CT scan significantly improved image quality and allowed more accurate tracer quantitative analysis.

To accurately analyze longitudinal FDG preclinical PET brain scans, especially to quantitate image values in small brain structures, an automated MRI template-based volume of interest (VOI) analysis method has been established. The method was applied to longitudinal rat brain FDG PET images to evaluate regional cerebral metabolic change that could not be done without such a template-based analysis methodology. A 6-month study in normal young adult rats showed stable FDG uptake in sensorimotor cortex and lateral prefrontal cortex, a linear decline of relative FDG uptake in striatum, hippocampus and medial prefrontal cortex, while a linear increase in the relative FDG uptake was observed in cerebellum and brain stem. This linear progressive change in regional brain metabolism is first elucidated in normal young adult rats. The method was also applied to rat brain FDG PET images to evaluate the acute and long-term effects of chemotherapy on regional cerebral metabolism.
The dissertation of Hu Ye is approved.

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I dedicate this dissertation to my husband Dr. Xiang Li, my mother Prof. Xingming Hu, my father Prof. Zhizhen Ye, my maternal grandparents Canshi Hu, Peixuan Zhou and my paternal grandparents Yiquan Ye, Cailiu Chen for their love and continuous support.
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Acknowledgement

First and foremost, I would like to express my sincerest appreciation to my dissertation advisor Dr. Sung-Cheng (Henry) Huang for his guidance and support. Dr. Huang has solid knowledge background, great insight and rigorous attitudes in science and engineering. I would also like to express my gratitude to my committee member and advisor in the rat brain project, Dr. Daniel Silverman. I am always grateful to Dr. Huang and Dr. Silverman for their understanding, tolerance, kindness and encouragement during my toughest time. I also thank my other committee members Dr. Jorge Barrio, Dr. Arion Chatziioannou and Dr. Elliot Landaw.

I would like to thank Dr. Magnus Dahlbom, Dr. Nicole Detorie and Mr. John Williams for the help with the Hoffman phantom study, Dr. Linda Nelson, Dr. Gary Small and Dr. Vladmir Kepe for patient data collection. I also thank Dr. David Stout, Dr. Waldemar Lando, Dr. John David and Mr. Darin Williams for their assistance in performing the rat PET studies, Dr. Andrew Frew for his assistance in performing the rat MRI studies.

I would like to express my special appreciation to all my lab mates and co-workers, Dr. Wei Sha, Dr. Mirwais Wardak, Dr. Koon-Pong Wong, Dr. Kenji Hirata, Mr. Weber Shao, Dr. Amy Yu, Mr. Moses Wilks, Dr. Xiujuan Zheng, Mr. Andrew Surmak, Ms. Regina Ahn, Mr. Stefan Nguyen, Ms. An Nguyen and Ms. Beata Durcanova. It has been wonderful to work with all of you.

I would also like to thank Dr. Michael Phelps, the chair of Pharmacology, and Dr. Greg Payne, the director of ACCESS program as well as all the student affair officers of these two programs for providing excellent academic environment. I am grateful to all the friends I met in ACCESS Program, Pharmacology and student organizations on UCLA campus. All those fun activities are colorful memories and the friendships are cherish fortunes.
I also would like to express my gratitude to Dr. Charles Glaus, Dr. Sharon Ungersma, Dr. Pedro Beltran, Mr. Tim Kazules and Ms. Brittany Yerby in Research Imaging Sciences at Amgen Inc. for granting me the opportunity to work as a graduate intern there. It was a wonderful experience that I could apply the knowledge and skills I developed during this dissertation research in industry. I also thank them for their help and support during my internship.

My parents always give me love and support and they are always my role models for their hard working and persistence. My parents had to leave school after they finished high school, however they never forgot their college dreams to further pursue knowledge when they were away from school. After the resume of Chinese college entrance examination in 1977, they made great efforts to perform excellently in the exam and finally got into Department of Electrical Engineering at Zhejiang University. Later in their life, they always gracefully faced challenges in and bravely conquered difficulties just like what they did to make their college dreams come true. I was born when my father was still a Ph.D. student in Optical Engineering at Zhejiang University. When I was a little girl, everyone told me that “you would become a doctor like your father”. I am very proud that, after this dissertation get approved, the prophecy will come true.

I still remember that I wrote “I thank my friend Xiang Li for his help and support” in my bachelor thesis’ acknowledgement. I am very lucky to have you always standing by me since then. I am also proud of you for your success in pursuing your Ph.D. degree and in your research work. Thank you for all the things you have done for me in the past eight years. Especially, thank you for the more than a hundred thousand mile flights back and forth between Los Angeles and Niskayuna during the last two years. We grew up together, share the tears and smiles together. You are the best husband and always my best friend.
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Chapter 1. Background and Introduction

1.1 Dissertation Research Aims

Quantitative analytic methods are needed to take full advantage of the PET/CT dynamic scanner in acquiring physiologic parameters. Large errors in the PET images due to head movement may yield incorrect biological information. On PET only scanner, Movement Correction (MC) by motion tracking system or with image-based software realignment methods, have been reported (1,2). The motion tracking system for MC, though validated on phantom scans, has not been that successful in real human scans and was outperformed by image-based method. Head movement during a dynamic brain PET/CT scan results in mismatch between CT and dynamic PET images. It can cause artifacts in CT-based attenuation corrected PET images, thus affecting both the qualitative and quantitative aspects of the dynamic PET images and the derived parametric images. In my dissertation research, an automated retrospective image-based MC has been developed for PET/CT scanner and evaluated on Hoffman phantom and patients’ data.

Until small animal dedicated PET was developed, PET has not been available for small animal models because of resolution limitations. The non-invasive and quantitative imaging of biological function with small animal PET in small animals is increasingly being used. The purpose of VOI analysis is to calculate the distribution of pixel values within delineated tissue structures. This is especially a challenge for preclinical longitudinal studies. Due to the resolution restriction, some small brain region such as lateral prefrontal region is hardly recognizable visually. Manually defined VOI may induce big variation among animals and in repeated scans. Thus, a MRI template-based VOI analysis method can not only avoid the tedious and time consuming procedure but also reduce subjective biases. This method was applied to a set of small animal PET studies to
demonstrate its usefulness for quantitative longitudinal rat brain PET studies for answering challenging biological questions. In my dissertation research, longitudinal cerebral metabolic changes in normal young-adult rats were investigated. Symptoms of cognitive impairment following chemotherapy for cancer have been found prevalent (3), but have not been systematically evaluated. In this dissertation study, acute and long-term effects of breast cancer chemotherapy were investigated in longitudinal rat studies using small animal PET.

1.2 Positron Emission Tomography (PET)

Positron emission tomography (PET) is a nuclear medicine imaging technique that produces a three-dimensional (3D) image and reflects functional process in the living body. Biologically active molecules are labeled with positron emitting radionuclide (tracers) and imaged with PET. The system locates the presence of tracers by detecting pairs of gamma photons emitted indirectly by the position emitting radionuclides and records the data over a period of time in a sinogram. 3D images of tracer concentration are then reconstructed from the sinogram mathematical algorithms implemented with computer software (Fig. 1.1).

1.2.1 Positron Emission and Annihilation

Positron Emission

A positron is a particle which has same mass but opposite electric charges with electron. It could be generated from positron emission or pair production. Positron emission is one of the ways that an unstable nuclide with an excess of protons may decay. In this radioactive decay process, a proton (p) in nucleus is converted into a neutron (n), a positron (β⁺) and an electron-type neutrino (Ve) (4).

\[ p \rightarrow n + \beta^+ + V_e \quad \text{Eq.1.1} \]

The positron is ejected from nucleus along with the neutrino. Positron decay is denoted as
\[ ^A_ZX \rightarrow ^{A-1}_{Z-1}Y + \beta^+ + V_e \quad \text{Eq.1.2} \]

Where \(^A_ZX\) denotes the parent nuclide with mass \(A\) and atomic number \(Z\) and \(^{A-1}_{Z-1}Y\) denotes the daughter nuclide with same mass as the parent nuclide and decreased atomic number by 1. Neutrino has no electric charge and very small mass. The net energy released during positron emission is shared by the daughter nucleus, the positron and the neutrino. Positrons are emitted with a range of energies, from zero up to a maximum endpoint energy \(E_{\text{max}}\), while the mean energy is about 1/3 of \(E_{\text{max}}\) (4).

**Annihilation**

After travelling up to a few millimeters in soft tissue the positron encounters an electron with high probability. The encounter annihilates them both, producing a pair of 511KeV photons moving in opposite directions (Fig. 1.1A). The momentum of annihilated electron and positron is very close to zero so that the net momentum of two opposite traveling photons is zero. The energy released from the mass of the electron and the positron is equal to 1.022MeV and is converted into two annihilation photons’ electromagnetic energy (4). So both the laws of conservation of energy and momentum are obeyed in the process of annihilation. It is possible that more than 2 photons are emitted, but only occurs in about 0.003% of the annihilation.

**Positron Range and Non-collinearity**

Positron range and non-collinearity of the two opposite 511 KeV photons are the two effects that limit the ultimate resolution of PET. Generally speaking, the larger the energy with emitted positron, further the distance positron may travel before annihilation (positron range). Positron range is isotope-dependent and lead to errors in determining the line which a positron emitting radionuclide is to be determined. The non-collinearity is due to a small net momentum of positron and electron that are not completely at rest when they annihilate. Thus the annihilation photons are
emitted with a distribution of deviation from 180°, but not exactly 180°. The distribution is roughly a Gaussian distribution in shape, with a full width at half maximum (FWHM) of ~0.5°. This non-collinearity effect, unlike positron range, is radionuclide independent but also blur the reconstructed PET image. However, it should be pointed out that these two effects’ influence on spatial resolution in current clinic PET scanner is relatively small compared to that by the detectors (4).

**Isotopes for PET**

In addition, proton-rich radionuclides also can decay by electron capture. Decay by positron emission competes with electron capture, but usually being the dominant process in low Z nuclei. Generally speaking, radionuclides that decay predominantly by positron emission but not by electron capture (i.e., they have a high positron branching fraction) are preferred for PET imaging. However, other parameters such as half-life, positron range and ease of synthesis also needed to be considered when designing a tracer.

The positron emission and annihilation are the basis of PET imaging. The positron emitting radionuclides that are used for PET include $^{82}$Rb, $^{15}$O, $^{13}$N, $^{11}$C, $^{18}$F, $^{64}$Cu, $^{86}$Y, $^{76}$Br, $^{89}$Zr and $^{124}$I, which have half-lives of 78s, 2.1 min, 9.97min, 20.4 min, 109 min, 12.7 hr, 14.7 hr, 16.2 hr, 78.5 hr and 4.18 d respectively (5). Table 1.1 lists some of the properties of radionuclides that are commonly used in PET today.

1.2.2 PET Tracers

**Design of PET Tracers**

PET tracers are position emitting nucleotides labeled molecules with certain biological functions. They are designed to diagnose disease, monitor disease progression, track therapeutic response and enhance our knowledge of physiology and pathophysiology. PET imaging with its
tracers is expected to play an increasingly important role in personalized medicine and drug development (6).

In order to evaluate a particular biological system or physiological function with PET, it is important that the PET radiotracer selectively bind to markers or target a pathway of interest. And taking advantage of PET’s high sensitivity, the amount of tracer introduced into a living body is usually low enough that the inherent physiological system won’t be influenced. There are several types of tracers:

1) Direct radiolabeled version of a cold nature or synthetic compound (e.g. substitute the stable $^{12}$C in a compound with radioactive $^{11}$C);

2) Analogue of a cold nature or synthetic compound (e.g. substitute the hydroxyl group in a compound with $^{18}$F);

3) A biological functional compound attached with radioisotope atoms (7).

For the latter two tracers, the similarity of biochemical behavior between the radioisotope labeled version and original compound needs to be examined.

The half-life of chosen isotope needs to be matched with the pharmacokinetics half-life of the tracer. $^{82}$Rb, $^{15}$O, $^{13}$N, $^{11}$C, $^{18}$F are used to synthesize simple tracers or labeled small molecules that have shorter pharmacokinetics half-life while $^{64}$Cu, $^{86}$Y, $^{76}$Br, $^{89}$Zr and $^{124}$I are used to label peptides and proteins which stay much longer in the circulation in the living animal body. Up to date, four out of six FDA approved PET tracers are $^{18}$F based.

$^{18}$F and FDG

$^{18}$F has a moderate 110 minute half-life which not only makes it convenient for radiopharmaceutical synthesis but also allows centralized production to minimize the necessity of on-site cyclotron. $^{18}$F also has a high positron branching fraction (97%) and a relatively low mean
positron energy (0.64 MeV) (4). Those characteristics make $^{18}$F a popular PET isotope. In an $^{18}$F labeled tracer, usually $^{18}$F replaces one or more hydroxyl groups of the original molecule while the new molecule still keeps most of its bio-function due to similar steric and electrostatic properties. There may still be differences between the tracer molecule and the original molecule especially when the substituted hydroxyl group plays critical roles in certain steps of the targeted pathways, for example the hydroxyl group is required to be recognized by an enzyme to further metabolize the molecule. Actually, sometimes those differences between the tracer and the original molecule are favored for functional imaging purpose.

$^{18}$F labeled glucose analog, 2-deoxy-2-($^{18}$F)fluoro-D-glucose (FDG) is FDA approved and the most commonly used PET tracer in the clinic for oncologic, neurologic and cardiologic applications. It is used in evaluating glucose metabolism. FDG is currently the only PET radiopharmaceutical used for routine cancer imaging. FDG-PET was a research device until 1999 because there was no reimbursement by Medicare or any other payer worldwide. FDG-PET was approved by FDA in 2000 and this major breakthrough boosted rapid incorporation of PET in nuclear medicine practice especially in oncology. Ido et al. at the Brookhaven National Laboratory were the first to describe the synthesis of FDG labeled with $^{18}$F in 1976 (8). The first $^{18}$F-FDG human PET scans were performed at UCLA in 1977 by Phelps et al. (9,10). In 1989, Barrio et al. developed the first automated chemistry module for synthesizing $^{18}$F-FDG (11).

