MULTIMODALITY IMAGES ANALYSIS FOR PHOTODYNAMIC THERAPY OF PROSTATE CANCER IN MOUSE MODELS

by

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
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<tr>
<td>CG</td>
<td>Conjugate Gradient</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>DCE-MRI</td>
<td>Dynamic Contrast Enhanced MRI</td>
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<tr>
<td>DRE</td>
<td>Digital Rectal Examination</td>
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<tr>
<td>DW-MRI</td>
<td>Diffusion-Weighted MRI</td>
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<tr>
<td>EM</td>
<td>Expectation Maximization</td>
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<tr>
<td>FCM</td>
<td>Fuzzy C-Means</td>
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<td>FDG</td>
<td>Fluorodeoxyglucose</td>
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<td>FWHM</td>
<td>Full Width At Half Maximum</td>
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<td>GM</td>
<td>Gray Matter</td>
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<td>GTM</td>
<td>Geometry Transfer Matrix</td>
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<tr>
<td>H&amp;E</td>
<td>Hematoxylin And Eosin</td>
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<tr>
<td>ICP</td>
<td>Iterative Closest Point</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MRF</td>
<td>Markov Random Field</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
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<td>MsFCM</td>
<td>Multiscale Fuzzy C-Means</td>
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<tr>
<td>PDT</td>
<td>Photodynamic Therapy</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PSA</td>
<td>Prostate Specific Antigen</td>
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<td>PVC</td>
<td>Partial Volume Correction</td>
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<td>PVE</td>
<td>Partial Volume Effect</td>
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<td>ROI</td>
<td>Region Of Interest</td>
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<td>RPM</td>
<td>Robust Surface Matching</td>
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<td>TPS</td>
<td>Thin Plate Spline</td>
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<tr>
<td>TRUS</td>
<td>Transrectal Ultrasonography</td>
</tr>
<tr>
<td>WM</td>
<td>White Matter</td>
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MULTIMODALITY IMAGES ANALYSIS FOR PHOTODYNAMIC THERAPY OF PROSTATE CANCER IN MOUSE MODELS

Abstract

by

HESHENG WANG

Prostate cancer is the second leading cause of cancer mortality for males in the United States. Treatment of prostate cancer using photodynamic therapy (PDT) with a second-generation photosensitizing drug, phthalocyanine Pc 4, is investigated in animal models. The research focuses on quantitative assessment of the early therapeutic response of the prostate tumor to Pc 4-PDT. The tumor therapeutic response was imaged by magnetic resonance imaging (MRI) and positron emission tomography (PET) at different time points relative to the treatment. Diffusion-weighted MRI shows increasing apparent diffusion coefficients 24 hours after the treatment, and thus provides a potential imaging parameter for monitoring early tumor response. A multiscale MR image classification method was developed to classify tumor MR images into necrotic and viable regions, wherein, treatment effect was quantified by necrosis measurement. In order to follow up tumor response, a B-spline based surface registration algorithm was proposed to align the mouse body imaged at different time points, and so allowed tracking tumor response at pixel level. $^{11}$C choline PET shows choline uptake of prostate tumor decreasing 48 hours
after the treatment, but the quantification is limited by PET partial volume effect. The effect was corrected by a new PET partial volume correction algorithm using edge information from MRI. The study offers image analysis techniques to monitor tumor response at region and pixel levels and improves quantification of treatment outcome using MRI and PET. The research will expand understanding of the dynamic processing in prostate tumors to Pc 4-PDT. The imaging techniques and analysis algorithms might provide useful tools for monitoring therapeutic efficacy and optimizing treatment planning in potential applications.
Chapter 1. Prostate Cancer Treatment and Photodynamic Therapy

Prostate cancer is the most common non-skin malignancy in American men, and is the second leading cause of cancer mortality among them (Baade et al. 2009). In 2008, Prostate cancer affects one in 6 men during lifetime, and causes death in one out 35 men. There were about 186,320 new cases of prostate cancer in the United States, and about 28,660 men died of the disease (Jemal et al. 2008). Prostate cancer originates from DNA changes in the prostate cells (Witte 2009). High level hormones or androgens have been reported to play a role in prostate cancer risk in some males. Other risk factors such as controllable diet or lifestyles, and uncontrollable race or family history also link to prostate cancer (Uleckiene et al. 2008; Williams and Powell 2009).

1.1. Prostate cancer diagnosis

Prostate cancer can be detected in early stage by testing the concentration of prostate specific antigen (PSA) in blood. Increasing PSA level escalates the risks of prostate cancer (Schroder 2009). However, PSA level can change due to many reasons other than cancers, and gives little information about cancer position and cancer stage. Early prostate cancer can be found by a digital rectal exam (DRE) because doctors can feel bumps or hard place on the prostate through DRE (Smart 2008). More accurate diagnosis and location of prostate cancer are enabled by medical imaging techniques (Akin and Hricak 2007; Fuchsjager et al. 2008).

Transrectal ultrasonography (TRUS) images a prostate by picking up echoes generated from sound waves transmitting in the prostate (Smart 2008). TRUS is routinely used to guide biopsy needles for prostate biopsy. However, it shows low level of
sensitivity and specificity for cancer diagnosis compared with biopsy data or radical prostatectomy specimen. Generally, the widely used imaging modality, computed tomography (CT), has limited value for prostate cancer diagnosis because of its difficulty in distinguishing soft tissue. CT often overestimates the size of the prostate and misses nodules within the prostate.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have been increasingly used for prostate cancer diagnosis and staging (Mueller-Lisse and Scherr 2007; Fuchsjager et al. 2008). MRI presents excellent soft-tissue contrast and is often able to identify tumor invading in nodules and capsular or seminal vesicle. Recently, high field (3 Tesla) MRI can produce higher spatial resolution and improve anatomical differentiation (Torricelli et al. 2008; Cornfeld and Weinreb 2007). With appropriate imaging parameters, endorectal MRI significantly improves the detection of extracapsular cancer extension. Endorectal MRI also shows high sensitivity (95%) and specificity (100%) for imaging cancer recurrence after radical prostatectomy (Sella et al. 2004). Gadolinium contrast enhanced MRI (Gd CE-MRI) shows prostate cancer is enhanced more rapidly to a greater degree than normal tissue. It can provide relatively high sensitivity (90%) and specificity (88%) for detecting prostate tumors greater than 0.5 cc by correlating with radical prostatectomy finding (Villers et al. 2006). Using nanoparticle-enhanced MRI, high sensitivity (90.5%) and specificity (97.8%) have achieved for detection of metastatic lymph nodes (Harisinghani et al. 2003). MRS images biochemical and metabolic compounds in tissue, and thereby may be helpful for both tumor grading and determination of the aggressiveness (Casciani and Gualdi 2006). Prostate cancer has higher cell proliferation rate and greater cellular density than normal
gland tissue. So MRS shows higher concentration of choline, but less amount of citrate usage. This measurement enables accurate location of cancer tissue and also is valuable for visualizing recurrence after radiation therapy (Purohit et al. 2003; Koutcher et al. 2000). MRS data can be fused with anatomic images to localize tumors. Unfortunately, MRS has relatively low spatial resolution compared with MRI and leads to difficulty in detecting small infiltrating tumors.

A PET tracer is formed by unstable radioactive isotopes integrated with molecules which are able to be consumed or concentrated in cells via interrogated metabolic activity. PET images the tracer distribution in the body so offers functional information of the tissue metabolizing the molecules. PET with $^{18}$F-Fluorodeoxyglucose (FDG) has been clinically used for cancer staging, metastases detection and evaluation of therapeutic response. But $^{18}$F-FDG accumulation in primary prostate cancers is generally low and may not differentiate cancers from benign prostatic hyperplasia (BPH) and normal glands (Jadvar et al. 2008). FDG PET may be useful for evaluation of advanced disease with active metastases and recurrent tumors. $^{11}$C-choline was proven to play a significant role for detection of prostate cancer relapse site. Choline is an essential element of phospholipids in cell membrane and up-regulated in cancer cells. So choline uptake is related to the increased tumor proliferative activity. Choline PET can accurately detect and locate major area with prostate tumors and differentiate them from normal prostate tissue and hyperplasia (Rinnab et al. 2009). $^{11}$C-Acetate also shows marked uptake in prostate cancer and is another promising tracer for imaging prostate cancer and its metastases (Soloviev et al. 2008; Morris and Scher 2007). Single photon emission computed tomography (SPECT) provides 3D information through radiolabeled target-
specific markers using gamma rays. Indium-111 capromab pendetide SPECT has been used for staging and detection of prostate cancer recurrence (Haseman et al. 2000), and it is particularly valuable for determining the disease spreading to distant metastatic sites (Sodee et al. 2005).