**Beyond FDG**

In oncology, besides FDG, there are several other radioactive small molecule tracers that are under intensive research and to target various pathways. For example $^{11}$C-Thymidine and $^{18}$F-fluorothymidine (FLT) are designed to evaluate DNA synthesis and cellular proliferation level, $^{18}$F-fluoroestradiol (FES) is aim to bind to estrogen receptor (ER) and detect ER positive breast
cancer, \(^{18}\text{F}\)-fluoromisonidazole (FMISO) is invented to assess tumor hypoxia, and L-3,4-dihydroxy-6-(\(^{18}\text{F}\))fluorophenylalanine (FDOPA) and \(^{11}\text{C}\)-L-methionine are developed to characterize amino acid transport and protein synthesis (12). \(^{18}\text{F}\)-FDOPA as an \(^{18}\text{F}\)-labeled dopamine precursor, was also aim to help with diagnosis of Parkinson disease in neurology. In cardiology, besides using FDG to exam myocardial viability, \(^{82}\text{Rb}\)-RbCl and \(^{13}\text{N}\)-NH\(_3\) are approved by FDA and used in clinic to assess myocardial perfusion (13). Research groups at UCLA dedicated in new tracer development. 2-\{(1-\{(2-\(^{18}\text{F}\))-fluoroethyl\})(methyl)amino\}-2-naphthylgethylidene)-malononitrile (FDDNP) is a UCLA developed β-amyloid and τ-protein specific PET tracer. More details about FDG and FDDNP will be introduced in later sections.

Until now, all FDA approved PET tracers are small molecule tracers, positron emitting radionuclides labeled monoclonal antibodies (PET mAb tracers) are still in the translating status. As of 28 January, 2014, 31 monoclonal antibodies (mAbs) are approved by FDA and used for treatment in clinic, especially in oncology (drug voluntarily withdrawn from market are excluded) (14), and hundreds of mAbs are under research or in clinical trials nowadays. Immuno-PET, the tracking and quantification of mAbs using PET with mAbs PET tracers is an exciting innovation to increase the comprehension of in vivo behavior and efficacy of mAbs both in basic research, drug development and in clinic. Immuno-PET will be an important tool for assessing the target status, evaluating mAbs pharmacokinetics and bio-distribution, exploring off-target binding and optimizing mAbs dose and scheduling, and guiding FDA-approved mAbs treatment. While there are five FDA approved γ-emitting radionuclides (\(^{99m}\text{Tc}\) and \(^{111}\text{In}\)) labeled murine mAbs imaging tracer for single photon emission computerized tomography (SPECT, another type of nuclear medicine imaging modality), Immuno-PET has recently reached maturity in terms of technical development and is now entering the phase of broad-scale clinical evaluations. The clinical trials
of mAbs PET tracers were first launched in Europe Union and led the first PET $^{89}$Zr labeled mAbs tracer clinical trial in 2008. Beside mAbs PET tracers, tyrosine kinase inhibitors (TKIs) PET tracers also received lots of attentions (15-17).

1.2.3 Coincidence Detection and PET Instrumentation

In PET scanning, radioactive tracer is first injected into human/animal body, the tracer is delivered to tissue by blood flow and undergoes $\beta^+$ decay to produce positrons. After travelling up to a few millimeters the positron encounters an electron. The encounter annihilates them both, producing a pair of annihilated 511KeV photons moving in opposite directions (Fig. 1.1). The pair of photons is detected when they reach gamma photon detectors almost at the same time in the scanning device, and it is called coincidence detection. Hence it is possible to localize the annihilation event along a straight line of coincidence between two detectors, which is formally named as the line of response or LOR (Fig. 1.1B).

*Gamma Photon Detectors*

The gamma photon detector is the basic unit of a PET scanner; it converts an arriving 511 KeV photon into an electrical signal (e.g. an electrical current pulse). The detectors are expected to have high efficiency for 511 KeV photon detection and precision of photon localization to provide high signal to noise ratio and spatial resolution in the PET images. To achieve the latter, the detector either has a small size or position-sensing capability. In addition, a fast response time, sometimes refer as the timing resolution (on the order of 2-6 ns) is another important detector property (4).

In today’s commercial PET scanner, scintillator detectors are widely used gamma photon detectors (4). And a common design is called “Block Detector”, which was originally proposed by Casey and Nutt (18). The block detectors consist of a large dense crystalline scintillator (usually around 4cm×4cm in area with a depth of 2-3cm) which is segmented into an n×n array of elements
and the light output collected with four photomultipliers (PMTs) (Fig. 1.2). The transparent scintillator crystal emit visible light due to the gamma photon’s energy deposited in it. In general, the shorter the light decay time and the more light produced, the faster the detector (19). Common scintillator materials include bismuth germanate (BGO), lutetium oxyorthosilicate (LSO), and gadolinium oxyorthosilicate (GSO). Their properties are listed in Table 2.2. PMTs convert scintillation light emitted from scintillator into electrical current. The design of scintillator segmentation and 4 PMTs enable the localization of where gamma photon interaction happened.

The PET system of Biograph TruePoint 64 PET/CT (Siemens Inc.) consists of four rings of 48 LSO detector blocks, and each block (52mm×52mm in area with 20mm in depth) is divided into 13×13 crystal segmentations. The diameter of the ring is 842 mm, while the axial and transaxial field of view (FOV) of this model is 216 mm and 605mm, respectively. The axial and transaxial resolution is 5.9 and 5.5 mm respectively (20). This model of scanner was used for all of the Hoffman phantom and patient studies in this dissertation research.

**Coincidence Detection**

PET determines the LOR between two detectors by coincidence detection of two 511 KeV gamma photons. Two annihilation photons are considered to be in coincidence if they are detected within a specific time interval known as the coincidence timing window. A typical timing window is set to be 2 to 3 time of the detector time resolution, leading to a range of 4 to 18ns for different detectors (4).

However, not all the coincidence detection indicates positron annihilation, and only true coincidences should be recorded. The true coincidence is defined as the detection of two 511KeV annihilation photons originate from the same radioactive decay and keep the original direction and energy. There are three major types of events which may contaminate coincidence measurement:
scattered, random and multiple coincidences (Fig. 1.3). When one or both photons of an annihilation event change direction due to Compton scatter, this coincidence is called scattered coincidence and leads to mis-positioning of the LOR. The distribution of scattered events depends on the radioactivity distribution and scatter medium (i.e. patient) shape. Recorded scattered events may account for 15% to well over 50% of recorded coincidence events, so scatter correction are necessary. When two unrelated annihilation are recorded, it becomes a random coincidence. Random coincidence rate is proportional to the square of total activity in the field of view and the timing window. Thus optimizing injection dose and timing window are important to control random coincidence rate. Multiple coincidence refers to the situation that three or more photons are detected simultaneously due to multiple annihilation events taking place in a short time. Multiple annihilation events are usually discarded (4,21).

The raw data collected by a PET scanner, so called sinogram, records the activity integral over a period of time along LORs at different positions and angles. After detector normalization, scatter, attenuation correction (will be introduced in Chapter 2.2), the PET image is reconstructed from the corrected sinogram and reveals the distribution of radioactive tracer in human/animal bodies. The intensity of a voxel in a reconstructed PET image represents the average radioactivity in the corresponding region in a living animal body.

1.2.4 Image Reconstruction

Image reconstruction is the essential step for generating the cross-section image of radioactivity distribution in the object that is being scanned (PET slice). A sinogram is a 2D matrix which record the sum (projection) of activity along LORs during the recording period by plotting each LOR as function of its angular orientation versus its displacement from center of gantry (22). Each row in the sinogram is the projection of that slice viewing at the particular angle associated
with that row. Each point in the sinogram represents the sum of activity along a LOR connecting two detector elements. The image reconstruction process aims to recover the activity distribution from the 2D sinogram (Fig. 1.4).

There are two basic approaches of image reconstruction: filtered backprojection (FBP) and iterative reconstruction methods. Filtered backprojection is an analytic approach which applies a filter to the projection data in the Fourier domain and then backprojects the filtered data into image space (23). Iterative methods model the PET data collection process (forward projection) and try to find the image that is most consistent with the measured data in a series of successive iterations. To maximize consistency (convergence) between the estimated and measured projections, various approaches have been adopted by different algorithms, such as, maximizing likelihood (ML), maximizing likelihood under a Poisson data model (Expectation-maximization, EM) and maximizing a posteriori (MAP). Iterative methods allow for a more accurate modeling of the data-acquisition system (e.g. the detector configuration) as well as the statistical noise in the emission images. Iterative methods are computationally intensive and a number of approximations have been developed to accelerate the process. Ordered subsets expectation maximization (OSEM) is an EM based accelerating algorithm, in which only a subset of the projection angels are used in any one iteration (4). OSEM was chosen for image reconstruction from the Biograph 64 PET/CT for all the Hoffman phantom and patient studies.

1.3 Multimodal Imaging

CT and MR imaging are used primarily for anatomic imaging purposes; even though both modalities can be used, to varying degrees, for physiologic assessments. MR imaging has the capability to make molecular imaging assessments. PET, on the other hand, provides less anatomic information and is primarily molecular and, to a lesser degree, a physiologic imaging technique.
Multimodal imaging could combine the features of two or more modalities to better facilitate diagnostics.

1.3.1 PET/CT

In the 1990s, PET was applied more frequently in oncology, when it became obvious that, in many malignant tumors, PET is superior in the detection of metastases compared to CT. However, the insufficient capability of PET to yield adequate spatial information on the detected lesions also became obvious. CT images were usually used to add the spatial information. PET and CT information together increased tumor staging accuracy and influenced treatment arrangement (24). PET and CT images were first combined in a side-by-side manner and with software-based fusion. After 2000, combined PET/CT scanners, which integrate both modalities on the same scanner, became more popular. The combined PET/CT was introduced commercially in 2001, and the global market of stand-alone PET continuously declined after that. While stand-alone PET scanners were about one thirds among all newly installed PET scanners (including both PET stand-alone and PET/CT scanners) in 2003 (25), the fraction was reduced to 10% in 2012.

The idea to combine the two modalities on the same device was first introduced in early 1990s. However, the first prototype wasn’t introduced until 1998. In contrast to the more integrated approach of the initial prototype, the sequential (tandem) design (Fig. 1.5A) was adopted, but different designs were adopted among different manufactures (25). During a study, the patient passed first through the CT scanner and then into the imaging field of the PET scanner. A 64 slice spiral CT was in a Biograph 64 PET/CT system (20).

Besides offering structure information, CT provided attenuation matrix for PET attenuation correction in PET/CT scanner and eliminated the need for a separate, lengthy PET transmission scan (25,26). It largely reduced the time to generating attenuation matrix compared to PET stand-
alone scanner, while still keeping the accuracy of attenuation correction. CT based attenuation correction will be introduced in Chapter 2.2. However, due to the sequential scan method adopted by commercial scanners, there is still possible movement between PET and CT scans, especially for patients scanned with a relatively long duration. In this dissertation, an automated retrospective image-based movement correction method was developed to address this problem.

1.3.2 PET and MRI

MRI has not only high resolution but also superior soft tissue contrast compared to CT. MRI scans are able to differentiate grey matter and white matter in brain tissue, thus making this modality very useful for brain imaging.

Unlike PET/CT’s sequential design, popular PET/MRI design adopts a gantry sharing design and enable simultaneous data acquisition of both modalities (Fig. 1.5 B). Traditional PMTs are very sensitive to magnetic field thus not able to work probably within or near an MRI system. Alternate to PMTs, silicon photomultipliers (SiPMs) and avalanche photodiodes (APDs) are solid state photon detectors. APDs provide internal amplification of the signal and lead to equivalent performance with PMTs. APDs are very compact devices, and most importantly, they are insensitive to magnetic field, thus may replace PMTs in a combined PET/MRI scanner (4). The combined PET/MRI was not fully commercialized yet due to technical difficulty, however, the benefits of using both modalities in diagnosis have already been discussed; examples have been shown in oncology and neurology. In this dissertation, more examples of the synergy of using both modalities in preclinical studies will be shown.

1.4 Brain PET Imaging

Hidden under skull, there are fewer minimally invasive biopsy procedures for brain compared to some other tissues such as breast, liver and prostate. Thus non-invasive neural imaging plays an
important role in diagnosis and treatment plan. In addition, targeted neuro-tracers are valuable tools for compound evaluation and dose selection and have translational potential for drug development. This section reviews two neurological applications: neurodegenerative disease and neural impairment after drug intervention.

1.4.1 Neurodegenerative Disease

Neurodegenerative diseases are defined as hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. These disorders are often associated with atrophy of the affected central or peripheral structures of the nervous system. They include diseases such as Alzheimer disease (AD), Parkinson's disease (PD), Dementia with Lewy bodies (DLB), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), prion diseases, hereditary ataxias, Dentatorubral-pallidoluysian atrophy (DRPLA), Wilson's disease (WD) and others (27).

Given the growing elderly population in the United States, Europe, Japan and China, the number of individuals with neurodegenerative disease is expected to significantly increase. This kind of increase will place a significant burden on healthcare systems, increase economic cost and influence quality of lives. Early imaging and diagnosis of neurodegenerative disease such as AD leads to early medication, changes in patient care and better outcomes (28).

As introduced in previous sections, FDG, an $^{18}$F labeled glucose analog, is most commonly used in nuclear medicine clinics today. The pattern of brain FDG scan was used to help with neurodegenerative disease diagnosis (29). For example, PET scans in AD patients typically show bi-parietal and bi-temporal hypo-metabolism, with characteristic sparing of the sensorimotor cortex (30).
18F-FDDNP was among the first radiolabeled PET molecular imaging probes to be successfully applied to the in vivo visualization of AD’s hallmarks β-amyloid plaques and τ neurofibrillary tangles (NFTs) in AD patients (31). It is a hydrophobic molecule and contains a naphthalene ring system (i.e., two benzene rings fused together) in its structure. 18F-FDDNP is short for its full chemical name: 2-([2,6-fluoroethyl](methyl)amino)-2-naphthyl-ethylidene)malononitrile). It was developed by Jorge R. Barrio and his colleagues at UCLA (31). FDDNP was also used to study other diseases which involved β-amyloid plaques or NFTs in disease progression, for example, Down Syndrome (DS), mild cognitive impairment (MCI) and Progressive supranuclear palsy (PSP). DS patients have an extra copy of genetic material on the 21st chromosome. Middle-aged adults with DS exhibit the same neuropathological hallmarks with AD. DS has been proposed as a model for the study of AD. Amnestic MCI patients may be in a transitional stage of evolving Alzheimer's disease and exhibit AD hallmarks as well. PSP patients have straight filament of tau protein deposit in the cortex and brain stem.

1.4.2 Evaluation of Neural Impairment after Intervention

As introduced in previous sections, PET imaging with FDG has been used to image alterations in glucose metabolism in brain in the field of clinical diagnosis. Recently FDG PET scan has been used to evaluate neural impairment after drug intervention. Shirakawa et al. tested the potential of FDG PET scan in evaluating neurotoxicity induced by N-methyl-d-aspartate (NMDA) receptor antagonist in rats and monkeys (32). Brownell et al. used longitudinal FDG PET to explore acute and long-term effects of neurotoxicity induced by 3-Nitropropionic acid-induced neurotoxicity (33).

Acute and long term neurotoxic effects of chemotherapy were evaluated in patients with PET/CT scan recently. Besides FDG scan which was used to evaluate glucose metabolism, [15O]
water PET scan was acquired during performance of control and memory-related tasks to evaluate cognition-related cerebral blood flow. Specific alterations in activity of frontal cortex, cerebellum, and basal ganglia in breast cancer survivors were documented by functional neuroimaging 5-10 years after completion of chemotherapy (3).

1.5 PET Tracer Kinetic Modeling

The unique capability of emission tomography is derived from its intrinsic use of radio-tracers to trace biological pathways, and the availability of many radio-tracers for a wide range of biological functions in tissues/cells. Since the use of tracers to indicate biological function is based on the principle of tracer kinetics, understanding of the principle is critical for the interpretation of the image/measurements and for guiding the development of new radio-tracers (34).

The most basic data acquisition protocol in PET is the collection of a single sinogram for a static frame over a period of time. For a dynamic PET scan, the data are collected as a sequence of dynamic time frames, where the PET images provide information about the changes in activity concentration distribution over time (4,7). Kinetic compartmental modeling was first developed for dynamic FDG PET scanning to determine the local cerebral metabolic rate of glucose in human. Later, it was transplanted to estimate the local target intensity in ligand-binding image, for example, evaluating intensity of amyloid and neurofibrillary for dynamic FDDNP PET scanning use.

1.5.1 Compartment Model, Graphical Analysis and Semi Quantification for FDG

Compartmental Models are used to describe glucose and FDG kinetics in human body (Fig. 1.6). The concentrations of FDG in plasma, FDG in the brain tissue and FDG-P in the brain tissue are $C_{\text{Plasma}}^*$, $C_E^*$ and $C_M^*$, respectively. The concentration of glucose in plasma, glucose in the brain tissue and glucose-6-P concentrations in the brain tissue are $C_{\text{Plasma}}$, $C_E$ and $C_M$. The symbols
with asterisk are used to denote quantities of FDG or FDG-6-P, and those without the asterisk denote the quantities of natural substrates (10).

The compartments can be described with a set of ordinary differential equations with some appropriate assumptions:

\[ \frac{d}{dt} C_E^* = K_1^* C_{Plasma}^* - (k_2^* + k_3^*) C_E^* + k_4^* C_M^* \]  
Eq. 1.3

\[ \frac{d}{dt} C_M^* = k_3^* C_E^* - k_4^* C_M^* \]  
Eq. 1.4

The unit of \( C_p^* \) is µCi/ml and, unit of \( C_E^* \) and \( C_M^* \) is usually µCi/g. The unit of \( K_1^* \) is ml (min\(^{-1}\) g\(^{-1}\)) while the unit of \( k_2^*, k_3^* \) and \( k_4^* \) is min\(^{-1}\).

The measured time-activity curve from a tissue ROI in the PET image can be defined in terms of the model solutions for each compartment as shown in Eq. 1.5:

\[ C_{Tissue}^*(t) = C_E^*(t) + C_M^*(t) + V_{Plasma} C_{Plasma}^*(t) \]  
Eq. 1.5

where \( V_p \) is the vascular volume and \( C_{Plasma}^*(t) \) is the blood radioactivity, which includes the authentic tracer and its labeled metabolites. With input function \( C_{Plasma}^*(t) \) measured directly through blood samples or image derived methods, the parameters \( K_1^*, k_2^*, k_3^* \) and \( k_4^* \) in the model solution for the tissue time-activity curve are optimized until the sum of squared differences between the model solution and the measured tissue time-activity curve from the dynamic PET image is minimized. The estimated model parameters are then outputted and analyzed. Various software packages, have been developed over the years to perform tracer kinetic analysis (35-39); among them include KIS, SAAM II and PMOD.

Tissue FDG influx rate \( (K_i^*) \) can be calculated as

\[ K_i^* = \frac{K_1^* \times k_2^*}{k_2^* + k_3^*} \]  
Eq. 1.6

The unit of \( K_i^* \) is ml (min\(^{-1}\) g\(^{-1}\)) , the same with \( K_1^* \). Glucose metabolic rate is
\[ MR_{\text{Glu}} = \frac{[\text{Glu}]}{L\text{C}} \times K_i^* \quad \text{Eq. 1.7} \]

The Lumped Constant (LC) is a correction factor used to infer glucose metabolic rate (MR\text{Glu}) from FDG metabolic rate (MR\text{FDG}).

\( K_i^* \) can also be estimated with Patlak graphical analysis using Eq. 1.8. This method will be used in this dissertation to estimate FDG influx rate graphically:

\[
\frac{\int_0^T c_{\text{Tissue}}^*(t)\,dt}{c_{\text{Tissue}}^*(T)} = K_i^* \times \frac{\int_0^T c_{\text{Plasma}}^*(t)\,dt}{c_{\text{Tissue}}^*(T)} + \text{const} \quad \text{Eq. 1.8}
\]

The standardized uptake value (SUV) is a semi-quantitative measure that is often used for describing PET data. The formula for the SUV is given by

\[ \text{SUV} = \frac{\text{Radioactivity in VOI}}{\text{Injection Activity/Body Weight}} \quad \text{Eq. 1.9} \]

Semi quantitative methods are frequently used in clinical studies (40-42).

### 1.5.2 Compartment Model and Graphical Analysis for Ligand Binding Tracer

A compartment model for ligand binding tracer is described in Fig. 1.7. Distribution Volume (DV) in tissue is defined as the ratio between tissue concentration and blood concentration at steady state:

\[ DV = \frac{c_{\text{Tissue}}(\infty)}{c_{\text{Plasma}}(\infty)} \quad \text{Eq. 1.10} \]

For regions with specific binding:

\[ DV_{\text{Tissue}} = (K_1/k_2)(1 + k_3/k_4) \quad \text{Eq. 1.11} \]

For regions without specific binding (reference region: Cerebellum for AD):

\[ DV_{\text{Reference}} = (K_1/k_2) \quad \text{Eq. 1.12} \]

For regions without specific binding (reference region: Cerebellum for AD), Distribution Volume Ratio (DVR) is defined as:
\[ DVR = \frac{DV_{\text{tissue}}}{DV_{\text{reference}}} \quad \text{Eq. 1.13} \]

Logan et al. developed a graphic analysis to get DVR from dynamic PET scan without using input function, where \( C_{\text{tissue}} \) represents the tracer radioactivity concentration in the target tissue, \( C_{\text{Reference}} \) represents the concentration at reference region. The DVR is the slope of Eq.1.14 (43). This method was applied to estimate FDDNP DVR with cerebellum as reference region (44) and will be used in this dissertation.

\[ \frac{\int_0^T C_{\text{tissue}}(t) dt}{C_{\text{tissue}}(T)} = DVR \times \frac{\int_0^T C_{\text{Reference}}(t) dt}{C_{\text{tissue}}(T)} + \text{const} \quad \text{Eq. 1.14} \]

1.6 Small Animal Imaging with PET

1.6.1 Enhanced Resolution

The resolution measured by full width half maximum (FWHM) of clinical PET/CT is around 5mm, while the small animal dedicated PET scanners have about 1.5 mm resolution at the center of field of view. Before small animal dedicated PET scanners were invented, non-human PET research studies were limited to large animals such as dogs and primates due to insufficient resolution. The invention of small animal dedicated PET systems enabled PET studies using small animals such as mice and rats, thus expanding preclinical non-invasive functional imaging to rodent models of disease (45).

1.6.2 Applications in Preclinical Oncological and Neurological Studies

Small-animal dedicated PET was extensively used in cancer research, partially due to the abundant rodent models in the area of cancer biology. FDG and FLT imaging are used to monitor tumor responses to therapies by examining metabolism and proliferation respectively. Positron emitting isotope, such as \(^{64}\text{Cu},^{89}\text{Zr}\) and \(^{124}\text{I}\), labeled intact and engineered antibodies and tyrosine kinase were used for the preclinical phase in drug development (45).
There are a few rodent models in neurological disease study. For example, A PD rodent was modeled by intracerebral injection of 6-hydroxydopamine (6-OHDA) which induced unilateral destruction of catecholaminergic neurons. Recently, works by Casteels et al. and Silva et al. have demonstrated that FDG PET imaging in the rat 6-OHDA PD model is feasible and indicated reduced glucose metabolism in destructed substantia nigra (SN) (46-48). FDG PET scan was also used in evaluating neurotoxicity induced by NMDA receptor antagonist in rats (32). Besides FDG, targeted PET neurotracers which are valuable tools for lead compound evaluation, dose selection and off target binding are also employed in preclinical phase of drug development.

Additionally, both quantitative methods such as local cerebral glucose utilization rate and tumor glucose utilization rate, and semi quantitative methods such as SUV and normalized SUV to a reference region (SUVR) have been used for FDG PET scan in rodents (46, 48-50).

1.6.3 Critical Issues in PET Measurement of the Rat Brain

    Since PET imaging is non-invasive, it enables repetitive measurements for longitudinal studies in the same animal. However, the changes associated with normal aging and repeated handling in the animals needs to be further investigated. Besides that, compared to clinical studies, there are some factors that are unique to pre-clinical studies that need to be considered to help keep experimentation reliable and consistent. Factors including anesthetic agents, injection routes, diet conditions, living conditions and blood glucose levels may interfere PET measurement.
1.7 Figures

Fig. 1.1 Principles of PET using FDG PET scan as an example. A. FDG is an $^{18}$F labeled glucose analog; positrons are emitted from the decay of $^{18}$F. An annihilation happens when a positron encounters an electron, and producing a pair of 511KeV photons moving in opposite directions. B. The pair of photons are detected when they reach a pair of gamma photon detectors almost at the same time in the PET device, and it is called coincidence detection. The annihilation event can be positioned along a straight line between these two detectors; the line is formally named as the line of response or LOR. C. The angles and position of LORs are recorded for a period of time into sinogram and reconstructed into a PET image. Here shows a single 6-mm-thick coronal plane in woman with bilateral metastasis to lung (arrow) from previous ovarian cancer that was surgically resected. Black is highest FDG uptake in image. (Reprint by permission of SNMMI from Phelps ME. PET: The Merging of Biology and Imaging into Molecular Imaging J Nucl Med. 2000;41:661-681.)
Fig. 1.2 Block detector configuration. The block detectors consist of a large dense crystalline scintillator (usually around 4cm×4cm in area with a depth of 2-3cm) which is segmented into an n×n array of elements and four photomultipliers (PMTs). The transparent scintillator crystal emit visible light promotional to the gamma photon’s energy deposited in it. PMTs convert scintillation light emitted from scintillator into electrical current. The design of scintillator segmentation and 4 PMTs enable locate the element where gamma photon interaction happened.
Fig. 1.3 True, scattered, and random coincidence detections. The true coincidence is defined as the detection of two 511KeV annihilation photons originated from the same radioactive decay and kept the original direction and energy. Only the true coincidences carry spatial information regarding the distribution of the radiotracer. In a scattered coincidence, one or both of the annihilation photons change direction due to Compton scatter prior to detection. This results in a mispositioning of the LOR. A random coincidence is generated by two photons originating from two separate annihilations. Random coincidence detections form a background in the data that needs to be subtracted. Corrections for random and scattered coincidence events must be applied to maximize quantitative accuracy. In a multiple coincidence event (not shown above), three or more photons are detected simultaneously. However, due to the ambiguity of where to position the multiple events, these events are normally discarded.
Fig. 1.4 The illustration of sinogram formation. Sinogram records the activity integral over a period of time along LORs at different angular orientation and displacements from center of gantry. A. Center of gantry is noted by X. Four LORs passing through a locus of interest (e.g. a tumor) are labeled A, B, C, and D. B. These 4 LORs are plotted on this sinogram as point A, B, C and D. The intensity of the point represent the activity integral along the corresponding LOR. Angular orientation is on y-axis and displacement from center of gantry is on x-axis. If all possible LORs that pass through this point are plotted, it maps out half of sine wave turned on its side as shown here. C. A sinogram of a brain scan is shown, it is composed of many sine waves. D. Reconstructed brain image corresponding to the sinogram in C is shown. (Reprint by permission of SNMMI from JNMT. Fahey FH. Data Acquisition in PET Imaging. J Nucl Med Technol. 2002;30(2):39-49.)
Fig. 1.5 PET/CT and PET/MRI. A. A type of sequential design of PET/CT with fixed cantilever point and floor-mounted rails. B. Gantry sharing design of PET/MRI. (Fig. 1.5A is reprinted by permission of SNMMI from Townsend DW. Dual-Modality Imaging: Combining Anatomy and Function. *J Nucl Med*. 2008;49(6):938-955)
Fig. 1.6 The compartment model for FDG. It consists of input functions as glucose and FDG plasma concentrations, compartments represent brain tissue ROI for free glucose and free FDG and compartments represent brain tissue ROI for glucose 6-phosphate and FDG 6-phosphate. While glucose-6-P can be metabolized further, FDG-6-P can only be hydrolyzed back to free FDG. The asterisk is used to denote quantities associated with FDG or FDG-6-P. Quantities for natural glucose or glucose-6-P are denoted with symbols without the asterisk.