1.2. Current prostate cancer treatment

Prostate cancer can be categorized as organ-confined (localized to the gland), locally advanced (a large prostate tumor or locally spread), or metastatic (distantly or widely spread) (Gomella et al. 2009). Unfortunately, a cure for metastatic prostate cancer is unavailable at the current time. The treatments for metastatic cancer are to slow the growth of the tumor and to relieve the symptoms of the patient. However, there are a number of treatment options available for organ-confined or locally advanced prostate cancers. If the prostate cancer is diagnosed and treated appropriately at an early stage, they will have a cure rate of over 90% (Jemal et al. 2008).

1.2.1. Radical prostatectomy

A radical prostatectomy is to surgically remove the entire prostate gland in order to treat prostate cancer (Hammad 2008). The American Cancer Society estimates the operation can attain a 90% cure rate nationwide when the cancer is confined to the prostate. The surgery typically makes an incision in the abdomen and removes the entire prostate gland from behind the pubic bone. The operation is complicated with risks of anesthesia, local bleeding, impotence (loss of sexual function) in 30%-70% of patients, and incontinence (loss of control of urination) in 3%-10% of patients. Impotence and incontinence may be alleviated using nerve-sparing techniques in the surgery.
1.2.2. *Hormone therapy*

Hormone therapy is to treat prostate cancer by decreasing the male hormone testosterone which stimulates the growth of cancerous prostatic cells. Testosterone normally is produced by the testes in response to stimulation from a hormonal signal called luteinizing hormone-releasing hormone (LH-RH). Therefore, hormone therapy can be accomplished by depriving testosterone via either removal of the source of testosterone or usage of medications to inhibit the release of LH-RH from brain. Other types of drug work against the male hormone by blocking the effect of testosterone itself on the prostate. Hormonal treatment usually effectively eliminates the cancer cell stimulation. But some prostate tumors grow independent of testosterone; therefore it does not always respond to the treatment. The side effects of hormone treatment are enlarged breasts, flushing and impotence (Suzuki *et al.* 2008).

1.2.3. *Radiation therapy*

Radiation therapy damages or kills cancer cells by radiation beams. Clinical trials with organ-confined prostate cancers have shown radiation therapy resulted in a rate of survival at 10 years that is comparable to that of radical prostatectomy (Eichler 2007). The most common type of radiation therapy is external beam radiotherapy which targets x-ray beams to the tumor areas mapped by radiological images. The other radiation based method is called brachytherapy. The treatment inserts tiny metal pellets containing radioactive iodine or palladium into proper locations in a prostate. The seeds kill the cancer cells by continuously giving off radiation to the immediate surrounding area for
several months. Incontinence and impotence can occur as results of radiation therapy, and late recurrence of the cancer sometimes arises.

1.2.4. Cryotherapy

Cryotherapy kills prostate cancer by freezing tumor cells, which is implemented using needles to infuse freezing liquid (liquid nitrogen or argon) into the prostate gland (Merrick et al. 2005). Temperatures lower than -40°C are required to completely destroy cells. Transrectal ultrasonography is typically employed for real-time monitoring and guiding the needles to the proper sites. The treatment possibly induces erectile dysfunction, incontinence, urethral sloughing and rectal injury.

1.2.5. Chemotherapy

Chemotherapy treats prostate cancer using anti-cancer drugs (Chowdhury et al. 2007). It is frequently used for advanced metastatic prostate cancers to relieve symptoms since a cure is unattainable at this time. Paciltaxel (Taxol) and docetaxel (Taxotere) have shown the potential to prolong the lives of men with prostate cancer that no long responds to hormonal therapy (Petrylak 1999). Suramin is one of the newer chemotherapeutic agents for androgen independent prostate cancers (Garcia-Schurmann et al. 1999). Chemotherapy is commonly associated with weakness, nausea, hair loss, and suppression of the bone marrow. The suppression of bone marrow decreases red blood cells, white blood cells, and platelets (Adler 2007).
1.2.6. *High-intensity focused ultrasound*