Fig. 1.7 Target tissue and reference tissue compartment models for a ligand binding tracer, where ‘F+NS’ represents ‘free and non-specific’.
### Table 1.1 Select List of Radionuclides That Decay by Positron Emission and Are Relevant to PET Imaging

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>$E_{\text{max}}$(Mev)</th>
<th>$\beta^+\text{ Branching Fraction}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{11}\text{C}$</td>
<td>20.4 min</td>
<td>0.96</td>
<td>1.00</td>
</tr>
<tr>
<td>$^{13}\text{N}$</td>
<td>9.97 min</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td>$^{15}\text{O}$</td>
<td>122 s</td>
<td>1.73</td>
<td>1.00</td>
</tr>
<tr>
<td>$^{18}\text{F}$</td>
<td>109.8 min</td>
<td>0.63</td>
<td>0.97</td>
</tr>
<tr>
<td>$^{64}\text{Cu}$</td>
<td>12.7 h</td>
<td>0.65</td>
<td>0.29</td>
</tr>
<tr>
<td>$^{76}\text{Br}$</td>
<td>16.2 h</td>
<td>Various</td>
<td>0.56</td>
</tr>
<tr>
<td>$^{82}\text{Rb}$</td>
<td>1.27 min</td>
<td>2.60, 3.38</td>
<td>0.96</td>
</tr>
<tr>
<td>$^{124}\text{I}$</td>
<td>4.17 d</td>
<td>1.53, 2.14</td>
<td>0.23</td>
</tr>
</tbody>
</table>

### Table 1.2 Properties of scintillator materials used for gamma-ray detection at 511 KeV.

<table>
<thead>
<tr>
<th>Scintillator</th>
<th>Density (g/cc)</th>
<th>Decay Time (ns)</th>
<th>Effective Atomic Number</th>
<th>Relative Light Yield (with respect to NaI)</th>
<th>Linear Attenuation Coefficient (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaI$^*$</td>
<td>3.67</td>
<td>230</td>
<td>51</td>
<td>100%</td>
<td>0.34</td>
</tr>
<tr>
<td>BGO</td>
<td>7.13</td>
<td>300</td>
<td>74</td>
<td>15%</td>
<td>0.95</td>
</tr>
<tr>
<td>LSO</td>
<td>7.40</td>
<td>40</td>
<td>66</td>
<td>75%</td>
<td>0.88</td>
</tr>
<tr>
<td>GSO</td>
<td>6.71</td>
<td>60</td>
<td>59</td>
<td>25%</td>
<td>0.70</td>
</tr>
</tbody>
</table>

$^*$Sodium iodide crystal contains a trace amount of thallium.
Chapter 2. An Automated Movement Correction (MC) Procedure

2.1 Introduction

As introduced in Chapter 1, PET/CT is widely used in the clinic for oncological, neurologic and cardiologic applications. CT image of PET/CT study not only provides anatomical information but also is used for attenuation correction (AC) (26) and scatter correction (SC) (51) to replace the transmission scan in PET-only scans. Typical dynamic PET scans with molecular imaging probes usually last for over one hour. Head movement is frequently observed during this time. Even for patients with belt constraint on the head, significant movement is obvious in late time frames. The problem is especially serious for elderly patients and patients with movement disorders.

The effects of head movements on attenuation correction and scatter correction on stand-alone PET scans have been investigated (2,52). Generally, head movements during PET/CT dynamic scan may create artifacts in scatter corrected and attenuation corrected PET images due to the mismatch between CT and PET images. The artifacts such as uneven tracer distribution cannot be eliminated by simply realigning the PET images in different dynamic frames to a reference frame or to the CT image alone (2). Moreover, time activity curves (TACs) of different regions are usually extracted from dynamic PET images and are then used for kinetic modeling to calculate physiologic parameters such as distribution volume ratio (DVR) for FDDNP scans and metabolic flux rate (Ki) for FDG scans (43,44). The errors in the PET images may create incorrect physiologic parameters (53,54).

The aim of this study is to enhance the dynamic PET images in both qualitative and quantitative aspects using an automated retrospective image-based MC method. This MC method not only corrects movements between CT and dynamic PET frames but also corrects movements
during the dynamic PET scan. This method was first validated on phantom scans and the effect of MC was then demonstrated on a few dynamic FDDNP and FDG PET scans on patients.

2.2 Attenuation Correction

In order to generate accurate PET image, after sinogram is acquired, several corrections are need to apply on sinogram before reconstruction. Attenuation correction is one of them and has the most significant impact on PET image data (55). Photons that travel through tissues undergo attenuation, predominantly through Compton interaction and also through photoelectric effect (4). Consider an annihilation event which happens at point B in the subject and two annihilation photons travel towards detector a and detector b (Fig. 2.1). If the LOR between detectors a and b crosses the object through point A to point C, and the attenuation coefficient for 511 KeV photons on position x along LOR within the object is $\mu_{511\text{KeV}}(x)$, then the probability that both photons will escape the object and arrive the pair of detectors ($P_n$) is shown in Eq. 2.1. $p_a$ and $p_b$ are the probability that each of the two annihilations from point B will escape from the object and arrive detectors (assume air has little attenuation effect on 511KeV photons), and $P_n$ is the product of $p_a$ and $p_b$. It turns out that $P_n$ is independent of the position of point B along AC but only dependent on AC itself. The photon attenuation inducing lower signal in depth of the body is needed to be corrected. The attenuation correction factor (ACF) for the LOR across points A and C is simply

$$(p_a \times p_b)^{-1},$$

which equals to

$$P_n = p_a \cdot p_b = e^{\int_{A}^{B} \mu_{511\text{KeV}}(x)dx} \cdot e^{\int_{B}^{C} \mu_{511\text{KeV}}(x)dx} = e^{\int_{A}^{C} \mu_{511\text{KeV}}(x)dx}$$

Eq. 2.1

Consider a uniformly radioactive cylinder phantom, without attenuation correction, the central portion of the cylinder appears to have lower activity than the outer edge because photons coming
from the center of the cylinder must pass through more material to reach the detectors than photons at the edge.

In PET/CT scanner, for X-ray CT image, the relation of X-ray intensity arriving at detectors with source intensity is shown in Eq. 2.2.

\[
\frac{I}{I_0} = e^{-\int_{C}^{A} \mu_{X-Ray}(x) \, dx}
\]

\[\text{Eq. 2.2}\]

By finding out the relation between the attenuation coefficient for 511KeV photons (\(\mu_{511\text{KeV}}\)) and for X-ray photons (\(\mu_{X-Ray}\)), ACF of each LOR between a certain pair of detector can be converted from the CT image of the same subject and thus generate an attenuation correction factor matrix. Previous studies found a bilinear scaling function could be used to convert \(\mu_{X-Ray}\) into \(\mu_{511\text{KeV}}\): A single scaling factor which is independent of the peak kilovoltage (kVp) setting of the x-ray tube is applied to all the tissue with \(\mu_{X-Ray} < (1060\mu_{X-Ray,\text{water}}/1000)\) (i.e. Hounsfield unit (HU) <60, where \(\mu_{X-Ray,\text{water}}\) is water’s attenuation coefficient for X-ray and HU is a linear transformation (Eq. 2.3) of \(\mu_{X-Ray}\)); a higher scaling factor which is dependent of kVp is applied to the tissue with \(\mu_{X-Ray} > (1060\mu_{X-Ray,\text{water}}/1000)\) (i.e. HU>60) (25,56). The conversion process is described in Fig. 2.2.

\[
\text{HU} = 1000 \times \left( \frac{\mu_{X-Ray}}{\mu_{X-Ray,\text{water}}} - 1 \right)
\]

\[\text{Eq. 2.3}\]

The attenuation correction matrix is multiplied to sinogram to correct sinogram before reconstruction. Thus, movements during PET/CT dynamic scan may create artifacts in scatter corrected and attenuation corrected PET images due to the mismatch between CT and PET images.

2.3 Movement Correction Methods

Considerable work has been conducted to reduce motion artifacts on PET-only scanners. Two general approaches-by motion tracking system (1) or with image-based realignment-have been
investigated to realign the transmission image to emission frame for more accurate scatter and attenuation corrections (2,57,58). An image-based MC method for the PET-only scanner has been developed for dynamic brain PET scans previously and has been shown to be practical and easy to use in several applications (2,59-61). Movement correction (MC) based on motion tracking systems such as the Polaris has been shown to be very accurate for phantom scans. However, because it is difficult to fix the motion tracker on human subject properly, image-based movement correction was found to outperform the other approach in a majority of situations (1).

2.4 Co-registration Algorithms

Consider two images, a target image \( R \) and a source image \( S \), each containing an object of interest (e.g. brain images). The aim of a co-registration is to find a transformation \( t_\alpha \) to transform the image \( S \) so that the objects within the two images are aligned. The image based MC procedure includes steps to align CT to PET and PET to PET. The accuracy of co-registration between PET and CT images and among PET images themselves is critical for image-based movement correction method. The co-registration in our proposed MC method was done by a software package called ‘Volume Imaging in Neurological Research, Co-Registration and ROIs included’ (Vinci, Max-Planck-Institut für neurologische Forschung, Köln) (62); its co-registration algorithm is briefly introduced below (63,64). To co-register two images, an optimization procedure tries different transformations and evaluates each of these parameters using a similarity metric (63-65).

2.4.1 Transformation

The transformation applied to co-register the images can be categorized according to the degrees of freedom. Rigid-body transformation is defined as one that includes only translations and rotations. It is described by six parameters: three translations along the \( x, y, \) and \( z \) axes, and three rotations around each of the respective axes. The shape of a human head changes little with
movement because of the skull, so rigid-body transformations were used to estimate the relative head positions during a dynamic PET/CT brain scan. Affine transformations add scaling and shearing to rigid-body transformations and are useful for inter-subject co-registration. Both rigid and affine transformations are linear transformation, a global similarity metric can be used to optimize linear transformations. Curved Transformations (i.e. warping) which allow the mapping of straight lines to curves are non-linear transformations. A global or local similarity metric can be used to optimize it (64,65). Fig. 2.3 shows application of these three types of transformations on a rectangular grid.

2.4.2 Similarity Metrics

The cost function, or similarity metric, evaluates the quality of alignment of the object in image R and the object in the transformed image \( t_a(S) \). The goal is to find a transformation for which the similarity measure is maximized.

The similarity metrics presented in the following sections work on pairs of corresponding voxels \((v, t_a(v))\) where \(v \in R\) and \(t_a(v) \in S\). Only voxels in the overlap of \(R\) and \(S\) are taken into account. To simplify definitions of similarity measures, we will define a sub-image \(R^0\) as the part of the image \(R\) that overlaps with the transformed image \(S\). \(R(v)\) represents the image intensity value of \(R\) in pixel \(v\) and \(S(t_a(v))\) represent the image intensity value of \(S\) in corresponding pixel \(t_a(v)\). An abbreviation \(S_a(v)\) will be used in place of \(S(t_a(v))\) in the following sections.

**Cross Correlation and Correlation Coefficient**

When both the target and source images are of the same modality (e.g. both are PET images), they may have a similar appearance and pattern. Similarity of such images can be measured by a direct comparison of image intensity values in corresponding voxels (64); cross correlation and correlation coefficient are two of the metric.
The cross correlation (C) is defined as the sum of the product of all corresponding voxel pairs in the image overlap (Eq.2.4) (64)

\[ C(R, S_\alpha) = \sum_{v \in R^c} R(v) \cdot S_\alpha(v) \quad \text{Eq. 2.4} \]

The metric tends to prefer transformation that produce a larger overlap or overlaps with higher intensity values even though other transformations might give a better alignment. It could be problematic especially when it is used as a global similarity metric. To overcome these problems, correlation coefficient (CC) is proposed as a similarity metric and defined in Eq. 2.5 (64)

\[ CC(R, S_\alpha) = \frac{\sum_{v \in R^c} (R(v) - \bar{R}) \cdot (S_\alpha(v) - \bar{S})}{\sqrt{\sum_{v \in R^c} (R(v) - \bar{R})^2} \cdot \sqrt{\sum_{v \in R^c} (S_\alpha(v) - \bar{S})^2}} \quad \text{Eq. 2.5} \]

where \( \bar{R} = \frac{1}{N} \sum_{v \in R^c} R(v) \) and \( \bar{S} = \frac{1}{N} \sum_{v \in R^c} S_\alpha(v) \) is the average image intensity value of images R and S in their overlapped region, and N is the number of pixels in the overlapped region. In practice, Vinci uses a squared version of the correlation coefficient CC^2 in order to avoid the computation of taking the square roots.

**Mutual Information**

Cross correlation and correlation coefficient are not suitable for multimodal co-registration, more sophisticated methods must be used to estimate the similarity. One of the most successful metric is the mutual information (64). The introduction of mutual information as a registration criteria for multimodality images dates back to the 1990s (66-67). Based on the entropy concept as a measure for registration of multimodality medical images, mutual Information quickly appeared and gained great success in rigid co-registration of multimodality images.

For a random variable X, with a set of value as \{x_1, x_2, \ldots, x_m\} and \( p_X(i) \) as the probability of value \( x_i \), the Shannon entropy is defined as
The Shannon entropy was introduced as a measure of information of a message, telegraph and radio communication, sending Morse code or words. The Shannon entropy can also be computed for an image, in which case it focuses on the distribution of the image intensity values of the image instead of the probabilities of letters or words occurring (65).

The histogram is a method to describe the distribution of the image intensity values of an image and thus used to estimate the entropy of an image. The histogram of image R (H_R) maps image intensity value into equally spaced discrete bins which covers the image intensity range [Minimum Image Intensity of R (r_min), Maximum Image Intensity of R (r_max)]. B_R is the number of bins desired for the histogram and width of the bin thus turns out to be (r_max - r_min)/B_R. A voxel v with intensity R(v) falls into a Bin k given as:

\[ k = \left\lfloor \frac{R(v) - r_{\text{min}}}{r_{\text{max}} - r_{\text{min}}} \right\rfloor B_R \]  
Eq. 2.7

And the H_R(k) is the number of voxels which falls into Bin k, thus the probability of an intensity falling into bin k can be approximated as

\[ p_R(k) = \frac{H_R(k)}{\sum_{i=0}^{B_R-1} H_R(i)} \]  
Eq. 2.8

where \( \sum_{i=0}^{B_R-1} H_R(i) \) is the total voxels in image R. Thus the entropy of image R, according to Eq. 2.6, can be estimated as

\[ E(R) = -\sum_{k=0}^{B_R-1} p_R(k) \log p_R(k) \]  
Eq. 2.9

Joint histogram H_{R,S} of images R and S is an estimation of the joint intensity distribution of the two images. H_{R,S}(k, l) is the number of voxels v with intensity R(v) falling into bin k and
intensity $S(v)$ falling into bin $l$. The joint probability distribution of image intensity of Image $R$ and $S$ ($p_{R,S}$) which is estimated from $H_{R,S}$ is described in Eq. 2.10

$$p_{R,S}(k,l) = \frac{H_{R,S}(k,l)}{\sum_{i=0}^{B_{\text{R}}-1} \sum_{j=0}^{B_{\text{S}}-1} H_R(i,j)}$$  
Eq. 2.10

Thus the joint entropy of image $R$ and $S$ can be estimated as

$$E(R,S) = - \sum_{i=0}^{B_{\text{R}}-1} \sum_{j=0}^{B_{\text{S}}-1} p_{R,S}(k,l) \log p_{R,S}(k,l)$$  
Eq. 2.11

The joint histogram plot of two images changes when the alignment of the two images changes. If the images are correctly co-registered, the joint histogram will show certain clusters and minimum dispersion (Fig. 2.4) and thus the joint entropy is minimized (65).

For two images $R$ and $S$, the Mutual Information MI is defined as

$$MI(R,S) = E(R) + E(S) - E(R,S)$$  
Eq. 2.12

The co-registration of $R$ and $S$ is to maximize MI. In this definition, it is clear that MI includes both the marginal entropies ($E(R)$ and $E(S)$) and joint entropy of the two images.

Studholme et al. (68,69) have shown that with increasing mis-co-registration, which normally correlates with decreasing overlap, MI can actually increase. This can occur when the overlap increases and sum of marginal entropies increases faster than the joint entropy. Studholme et al. (69) proposed a normalized measure of MI, which is less sensitive to changes in overlap

$$NMI(R,S) = \frac{E(R) + E(S)}{E(R,S)}$$  
Eq. 2.13

Improvement was found in normalized mutual information for rigid registration of MR-CT and MR-PET images.

2.4.3 Optimization

Searching for a maximum of mutual information is performed using the downhill simplex optimization method in Vinci. Unlike many optimization methods, the downhill simplex method
does not require computation of the gradient of a similarity measure function. It has been proven to be superior in speed, accuracy and robustness to other often used optimization methods (62,63).

2.5 Automated Procedure for MC

The automated retrospective MC method consisted of two major parts. The first part coregistered CT image to each of the PET frame in order to correctly provide attenuation information for the second part. The second part then re-reconstructed attenuation-corrected (AC) PET frames and aligned each frame to a common reference frame. The automated procedure was only executed once for a dynamic scan and was not iterative. The over-view of the MC procedure is illustrated in Fig. 2.5.

The sinogram corrections including attenuation correction and image reconstruction are all done by an offline JSRecon7 package (Courtesy of Siemens Inc., Knoxville, TN). All the coregistration, realignment and reslicing procedures mentioned above are done by Vinci (62). Vinci is a C++ based software package and provides active python interface. The whole procedure is streamlined and automated using an in-house active python script (ActiveState Software Inc. Vancouver, BC, Canada).

Step 0: CT and all PET frames of a dynamic PET/CT scan were reconstructed from raw data. A PET frame was selected to serve as a reference frame. The standard for choosing the reference frame is the frame has a relatively high signal-to-noise ratio and shows clear anatomical features. In our study, the 1st frame for phantom study, 7th frame for FDDNP dynamic scan and 24th frame for FDG dynamic scan were chosen as the reference frames. For other frames, attenuation correction (AC) were not applied, for the reference frame, both AC and Non-AC PET image were reconstructed.

Part I (Fig. 2.6)
**Step 1:** The background including the patient bed and head holder was stationary while the patient’s head moved. Thus, the first step of the MC method was to segment out the patient’s head from background in CT image. This was done automatically by defining a contour around the patient head and setting the background outside contour as -1000 (CT value of air) with an in-house matlab (Mathwork Inc. Natick, MA) program.

**Step 2:** The original patient’s head CT image (CT\textsubscript{ORIGINAL}) generated in the previous step was then co-registered (as a rigid body) to an AC PET image of the reference frame. Step 2 produced a CT\textsubscript{REF} which was aligned to the reference PET frame and contained no stationary part (i.e. the background structure).

**Step 3:** The non-AC reference PET frame was then individually co-registered to all other non-reference non-AC PET frames to derive (n-1) transformation matrices, where n was the total number of frames in the dynamic scans.

**Step 4:** CT\textsubscript{REF} was then resliced (n-1) times with the (n-1) transformation matrices obtained from Step 3. Step 4 produced (n-1) head CT images which were co-registered to (n-1) PET frames separately and without stationary background.

**Step 5:** The background (removed in step 1), which contained stationary components, was added back to each CT frames obtained from Step 4 automatically with an in-house MATLAB program.

**Part II**

**Step 6:** Each AC PET frame was re-constructed with ordered subsets expectation maximization (OSEM, 6 iterations and 16 subsets; OSEM was introduced in Chapter 1.2.4). Before reconstruction, sinogram was attenuation corrected using co-registered CTs generated in Step 5.

**Step 7:** Reconstructed AC PET frames were realigned to the reference PET frame.
Fig. 2.1 Attenuation correction in PET imaging. If the LOR between detectors a and b crosses the object through point A to point C, and the attenuation coefficient for 511 KeV photons on position x along LOR within the object is $\mu_{511\text{KeV}}(x)$, then the probability that both photons will escape the object and arrive the pair of detectors ($P_n$) is

$$P_n = p_a \cdot p_b = e^{\int_A^B \mu_{511\text{KeV}}(x) \cdot dx} \cdot e^{\int_B^C \mu_{511\text{KeV}}(x) \cdot dx} = e^{\int_A^C \mu_{511\text{KeV}}(x) \cdot dx}$$

where $p_a$ and $p_b$ are the probabilities that each of the two annihilations from point B will escape from the object and arrive detectors (assume air has little attenuation effect on 511KeV photons), and $P_n$ is the product of $p_a$ and $p_b$. It turns out that $P_n$ is independent of the position of point B along AC but only dependent on AC itself.
Fig. 2.2 The procedure of converting a CT scan into an ACF matrix. Bilinear scaling function is used to convert CT numbers (HUs) to linear attenuation values at 511 KeV. CT scan at 70-KeV effective x-ray energy is resampled to resolution of PET scan. Voxel value \( m_{\text{CT}} \) in resampled CT image is scaled to \( m_{\text{PET}} \) at 511 KeV using range of bilinear function appropriate for \( m_{\text{CT}} \) value. Attenuation correction factors are generated by reprojecting m-map at 511 KeV. (Reprint by permission of SNMMI from Townsend DW. Dual-Modality Imaging: Combining Anatomy and Function. *J Nucl Med*. 2008;49(6):938-955)
Fig. 2.3 Geometric transformations of a regular rectangular grid.

Fig. 2.4 Joint histogram (128 x 128 bins) of a misaligned and aligned pair of PET and MR images. (Both Fig. 2.3 and Fig. 2.4 are reprinted from Dr. Jiri Cizek’s dissertation "Robust Algorithms for Registration of 3D Images of Human Brain" achieved at Kölner UniversitätssPulikationsServer (KUPS) http://kups.ub.uni-koeln.de/1444. Their reuses are permitted by KUPS for not-for-profit purposes http://kups.ub.uni-koeln.de/policies.html).
Fig. 2.5 Over-view of retrospective MC method for PET/CT dynamic scan proposed in this study.
Fig. 2.6 Illustration of Co-registration between a CT image and a dynamic PET frame.
Chapter 3. Evaluation of Co-registration Algorithm and MC Procedure in Phantom Study

3.1 Introduction

The co-registration algorithm and automated retrospective MC method were first evaluated and validated on a phantom study. A Hoffman Phantom is a 3D phantom filled with $^{18}$F (usually FDG) solution and commonly used to simulate cerebral blood flow and metabolic images for PET. It not only mimics the tracer distribution but also simulates the attenuation and scatter (70). Two Phantom studies were carried out to evaluate the MC procedure quantitatively and qualitatively.

3.2 Materials and Methods

3.2.1 Phantom Study with a Computer Controlled Motion Platform

The co-registration algorithm and automated retrospective MC method were first evaluated and validated on phantom studies. A Hoffman Phantom filled with $^{18}$F solution was scanned by a PET/CT scanner. To validate the accuracy of the co-registration algorithm used in the MC method, a computer controlled motion platform was used to conduct pre-set translational movements of the Hoffman Phantom in different PET frames, and the images were aligned to determine the amount of movements, which were then compared with the pre-set values. Twelve PET frames were acquired at different positions with first frame as the reference frame. One CT image (CT1) was acquired of the phantom at the same position of the first PET frame (FR1) and another CT image (CT12) was acquired with the phantom at the position of the last PET frame (FR12).

Table 3.1 shows the relative positions of the phantom during the 12 frames, in reference to that of FR1. The Displacement of one frame to the reference frame was defined as:

$$\text{Displacement} = \sqrt{\text{trans}_{x}^2 + \text{trans}_{y}^2 + \text{trans}_{z}^2} \quad \text{Eq. 3.1}$$
The co-registration of CT to PET used normalized mutual information as similarity metric, while the co-registration between PET frames used correlation coefficient as similarity. The co-registration was performed using Vinci software package. The accuracy of the co-registration was evaluated by the error between the pre-set displacement and the displacement calculated from the resulted co-registration parameters.

\[
Error = \sqrt{(\text{trans}_x - \text{trans}_{x\text{-coreg}})^2 + (\text{trans}_y - \text{trans}_{y\text{-coreg}})^2 + (\text{trans}_z - \text{trans}_{z\text{-coreg}})^2} \quad \text{Eq. 3.2}
\]

The MC procedure (described in Chapter 2.5) was evaluated by examining the differences among PET images that were attenuation corrected with different datasets - aligned, mismatched and movement corrected CT (See Fig. 3.2-3.4 for more details). It should be noted that the measures of Displacement and Error defined above were only for the location at the center of the image. Without rotational movements (like in the phantom experiment with platform movements), they are also applicable to everywhere in the phantom. However, if there are rotational changes, the actual amounts of location movements in most of the head are much larger than indicated by these measures.

3.2.2 Phantom Study with Movement in All Direction and Axes

In another phantom study which further validated the MC procedure, various movements of translation, rotation and their combinations were made manually in different dynamic scanning frames to simulate head movements among different frames. The resulted images were evaluated in terms of artifacts and the amount of quantitative errors in image values.

3.3 Results

3.3.1 Evaluation of Co-registration Algorithm in Phantom Study

Co-registration errors for PET images of different AC procedures are shown in Fig. 3.1. The errors of co-registration based on the PET images without AC was smaller than the errors based
on the PET images with AC (but without MC), but were similar to those based on the AC PET images after MC. The difference was contributed by attenuation correction artifact. However, the co-registration error was always smaller than the pixel size (Fig. 3.1 A). The errors of CT-to-PET co-registration based on the PET images without AC were slightly larger than those based on the AC PET images after MC (Fig. 3.1 B).

3.3.2 Evaluation of MC Procedure in Phantom Study

Difference maps were used to further evaluate and validate the MC procedure on the phantom studies. They showed that, the accuracy of co-registration (error < 2mm) was adequate to avoid introducing extra artifacts and the MC procedure eliminated artifacts due to large mismatch between PET and CT. In Fig. 3.2-3.4, all PET images were co-registered to the reference frame for comparison. The same color scale was applied across all PET frames. A difference color scale was applied to the difference maps, on which green denoted zero difference. Fig. 3.2 shows that for small misalignment (2mm in Z direction) between PET and CT, the AC artifact was not noticeable compared to the images with correctly aligned PET and CT. Panel A of Fig. 3.3 shows that the difference map between the PET frame (FR12) which was based on a misaligned CT (CT1) for AC and the reference frame (FR1) which used a correctly aligned CT (CT1). The difference map between FR12 and the same PET frame based on a MC aligned CT (CT12) is shown in the Panel B. The misalignment between PET and CT caused significant AC artifacts. The attenuation matrix did not add extra noise to the PET images. After MC, there were no noticeable difference, except noise, between PET frame AC with originally aligned CT and PET frame AC with MC CT (Fig. 3.4A).

Result of the application of the automated MC procedure to the second phantom study is shown in Fig. 3.5 that demonstrated the procedure’s ability to correct misalignment induced by head
rotation besides translation. Co-registration to the reference frame (column 1) only reduced the attenuation artifact (column 3). Mean left-right asymmetry decreased significantly (P<0.05) from 46.8% to 6.5% after MC (column 4).

3.4 Discussion

An image-based MC method for the PET-only scanner has been developed for dynamic brain PET scans previously and used in several applications (2,59,61). The MC method requires the co-registration of medical imaging between different imaging modalities and within the same modality. The co-registration for both inter- and intra-modality has been under intensive research during the past fifteen years. Maximum mutual information has been shown to be an excellent criterion for automated and accurate rigid-co-registration of intra-individual images from different modalities especially between PET and MRI in a variety of applications (63). Multiple software packages (e.g., SPM and Vinci (60,62)) use this similarity metric for medical image co-registration. In our implementation of the MC method for PET/CT scanning, we have adopted the use of Vinci for image co-registration, since the pre-processing procedures provided by Vinci, including thresholding, speeded up the processing and made the co-registration more robust (63).