High-intensity focused ultrasound (HIFU) obtains instantaneous and irreversible coagulative necrosis in targeted tissue through hyperthermia. Because of the anatomical position of the prostate, transrectal HIFU transducer and the gland can be within 5 cm without intervening structures, and be visible on ultrasonography. HIFU can lead to precise ablation of malignant tissue without increasing the apparent risk of metastatic spread. Magnetic resonance thermography is employed to monitor the treatment effect by measuring the temperature in the gland and nearby structures (Kirkham *et al.* 2008). Because focused ultrasound wave cannot transmit deeply in the prostate, the therapy is difficult in ablating the entire prostate and the anterior tumors. Transrectal ultrasound-guided HIFU is a promising treatment of prostate cancer, but additional evaluation and study are required for clinical use (Maestroni *et al.* 2008).

1.3. *Photodynamic therapy for prostate cancers*

Various side effects are associated with current prostate cancer treatments discussed above. Alternative or adjunctive therapies for treatment of primary or recurrent prostate cancers are of great medical and economic significance. It is important for a new treatment to accurately target cancers and spare normal organs, such as bladder, rectum, and nerves, in addition to reduce complications and improve the quality of a patient’s life. Photodynamic therapy (PDT) can treat target tissue with limited collateral damage, thereby spare normal prostate tissues and adjacent organs. Currently, PDT is used in the treatment of recurrent and primary malignancies of the skin, bladder, lung, esophageal
head, neck and eye diseases (Ficheux 2009; Juarranz et al. 2008). It might also be a promising treatment option for localized prostate cancers.

1.3.1. Photodynamic therapy

Photodynamic therapy (PDT) is an emerging option for cancer treatment. It destroys targeted tissues by the interaction of a light-sensitive photosensitizer and laser light of a specific wavelength in the presence of molecule oxygen in tissue (Juarranz et al. 2008; Moore et al. 1997). Both the photosensitizer and the laser light are inert by itself. PDT can be precisely localized at a targeted region due to its inherent dual selectivity: accumulation of the photosensitizer in target tissues and accurate light irradiation.

Figure 1-1 shows the physical process of photodynamic therapy (Sibata et al. 2000). The photosensitizer somewhat preferentially localizes in cancer region. Light irradiation activates the photosensitizer from the ground state ($S_0$) to an excited state ($S_1$). Molecules at $S_1$ state readily return to the ground state with fluorescence or vibration relaxation ($V_r$). In addition, some molecules transfer into triplet excited state ($T_1$) through intersystem crossing. The molecules react with ground state oxygen to produce singlet state oxygen (Type II reaction) and decay to ground state by phosphorescence. The photosensitizer at the excited state can also directly transfer a hydrogen atom to nearby substrates and form radicals (Type I reaction). The radicals react with oxygen to generate oxygenated products. The excited molecules might also undergo Type III reaction. These generated ROS oxidize various substrates in cells and directly cause cell necrosis or apoptosis.
Figure 1-1 The process of photodynamic therapy. Light activates photosensitizer from ground state ($S_0$) to excited state ($S_1$). The excited photosensitizers decay to the ground state by fluorescence, vibrational relaxation ($V_r$) or initiating the three type reactions. The generated reactive oxygen species cause cell necrosis and apoptosis by the cascade of biochemical events.

1.3.2. Light propagation in tissue

Light propagation in tissue is attenuated by light absorption and scattering (Wilson and Patterson 2008). Electrical charges of matter are forced to oscillate when light radiation is incident. The oscillation causes atoms or molecules collision, and dissipates incident energy as heat in the medium. The absorption process reduces intensity of the light beam traversing tissue and is measured by the absorption coefficient $u_a$ that describes the probability of a photon to be absorbed by the medium in a unit length. $u_a$ is determined by light wavelength and the concentration of light-absorbing molecules in tissue. Protein absorption of light increases when the light wavelength decreases, but water absorption increases when the wavelength becomes longer. Hemoglobin and water are the two most important molecules for light absorption at PDT wavelength range between 600 nm and 800 nm.
Light scattering arises from the oscillatory particles re-emitting light when incident light reaches the particles. Scattering coefficient $\mu_s$ measures the fraction of scattered light per unit distance in the medium. The scattering from individual atom or molecule happens in all directions but with different probabilities depending on the scattering angles. Anisotropy factor $g$ is the mean cosine of the scattering angles and characterizes the asymmetry of the scattering. More forward scattering will decrease light attenuation; but more light will be attenuated if most scattering is backward. An effective isotropic or reduced scattering coefficient accounts for the direction of light scattering and given as $\mu' = (1 - g) \mu_s$. $g$ tends to be constant at long wavelength, and the scattering coefficient $\mu_s$ decreases when the wavelength increases. Therefore, longer wavelength light will have less reduced scattering coefficient, and thus better light penetration in tissue (Wilson and Patterson 2008).