Hoffman phantom is a 3-D phantom commonly used to simulate cerebral blood flow and metabolic images for PET. It not only mimics the tracer distribution but also simulates the attenuation and scatter (70). The Hoffman phantom was used in this study to show that Vinci was adequate for cross-modality co-registration between PET and CT and for co-registration between PET frames (Fig. 3.1). For co-registration between PET and CT, the PET images with AC outperformed those without AC, even though there might be artifacts in PET with AC based on mismatched CTs. This could be due to the reason that there was stronger signal inside the brain PET image with AC. We found in our study that the co-registration reached sub-pixel accuracy.
when the mismatch, which was consistent with results reported by others before (71). In contrast, for co-registration within PET frames, the use of PET images without AC outperformed the use of PET images with AC. It was worth mentioning that, for PET images with AC after MC, the co-registration accuracy reached the same level as that for PET images without AC. This result indicated that the AC artifact influenced the co-registration accuracy among PET frames.
3.5 Figures

Fig. 3.1 Co-registration accuracy evaluated from phantom study A. PET to PET co-registration accuracy B. CT to PET co-registration accuracy.
Fig. 3.2 Comparison between difference maps. **Panel A**: the difference map between PET FR4 AC with a 2 mm mismatched CT (CT1) and PET FR1 AC with an aligned CT (CT1) (taken as the reference PET frame); **Panel B**: the difference map between PET FR12 AC with an aligned CT (CT12) and the reference.
Fig. 3.3 **Panel A**: the difference map between PET FR12 AC with a 10 mm mismatched CT (CT1) and the reference PET image; **Panel B**: the difference map between PET Frame12 AC with a 10 mm mismatched CT (CT1) and the same PET frame AC using the correctly aligned CT (CT12).
Fig. 3.4 **Panel A:** the difference map between PET FR12 AC with movement corrected CT1 and the reference image; **Panel B:** the difference map between PET FR12 AC with movement corrected CT1 and the same PET frame with the correctly aligned CT (CT12).
Fig. 3.5 Movement correction in PET/CT misalignment induced by head translation and rotation.
3.6 Table

Table 3.1 Relative positions of the Hoffman’s phantom for different CT scans and PET frames. The positions were in reference to that of FRAME1.

<table>
<thead>
<tr>
<th></th>
<th>Dynamic PET Scan</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CT1</td>
<td>FR1</td>
<td>FR2</td>
</tr>
<tr>
<td>Y' (mm)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Z' (mm)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Y: the direction perpendicular to the bed panel; Z: the direction parallel to the long axis of bed
Chapter 4. Evaluation of Co-registration Algorithm and MC Procedure in Patient FDG and FDDNP Dynamic Scans

4.1 Introduction

As introduced in Chapter 1, FDG is an $^{18}$F labeled glucose analog, most commonly used in nuclear medicine clinics today. The pattern of brain FDG scan was used to help with neurodegenerative disease (29). FDDNP is a hydrophobic molecular probe used for in vivo imaging of β-amyloid senile plaques (SPs) and neurofibrillary tangles (NFTs), the neuropathologic hallmarks of Alzheimer’s disease (AD) (72). So, FDDNP PET is useful for imaging SPs and TFTs to assess the likelihood that a patient would have AD. In patients with Down syndrome (DS), the SPs are also present in the cerebral cortex at ages as early as the 30s. They might have AD onset in their late 40s and early 50s. Only about 25% of patients with DS live past 60 years old, and most of them have evolved to AD by that time. FDDNP PET scan was thus also used on DS patients to study their SPs and NFTs deposition (59), which are also involved in the development of Mild Cognitive Impairment (MCI) and Progressive Supranuclear Palsy (PSP). In this study, the effects of head movement on tissue time-activity curves and parametric DVR and Ki images were addressed.

4.2 Materials and Methods

4.2.1 Patient Scans

The automated retrospective MC method was applied to the representative patient data and the effects of MC were evaluated. The patient dataset used included FDDNP and FDG dynamic scans on DS, AD, MCI and PSP patients. All patient studies were approved by the Human Subjects Protection Committee at UCLA, and written informed consents were obtained from all subjects.
In all these studies, a curved head-holder attached to the patient bed of the scanner and a constraint belt were used to help minimize the head movement of the patient. The same scanner with phantom study, a Biograph 64 TruePoint PET/CT scanner (Siemens, Inc., Knoxville, TN) was used. The CT scan was acquired first, and used for generating the matrix for attenuation correction. Tracer was injected intravenously as a bolus and a dynamic PET scan was initiated immediately thereafter. A moderate physical constraint through the use of a belt over the patient’s head was used during the entire scan (of over 60 min).

**Dynamic FDDNP PET/CT Scan Protocol:** Dynamic FDDNP PET/CT images were obtained from 11 subjects. $^{18}$F-FDDNP ($\sim3.7\times10^8$Bq) was synthesized using the methods previously reported (73). Total dynamic PET acquisition time was 65 min (6×30s, 4×3min, 10×5min).

**Dynamic FDG PET/CT Scan Protocol:** Dynamic FDG PET/CT images were obtained from 6 subjects. Total dynamic PET acquisition time was 60 min (9×5s, 3×15s, 3×30s, 1×2min, 5×5min, 3×10min).

### 4.2.2 Quantification

To evaluate the effects of MC on the biological quantitation of the dynamic PET imaging in human studies, the following processing steps were applied to the dynamic images to extract the biological parameters. The same procedures were applied to dynamic images without MC and with MC, and the results were compared. The effects of MC were examined in terms of artifacts and noise reduction.

**Region-of-Interest (ROI) Analysis:** ROIs were defined manually on frontal cortex, lateral temporal lobe, medial temporal lobe, parietal cortex, posterior cingulate and cerebellum.

**FDDNP Distribution Volume Ratio (DVR):** Distribution Volume (DV) in tissue was defined as the ratio between tissue concentration and blood concentration at steady state (Eq. 4.1):
With regions without specific binding (reference region: cerebellum for FDDNP scan), Distribution Volume Ratio (DVR) was defined as Eq. 4.2:

\[
DVR = \frac{DV_{\text{tissue}}}{DV_{\text{reference}}} \quad \text{Eq. 4.2}
\]

Logan analysis (43) was usually used to estimate the DVR as the slope in the linear range of the Logan plot (Eq. 4.3).

\[
\int_0^T \frac{C_{\text{tissue}}(t)dt}{C_{\text{tissue}}(T)} = DVR \times \int_0^T \frac{C_{\text{reference}}(t)dt}{C_{\text{tissue}}(T)} + \text{const} \quad \text{Eq. 4.3}
\]

DVR images were generated using Logan Analysis on the dynamic FDDNP scans (with and without MC).

**FDG flux rate \((K_i)\):** To calculate \(K_i\), image-derived input functions (IDIF) were obtained from the carotid artery (CA) VOI \((C_{\text{plasma}}(t))\) (74,75). The early images over the early 45-second period were summed. To define CA VOI, 5 mm diameter circles were manually defined on CA in three consecutive slices which had clearly visible carotid artery below the temporal lobe. TACs were extracted from CA VOI \((C_{\text{plasma}}(t))\) and used as IDIF; parametric images of the FDG uptake constant \((K_i)\) were generated using Patlak Analysis (Eq. 4.4) (76). Both IDIF and the \(K_i\) values from images with MC were compared with those without MC. (Note: In Chapter 4, for convenience, FDG concentrations \(C_{\text{plasma}}\) and \(C_{\text{plasma}}\) and flux rate \(K_i\) are NOT denoted with asterisk)

\[
\int_0^T \frac{C_{\text{tissue}}(t)dt}{C_{\text{tissue}}(T)} = K_i \times \int_0^T \frac{C_{\text{plasma}}(t)dt}{C_{\text{tissue}}(T)} + \text{const} \quad \text{Eq. 4.4}
\]
4.3 Results

4.3.1 Evaluation of MC Procedure in Patient FDDNP Dynamic Scan

If the displacement was larger than 5 mm (resolution of the PET/CT imaging system) or the rotation was greater than 4 degree, the movement would cause visually noticeable artifacts in the PET images. Half of the subjects had displacements over 5 mm in late frames (after 40 min) (Fig. 4.1). The z-direction translation and nodding were found the most common patient movements during a dynamic scan. Under-attenuation or over-attenuation correction artifacts were observed in frontal or parietal region due to mismatch in z direction.

Fig. 4.2 shows the improvement of the PET/CT images with proper AC after the automated MC procedure in a FDDNP PET/CT dynamic scan on a DS patient. Panel A shows the FDDNP PET images reconstructed based on a CT image that was misaligned (due to movement between CT and PET imaging). Panel B shows the same PET image attenuation corrected and reconstructed based on the CT image that was properly aligned post MC. Significant differences were seen between images in the Panels A and B in the figure.

Since the tissue TAC of FDDNP in the temporal region usually became linear later in time when plotted as a Logan plot, we used it to determine the time range for DVR image generation. Comparison before and after MC of TACs and their corresponding Logan plots for a medial temporal region in a patient is shown in Fig. 4.3. TAC and Logan plot showed less fluctuation after MC. The slope of the Logan plot increased by 7.5% after MC.

The slope of Logan plot 30 minutes after scan start was used to estimate the DVR value of each pixel and a DVR image was generated. Improvement in the DVR image after MC was also shown in Fig. 4.4 A for a patient who had head rotations and translations. Reduction in artifacts in the temporal region, and in left-right asymmetry after MC was easily noticeable. The same study
also showed better hemisphere separation, increased uptake in brain stem and occipital regions, and decreased uptake in frontal region after MC (Fig. 4.4 B).

In the study of 12 patients with FDDNP dynamic PET/CT scans, DVR values in the frontal lobe and medial temporal region were significantly different (p<0.05) before and after MC; the absolute difference of DVR before and after MC was 0.074 (8.1%) and 0.076 (6.9%) respectively (Fig. 4.5).

4.3.2 Evaluation of MC Procedure in Patient FDG Dynamic Scan

Movements were observed in all studied subjects in late PET frames with an average central displacement of 5.55±2.9 mm. Artifacts were observed in late dynamic frames and the $K_i$ image before MC, and were significantly reduced after MC as shown in Fig. 4.6 for a representative patient study. For IDIF from CA ROI, the area under the curve (AUC) and the AUC of the TAC between 30 and 60 minutes (tail AUC) were respectively changed by 33% and 57% in average after MC. The $K_i$ values were changed after MC in posterior cingulate, frontal, parietal and temporal regions by 44.6%, 46.4%, 53.2% and 48.3% in average, respectively. The coefficient of variation of $K_i$ value among the 6 subjects over those regions decreased from 68.4% to 20.9% after MC.

4.4 Discussion

Multiple static Hoffman phantom scans mentioned in Chapter 3 (Table 3.1, Fig. 3.5) did not exactly reflect the dynamic FDG patient scan, in which the radioactivity distribution changed over time, while Hoffman phantom only mimicked the FDG distribution in the brain at a single time point late after tracer injection. The evaluation of the MC method, was thus further performed on patient dataset of FDDNP and FDG scans (Fig. 4.1-4.6). In these evaluations with patient images, the effects of head movement and MC could not be evaluated in absolute terms. For example, in
the evaluation of their effects on the FDG Ki values, the exact percent changes should be interpreted with caution. Since no spillover and partial volume correction was applied in estimating the blood TACs, the input functions used in calculating the Ki values were not exactly correct. However, the results shown in Fig. 4.6, we believe, do represent the potentially large effects of head movement on the FDG Ki values that can be significantly reduced with the MC method presented in this study.

For the patients who have neurodegenerative disease or could not stay stationary during a dynamic study, even with a shortened dynamic scan (from two hours (2) to one hour) and with head belt for restraint, head movements were clearly noticed among half of the subjects studied (Fig. 4.1). Common head movements observed in this study included translation in the z-direction, rotation around the z-axis (left-right head rotation) and rotation around x axis (head nodding). With the back of the head as a pivoting point, forehead generally had a larger displacement compared to the back of the head, which was consistent with our previous report (2). Therefore, the frontal, parietal and temporal cortices that are important for AD evaluations (77,78) are particularly sensitive to head movements during dynamic PET scans (Fig. 4.2-4.5), and the automated MC method reported in this study is expected to improve the most for the dynamic PET/CT image results of these types of patients.

4.5 Conclusion

The retrospective image-based MC method described in this study is feasible for dynamic $^{18}$FDDNP and $^{18}$F-FDG PET/CT brain scans. It significantly improves the image quality and the measured tracer kinetics derived from dynamic PET/CT images. The MC thus should be applied if reliable DVR and Ki estimations on neurodegenerative patients are desired. The proposed MC method is also expected to be applicable to PET studies of patients with other disorders (e.g.
Parkinson’s disease) and to brain PET scans with other molecular imaging probes, but separate validation studies should be performed for each case.

4.6 Figures

Fig. 4.1 Displacement during dynamic FDDNP PET/CT scan: Half of the subjects have displacements over 5 mm in late frames (after 40 min).
Fig. 4.2 Improvement of a late PET/CT frame image after MC. **Panel A:** An FDDNP PET/CT study on a Down Syndrome patient with 10 mm movement in z direction in a late frame of a dynamic scan. **Panel B:** 3-D orthogonal images show the improvement of the PET/CT images with proper AC after MC. All the images are displayed with the same color scale and show clearly the quantitative differences in the PET images reconstructed with misaligned and aligned CT images.
Fig. 4.3 Time activity curve and Logan Plot of FDDNP kinetics in the medial temporal region of a subject who had large head movements during a dynamic FDDNP PET scan.
Fig. 4.4 A subject with both head nodding and shaking movements showed reduction in artifacts in the temporal region, reduction in left-right asymmetry, better hemisphere separation after MC. Panel A and B show the same subject at different cut position before and after MC.

Fig. 4.5 Boxplot for absolute difference of DVR in Regions of interest (ROIs) before and after MC.
Fig. 4.6 A. Orthogonal PET/CT images (before and after MC) of a late frame in a dynamic brain FDG scan of a DS patient; attenuation correction artifact in the right frontal region (pointed at by arrows) diminished after MC (lower row). B. Image Derived Input Function: After MC, a 21% drop in the AUC drop during 30-60 minutes was seen. C. The $K_i$ image: The $K_i$ values are seen to be more uniform (except the affected regions) and globally higher after MC. In this case, the lower input function accounted for the global increase in the $K_i$ values.
Chapter 5. An Automated MRI-Template Based Volume of Interest (VOI) Analysis for Rat Brain FDG PET Scan

5.1 Introduction

As introduced in Chapter 1, there are several critical yet unique issues in PET measurement of the rat brain. Besides good experiment design and animal handling, systematic analysis is necessary for minimizing subject biases in manual Volume of Interest (VOI) definition. Automated atlas-based image analysis system for PET and MRI studies of the rat brains have been developed. Several atlases were derived from cryo-sectioned animals and used for longitudinal studies (79,80). With the development of small animal dedicated MRI in the recent decade and the resulting advantages of excellent soft tissue contrast, several MRI templates and atlases were constructed and used for both PET and functional MRI VOI analysis (81, 82).

5.2 MRI Template

VOI analysis was based on a rat brain MRI template and atlas. The MRI template and atlas were adapted from Dr. Schwarz and Dr. Nie’s works (81,82). The atlas is a VOI set, which included whole brain and 7 brain regions (Lateral Prefrontal Cortex (LPC), Medial Prefrontal Cortex (MPC), Sensorimotor Cortex (SC), Striatum (S), Hippocampus (H), Cerebellum (C) and Brainstem (BS)) was predefined on the MRI template.

5.3 Automated Procedure

The MRI template was warped to individual MRIs to provide elastic mappings, then the transformations which rigidly co-registered a MRI image to FDG images of the same rat was calculated. The elastic mapping and the rigid transformation were applied to the atlas (the predefined VOI set) to map it to FDG images to extract FDG activity in each VOI (Fig. 5.1).
Absolute quantification parameters (e.g. cerebral glucose metabolism rate and FDG flux rate), Regional Standardized Uptake Value (SUV) or SUVR (normalized to a reference region, e.g. cerebellum, brain stem or whole brain) were then calculated. The software package used for elastic mapping was Advanced Normalization Tools (ANTs, Penn Image Computing & Science Lab at University of Pennsylvania, Philadelphia, PA) with the criterion of local cross correlation maximization (83). The rigid co-registration was done by Vinci (62) using the criterion of global normalized mutual information maximization. The whole procedure was streamlined and automated with an in-house active python script (ActiveState Software Inc. Vancouver, BC, Canada).

5.4 Figures

Fig. 5.1 A T2-weighted MRI template is warped to each rats individual MRI and elastic mappings are created. Each warped MRI is rigidly co-registered to FDG PET images of the same animal and transformations are obtained. The VOI set which includes 7 different regions is predefined on the template MRI. The elastic mapping and the rigid transformations are applied to the VOI set and mapping it to an individual FDG PET scan.
Chapter 6. Evaluation of Longitudinal Regional Changes in Normal Rat Brain Metabolism Using Automated MRI Template Based VOI Analysis

6.1 Introduction

Since PET imaging is non-invasive, this enables repetitive measurements for longitudinal studies in the same animal. However, the longitudinal changes due to normal aging, repeated handling and etc. has not been fully investigated. This information is very important because these changes need to be considered when designing a longitudinal study in order to properly understand the effects of any intervention. A previous study, which tried to cover this issue only compared the SUVRs within one week and one year apart (79). More time points are needed to add into the time course to better understand longitudinal changes. And this study was focused toward comparison between aged and young rats. It is important to understand the cerebral FDG uptake pattern change in aged rats for experiment design involved aged rat model. However, for other studies which do not want to include aging effects into the study design, the longitudinal change in desired model animal age range (e.g. young adult) is more relevant. This study focused on continuous longitudinal study of brain activity in young adult rats with the intention of aiding future experimental designs that were based on following the animal across a range of time.

In addition, compared to clinical studies, there are some factors that are unique to pre-clinical studies that need to be considered to keep experimentation consistent. Anesthesia is usually necessary for handling animals. Minimization of the anesthetic influence is very critical for animal studies, especially when assessing brain metabolism. The impact of a variety of common anesthetic agents on the rat brain was evaluated previously with both autoradiography and small animal
dedicated PET (4). The study evaluated the conscious FDG uptake during administration of anesthesia and arrived at an optimized a time for injection, such that FDG uptake disruption was minimal (84). The optimization, however, was focused only on injected anesthesia rather than inhaled anesthesia e.g. isoflurane; and the scanner had lower resolution than is in use today. Furthermore, the influence of the FDG injection route upon FDG uptake and dynamics was another important factor (79,85). Since intravenous tail vein injection in rodents needs intensive training, researchers often try to seek alternatives to replace intravenous injection (IV) with intraperitoneal injection (IP). However, there is typically a 10% possibility that IP injection might fail due to misinjection into the wrong location (e.g. bladder); thus avoiding such potential problems is crucial for conducting accurate longitudinal studies.

6.2 Materials and Methods

6.2.1 Animals

Ten Sprague-Dawley (SD) rats (Charles River, Portage, MI) were maintained in a strict defined-flora, pathogen-free environment in the AAALAC-accredited animal facilities at UCLA. Rats were housed in IVC cages (Innovive, San Diego, CA) with 1 inch corn cob bedding at 21°C, 70% humidity and a 12 hour light/dark cycle.

A group (Group 1) of five rats were used to study the effect of the anesthetic conditions and injection routes. After the anesthetic conditions and injection route were optimized, another group of five rats (Group 2) was studied longitudinally (Table 6.1). All animal experiments were performed in accordance with institutional guidelines and protocols approved by the Animal Research Committee of the University of California, Los Angeles, Los Angeles, CA.
6.2.2 FDG PET and CT Scan

Rats were imaged using a small-animal PET scanner (Inveon DPET, Siemens Preclinical Solutions, Knoxville, TN) running Inveon Acquisition Workplace (IAW) 1.5.0.28 for image acquisition and image reconstruction. Images were created using filtered backprojection (~1.5 mm) into a 128×128×159 matrix with a voxel size of 0.63×0.63×0.8 mm³. Attenuation and scatter corrections were applied based on a CT image. CT images were acquired immediately following the PET imaging using a small animal dedicated CT (MicroCAT II, Siemens Preclinical Solutions, Knoxville, TN). CT parameters were 70 kVp, 0.5 sec exposure, 360 steps and 2 mm Al filtration. Images were created using a modified Feldkamp process into a 194×194×176 image matrix with isotropic 0.50 mm voxels.

Rats were fasted overnight before the administration of FDG. Blood glucose levels were sampled from the tail vein before and after scanning. 37MBq (1 mCi) FDG was administrated either intravenously (IV) through the tail vein or intra-peritoneal (IP).

6.2.3 Anesthetic Conditions and Injection Routes Optimization

Five Sprague Dawley (SD) female rats of the Group 1 underwent scans under three conditions: 1) anesthetized FDG uptake with intravenous FDG injection (aIV), 2) conscious FDG uptake with intravenous FDG injection (cIV) and 3) conscious FDG uptake with intraperitoneal FDG injection (cIP). The scans under three different conditions were acquired on same animals at 20-week (5-month), 21-week and 22-week old time-points respectively. For anesthetized uptake, 2% isoflurane was provided to rats through the nose cone beginning 50 minutes before scan start. Scan data was acquired when administration of FDG intravenously via tail vein. For conscious uptake, FDG was injected either IV or IP. After 40 min of FDG uptake in an awake state, rats were anesthetized using isoflurane starting 10 minutes prior to scanning. A PET brain scan was
conducted at 50 min after FDG injection for both anesthetic and conscious rats, as shown in Fig. 6.1.

### 6.2.4 Longitudinal FDG Brain PET Scan

10-minute PET scans were acquired following conscious 40-minute FDG uptake at weeks 0, 1, 2, 4, 8, 12 and 24 from 5-month old. The age was roughly matched to 15 – 25 year old in human. The data was used to assess longitudinal changes and reproducibility of regional brain PET data over time, looking at both intra-individual and inter-individual variability (Fig. 6.2).

### 6.2.5 T2-weighted MRI Scan

To acquire more detailed structural information, T2-weighted MRI scans were obtained on a 7.0 T animal MRI scanner (70/16 PharmaScan, Bruker Biospin, Germany) with ParavVision 5.0 software, using a volume coil for radio frequency (RF) transmission and a quadrature surface coil for signal detection. The structural images were obtained with a rapid acquisition with relaxation enhancement (RARE) sequence (RARE factor = 6, repetition time (TR) = 10493 ms, echo time (TE) = 36 ms, matrix size 256 × 140 × 80, voxel size 0.2 × 0.2 × 0.4 mm3, no slice gap).

### 6.2.6 Standardized Uptake Value (SUV) and SUV Normalized to Whole Brain (SUVR)

Automatic VOI analysis was done as introduced in Chapter 5, radioactivity of each VOI was extracted.

Isoflurane and injection routes effect on regional FDG uptake was evaluated by SUV with the equation below:

\[
SUVR = \frac{Radioactivity \text{ in VOI}}{\text{Injection Activity/Body Weight}}
\]  
Eq. 6.1

SUVR was used evaluate longitudinal brain regional metabolic change calculated with the equation below:

\[
SUVR = \frac{Radioactivity \text{ in VOI}}{Radioactivity \text{ in whole brain}}
\]  
Eq. 6.2
6.3 Results

6.3.1 Effects of Isoflurane on FDG Uptake

Forebrain FDG uptake (SUV) under isoflurane was decreased by approximately 50% compared to the level of forebrain uptake in conscious rats. In contrast, cerebellar FDG uptake was less affected, decreasing by 30% of the conscious uptake levels (Fig. 6.3). The overwhelming effect of isoflurane on brain metabolism was clearly shown.

6.3.2 FDG Dynamics of Different Uptake Conditions and Injection Routes

SUV Time activity curves of three different animal handling methods (aIV, aIP and cIV) are shown in Fig. 6.4; time axis is “0” at time of FDG injection. As discussed above, anesthetic FDG administration and uptake led to substantially lower FDG brain uptake as assessed by SUV’s, especially for cerebellum. Comparing routes of administration, intravenous administration minimized within-group variability. With respect to time-course, relatively stable SUV’s were obtained by 50-60 minutes after injection for all regions, pointing to a suitable timeframe for assessing static image data.

6.3.3 Significant SUVR Change Longitudinally

Repeated measures ANOVA showed stable SUVR in sensorimotor cortex (SC) and lateral prefrontal cortex (LPC), but significant SUVR changes over time (p<0.01) in 5 other regions. Linear regression showed SUVR decreased with number of experiments (n) and number of weeks (w) in striatum (S) (-0.0096n+1.22 R2=0.78, -0.0023w+1.213 R2=0.77), hippocampus (H) (-0.010n+1.14 R2=0.88, -0.0026w+1.13 R2=0.84) and medial prefrontal cortex (MPC) (-0.015n+1.32 R2=0.98, -0.0036w+1.30 R2=0.86), while SUVR increased in cerebellum (C) (0.0060n+1.17 R2=0.34, 0.0014w+1.17 R2=0.30) and brain stem (BS) (0.010n+0.89 R2=0.95, 0.0025w+0.90 R2=0.84 (Table 6.2).
Longitudinal metabolic change in three representative brain regions is shown in Fig. 6.5. Different brain regions showed different trends in brain metabolism over time. In the forebrain regions, the medial prefrontal cortex, was gradually losing activity, while the sensorimotor cortex had no significant changes between the periods of 5 month-old to 11 month-old. However, the hindbrain region and brain stem had relatively increased activity compared to the whole brain region.

6.4 Discussion

6.4.1 Overview

Small animal dedicated PET solved the critical issue of inadequate resolution and thus allowed the application of this non-invasive imaging technology to longitudinal brain study in rodents. Although several factors that might influence FDG PET imaging in rodents had already been addressed in the past, such as anesthetic agents, injection routes, diet conditions and blood glucose levels, the stability of brain metabolism across time in a rodent remained to be addressed. It is important to be aware of natural longitudinal changes in a rodent’s brain, especially when conducting longitudinal interventional studies, since normal aging outcomes might be over-interpreted.

Several studies comparing the FDG uptake in young and aged rats have already been conducted and assessed by PET imaging. Previously, when comparing the brain metabolic activity of the same rats at the age of 4 and 16 months, notable FDG SUVR changes were observed: significant decrease in activity of the hippocampus and striatum, slight decrease in the activity of the cerebral cortex, and significant increase in the activity of cerebellum (79). The trend we found in young adult rats (5-11 months old) was accordance with their findings in hippocampus, striatum and cerebellum. But we followed the change with more time points and further elucidated linear
relation between SUVR and time. In addition, in our study we discovered the inhomogeneity in cortex by looking into refine VOIs: the medial frontal vortex displayed significant decreases in metabolic activity, while the activity of lateral prefrontal cortex and sensorimotor cortex remained stable across time. In another study, which compared the cerebral metabolic rate (CMR) of two groups rats at their 4 and 21 months respectively, similar results were noted in whole brain, cerebral cortex, striatum and cerebellum. Interestingly, however, this study noted a significant increase in the metabolic activity of hippocampus in aged groups compared to young groups (86). However, the rats used in this study were exposed to considerable amounts of the anesthetic isoflurane: the rats were continuously under 2% isoflurane during tail vein catheter install, a 20-min $^{11}$C tracer scan, 15-min break and a following 40-minute FDG dynamic scan. It is possible that the mentioned aging effect on FDG uptake might be overwhelmed by the use of anesthetics, and the observed increase in hippocampal CMR might at least could partially be a result of differing responses to isoflurane by the two age groups. Previous studies showed that hippocampus was considerably depressed by isoflurane (87) and this effect may not be uniform across aged groups. Actually in our study, older rats were observed to be less sensitive to isoflurane; or, it took older rats longer time to be fully anesthetized when exposed to the same amount of isoflurane (data not shown).

### 6.4.2 VOIs in MRI and PET

The resolution of MRI can reach as high as 50 μm for one pixel size, and it can be adjusted by setting scan parameters for the purpose of shortening scan time. The in-plane resolution of our rat brain for structural information is 200 μm, which is around one-fourth of PET pixel size and one-eighth of PET resolution (measured as FWHM). Thus some brain regions are visible and could be defined on MRI but are too small for small animal dedicated PET to have a good statistics (e.g., globus pallidus). The smallest region we examined was lateral frontal cortex, which were in
average 56 pixels in PET images. However, this region was very close to the olfactory bulb and was a small region at the tip of prefrontal cortex; the value of this region could be influenced by spillover from the olfactory bulb and the accuracy of attenuation correction.

6.4.3 SUV vs. SUVR

Since the effect of isoflurane was substantial and we wanted to avoid this confounding effect to study aging, static scans acquired with conscious uptake were the best alternative instead of dynamic imaging. Semi quantitative parameter SUVR was chosen to evaluate FDG uptake in long term experiment for the reasons listed in Table 6.3.

6.4.4 Other Factors that May Cause Longitudinal Brain Metabolic Change

The rats we chose were 5-month-old at the beginning of the experiment in order to examine the rats that were already mature but not significantly aged. Longitudinal changes in brain metabolism pattern evaluated by FDG were observed and showed time dependency in those young-adult rats. It has been shown that reduced cerebral glucose uptake is related to cerebral functional decline in both aged rats and aged humans (88, 89). It is interesting to note that our study observed this trend in young adult rats as well. However, we cannot fully rule out repeated handling and fasting effects. In addition, though the rats were exposed to isoflurane for a short time, repeated exposure to isoflurane is not fully understood. Recently it has been reported that isoflurane may cause changes similar to Alzheimer’s disease in rodents (90).
6.5 Figures

Fig. 6.1 PET scan design for different injection routes and uptake conditions.

Fig. 6.2 The timeline of longitudinal FDG brain PET scan. The red dots indicate the 7 repeated scans on the timeline.
Fig. 6.3 Brain images of rat with PET scan under anesthetized FDG uptake condition, PET scan under conscious FDG uptake condition, and T2-weighted MRI scan for structural information. The color bars show the SUV ranges.
Fig. 6.4 FDG dynamics **A.** in cerebrum and **B.** in cerebellum with different uptake conditions and injection routes: anesthetic FDG uptake with IV injection (aIV), conscious FDG uptake with IV injection (cIV), conscious FDG uptake with IP injection (cIP).
Fig. 6.5 Longitudinal SUVR change in three representative brain regions: medial prefrontal cortex (MPC), sensorimotor cortex (SC) and brain stem (BS).
6.6 Tables

Table 6.1 Uptake conditions and injection routes applied to each experimental group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Uptake Condition</th>
<th>Injection Route</th>
<th>Scan Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Anesthetized</td>
<td>Intravenous</td>
<td>20-week old</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conscious</td>
<td>Intravenous</td>
<td>21-week old</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conscious</td>
<td>Intraperitoneal</td>
<td>22-week old</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Conscious</td>
<td>Intravenous</td>
<td>20, 21, 22, 24, 28, 32, 44-week old</td>
</tr>
</tbody>
</table>

Table 6.2 Repeated measures ANOVA and linear regression of longitudinal SUVR in seven brain regions

<table>
<thead>
<tr>
<th>Repeated Anova</th>
<th>Linear Regression to Number of Experiment (n)</th>
<th>Linear Regression to Time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>Slope</td>
<td>$R^2$</td>
</tr>
<tr>
<td>MPC</td>
<td>4.80E-4</td>
<td>-1.52E-02</td>
</tr>
<tr>
<td>LPC</td>
<td>1.86E-1</td>
<td>-2.21E-03</td>
</tr>
<tr>
<td>SC</td>
<td>7.09E-1</td>
<td>-7.14E-04</td>
</tr>
<tr>
<td>S</td>
<td>1.83E-4</td>
<td>-9.32E-03</td>
</tr>
<tr>
<td>H</td>
<td>5.37E-5</td>
<td>-1.03E-02</td>
</tr>
<tr>
<td>C</td>
<td>2.89E-3</td>
<td>5.86E-03</td>
</tr>
<tr>
<td>BS</td>
<td>2.78E-4</td>
<td>1.04E-02</td>
</tr>
</tbody>
</table>
Table 6.3 Comparison between standardized uptake value (SUV) and normalized SUV (SUVR)

<table>
<thead>
<tr>
<th>Standardized uptake value (SUV)</th>
<th>Normalized SUV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial FDG SUV is influenced by FDG uptake in extracranial structures (e.g. Hadrian gland, skeletal muscles, tongue), even in the absence of a real difference in brain activity.</td>
<td>SUVR=SUV/reference SUV</td>
</tr>
<tr>
<td>Also influenced by blood glucose level, body weight/lean mass, blood-brain barrier penetrance, peripheral dose infiltration, etc.</td>
<td>Reference region: Whole brain uptake</td>
</tr>
<tr>
<td>Larger inter-animal variation (9.6-27.9%)</td>
<td>To the extent these factors influence tracer uptake across brain regions relatively uniformly, internal normalization minimizes these sources of variability</td>
</tr>
<tr>
<td></td>
<td>Smaller inter-animal variation (1.1-4.9%)</td>
</tr>
</tbody>
</table>
Chapter 7. Evaluation of Longitudinal Regional Changes in Post Chemotherapy Rat Brain Metabolism Using Automated MRI Template Based VOI Analysis

7.1 Introduction

It was found that although survivorship of cancer kept rising since 1990s, the quality of life of certain surviving patients was affected due to the symptoms of cognitive dysfunction (91). Cognitive complaints following cancer and cancer therapy are common. Many studies have investigated the effects of chemotherapy on the brain.

Symptoms of cognitive impairment following chemotherapy for cancer have been found to be prevalent in many studies. Early on, Schagen et al. reported that 28% of breast cancer patients suffered persistent cognitive dysfunction nearly two years after treatment with conventional chemotherapy (92). This problem has been confirmed in a number of subsequent prospective longitudinal studies such as the finding by Stewart that over 30% of women receiving cytotoxic chemotherapy for early stage breast cancer experienced reliable cognitive decline, compared with 12% of women receiving hormone therapy only, though not all investigators have documented such marked differences (93,94). Evidence has also accumulated over the past decade that prior exposure to chemotherapy as well as anti-estrogenic or estrogen-based therapies in humans is associated with altered brain metabolism (3,95-97), and in some cases with changes in brain structure (98,99). However, the underlying mechanisms remain unclear (100,101).

It appears that most cytotoxic agents do not enter most areas of the brain in high concentration when the layers of tissue comprising the blood-brain barrier (BBB) are healthy. However, BBB permeability can be altered by cancer, as well as therapies for cancer. Some of the chemotherapy
agents commonly used in treating cancer can have substantial neurotoxic effects, including increasing cell death and decreasing cell division in the sub-ventricular zone, the dentate gyrus of the hippocampus, and in the corpus callosum (102-104). Moreover, CNS progenitor cells in culture show greater vulnerability to those chemotherapy agents than do many cancer cell lines. Effectiveness of the barrier, however, depends upon the integrity and health of its constituents. Inflammation, for example, can render the barrier less effective, and disruption of its tight junctions or other components is involved in a number of neurologic disorders. It is less clear that to what degree systemic chemotherapies may diminish the functional and/or structural integrity of the barrier.

Systematic studies of cognitive and neurobiologic changes subsequent to chemotherapy for lymphoma, breast and other cancers have attracted substantial interest in the last few years. Little is known, however, concerning the contributions of individual compounds comprising cytotoxic chemotherapy regimens, to any observed ensuing neuropsychologic deficits. In particular, the alkylating agent, cyclophosphamide, is a very commonly used chemotherapy agent in chemotherapy regimens with demonstrated neurotoxicity but is rarely used as monotherapy. This study is aimed to systematically assess acute, sub-acute and long-term effect of cyclophosphamide on regional cerebral metabolism in rats.

7.2 Materials and Methods

7.2.1 Animals

Five Sprague-Dawley (SD) rats (Charles River, Portage, MI) were maintained in a strict defined-flora, pathogen-free environment in the AAALAC-accredited animal facilities at UCLA. Rats were housed in IVC cages (Innovive, San Diego, CA) with 1 inch corn cob bedding
at 21°C, 70% humidity and a 12 hour light/dark cycle. The experiment started at their five month old.

### 7.2.2 Cyclophosphamide Administration.

Cyclophosphamide (Medisca Inc., Plattsburgh, NY) was resolved in 0.9% saline with 25mg/ml under sterile environment at UCLA Pharmacy Technology laboratory. Cyclophosphamide was injected using 24G Terumo Surflo® I.V. catheter (Terumo Medical Corporation) though tail vein. Cyclophosphamide was administrated with dose escalation manner (low (30mg/kg), medium (100mg/kg) and high (200mg/kg)) in two weeks.

### 7.2.3 Longitudinal PET and CT scans

Each cyclophosphamide was followed by a PET scan 20 hour afterward to assess acute effects while the scan following the lowest cyclophosphamide was served as tight control. And additional scans were acquired at weeks 1, 2, 4, 8, 12 and 24 after the high dose to exam time-course and durability of observed effects (Fig. 7.1). All PET scans were acquired with a small-animal PET scanner (Inveon DPET, Siemens Preclinical Solutions, Knoxville, TN). Attenuation and scatter corrections were applied based on CT image. CT images were acquired immediately following the PET imaging using a MicroCAT (MicroCAT II, Siemens Preclinical Solutions, Knoxville, TN). PET and CT scan timeline (Fig. 7.2) was optimized and introduced in Chapter 6. Rats were fasted overnight before administration of FDG. 37MBq (1 mCi) FDG was administrated intravenously (IV) through the tail vein. After 40 min of FDG uptake in awake state, rat was anesthetized with 2% isoflurane 10 minute before PET scan, 20-min brain PET scans were acquired followed by a 10-min CT scan. Blood glucose levels were sampled from the tail vein right before and after scanning.
7.2.4 MRI Scans and VOI Analysis

For each animal, one MRI scan was acquired before all the PET and CT studies. T2-weighted MRI scans were obtained on a 7.0 T animal MRI scanner (70/16 PharmaScan, Bruker Biospin, Germany) with ParavVision 5.0 software, using a volume coil for radio frequency (RF) transmission and a quadrature surface coil for signal detection. The structural images were obtained with a rapid acquisition with relaxation enhancement (RARE) sequence (RARE factor = 6, repetition time (TR) = 10493 ms, echo time (TE) = 36 ms, matrix size 256 × 140 × 80, voxel size 0.2 × 0.2 × 0.4 mm3, no slice gap). An automated MRI template-based volume of interest (VOI) analysis was carried out to extract radioactivity value for whole brain and seven brain regions. Regional Standardized Uptake Values normalized to whole brain (SUVR) were then calculated for each region as described in Chapter 5 and 6.

7.3 Results

7.3.1 Regions with FDG Uptake Decline

Compared with scans that followed low dose cyclophosphamide, FDG uptake measure by SUVR significantly declined (p<0.05) in frontal associated cortex (AF) by 6%, lateral prefrontal cortex (LPC) by 4%, medial prefrontal cortex (MPC) by 5%, sensorimotor cortex (SC) by 4% and striatum (S) by 4% acutely (one week after highest dose). For most of these regions, the decline was acute and subacute, for striatum region the effect persisted until the end of the study (Fig. 7.3).

7.3.2 Regions with FDG Uptake Increase

Compared with scans that followed low dose cyclophosphamide FDG uptake measure by SUVR increased acutely in brainstem (BS) by 5% and cerebellum (C) 6%, and in the latter region the effect persisted until the end of the study (Fig. 7.4).
7.4 Figures

Fig. 7.1 Cyclophosphamide administration and PET scan schedule.

Fig. 7.2 Optimized FDG scan with conscious FDG uptake.
Fig. 7.3 Regions with FDG uptake decline: frontal associated cortex (AF), medial prefrontal cortex (MPF), lateral prefrontal cortex (LPC), sensorimotor cortex (SC) and striatum (S).
Fig. 7.4 Regions with FDG uptake increase: cerebellum (C) and brain stem (BS).

Error Bar:
Standard Error
N=5

Paired T-test with the scan in week 0
+ p<0.1
* p<0.05
Chapter 8. Future Direction

8.1 Movement Correction

Intra-frame movement was not taken into consideration, but intra-frame movement can also degrade image quality. For example, using a bootstrap method, unreliable PET scan or PET frame due to intra-scan movement can be picked out (105) and strategy to correct intra-scan movement needs to be further developed.

8.2 Longitudinal Rat Brain Studies

To fully assess the longitudinal dynamic changes of brain metabolism, it is necessary to follow the animals across their entire life span. Additional experiments will also be necessary to elucidate the long term effects of anesthetic exposure, repeated handling, housing and diet conditions on the FDG uptake in a rodent brain.

To further look into the causes of cerebral metabolic change after cyclophosphamide treatment, it is worth understanding possible brain exposure to cyclophosphamide under various circumstances. It can be estimated by measuring the permeability of the blood-brain barrier to an $^{18}$F labeled cyclophosphamide using small animal dynamic PET imaging, when cyclophosphamide is administered at different concentrations or co-administered in multi-drug regimens which are typically used in chemotherapy regimens for human patients. And this experiment can be further expanded using other $^{18}$F labeled chemotherapy agents. Results of these studies may help define the basis for alterations in cerebral metabolism that have been observed in patients undergoing treatment for cancer with various chemotherapy regimens. The studies may identify the specific agents that demonstrate toxicity in vivo under a variety of doses and time conditions, and quantitatively assess the extent to which direct exposure of brain tissue to the chemotherapy agents...
may occur under each of those conditions. It is thereby useful in formulating strategies to minimize or prevent associated impairment of brain function in the future.

In addition, comprehensive analysis including other brain regions or even pixel based analysis can be taken to better understand current data.
Reference


87. Wiegar BD, MacIver MB. Isoflurane depresses hippocampal CA1 glutamate nerve terminals without inhibiting fiber volleys. *BMC Neuroscience*. 2006;7(5).