Light attenuation in tissue results from the combination of absorption and scattering. It typically decays as $I = I_0 e^{-u_tL}$ along the propagating direction. The transport attenuation coefficient ($u_t$) is $u_t = u_a + (1 - g)u_s$, $I_0$ is incident intensity and $L$ is the transmitting length in the medium. However, the model assumes individual scattering does not significantly affect the others, and so the attenuation is summation of absorption and independent single scattering. The assumption becomes invalid when light with wavelength between 600-800 nm transmits in biological tissue, because multiple scattering dominantly occurs and the interaction of scattering waves between neighboring particles must be considered.

Radiative transfer theory was used to model the multiple scattering, which described transport of individual photon with absorption and scattering using radiative transfer
equation (Wilson and Patterson 1986). Typically, tissue scattering coefficient is one or
two orders of magnitude larger than the absorption coefficient for light of PDT. In this
case, light propagation can be considered as diffusion through scattering medium.
Derived from the radiative transfer equation and the diffusion assumption (Wilson and
Patterson 1986), transmitting light fluence is an exponential decay process with the depth
z in tissue and denoted as \( \Phi(z) = \Phi_0 \exp(-u_{\text{eff}} z) \). The effective attenuation coefficient
is given as \( u_{\text{eff}} = \sqrt{3u_a(u_a + u_s')} \approx \sqrt{3u_a u_s'} \). The effective penetration depth is given
as \( \delta = 1/u_{\text{eff}} \) which is the distance in tissue over which light fluence decreases to 1/e or
37% of the initial. The effective penetration depth is a function of tissue optical property
and light wavelength. Typically the effective penetration depth of 630 nm light in tissue
is about 2 to 3 mm; and it increases to 5 to 6 mm at longer wavelengths (700 to 800 nm)
(Hasan \textit{et al.} 2003).

Light fluence within tissue is also related to the incoming light irradiation that is
affected by the geometry of light illumination. There are several different geometries for
light irradiation in PDT which might be grouped into surface and interstitial irradiation
(Wilson and Patterson 1986). In surface irradiation, the light is external to the treated
region where light travels from the air or surrounding medium into the tissue. For lightly
pigmented tissue, the loss from this scattering could be greater than 50% at long
wavelength. The other type of light delivery is to directly put an optical fiber into tissue
so light is transmitted into tissue without an external interface with the tissue. In this case,
the light geometrically spreads into 3D volume from a point source. This causes an
additional fluence decreasing along the radial distance from the source. There is also
lateral light spreading due to scattering along the light tracks. This increases the beam diameter and can cause damage to nearby tissues.

1.3.3. *PDT treatment efficacy*

PDT efficacy is determined by three physical and biological processes: light propagation in tissue provides light for photosensitizer activation; ROS generation is determined by the presence of activated photosensitizers and oxygen; tumor ablation is associated with complicate biological pathway of cell impairment from ROS.

Light penetration describes light loss as a function of spatial distribution, but not the depth of PDT because treatment effect depends on the light fluence, i.e., the total light dose. The outcome of light-photosensitizer interaction is ROS generation, which generally is modeled by a bimolecular reaction (Josefsen and Boyle 2008). The reaction rate is governed by molecular diffusion which depends on the concentration of activated photosensitizer and O$_2$. Generated ROS is toxic to cells and leads to tissue destruction. There are three main mechanisms for cell damage (Dolmans *et al.* 2003). First, ROS can kill tumor cells directly by induced necrosis and apoptosis of cells. Second, ROS damages tumor-associated vasculature and leads to hypoxia for cell death. ROS is highly reactive and short half-life, so only organelles or molecules close to ROS generation (< 0.02 µm) are affected. The treatment effect is correlated with ROS distribution that is determined by non-homogenous distribution of the photosensitizers and oxygen. The third mechanism is PDT-induced immune response that is long term effect depending on the nature and intensity of the activated immune response.
The treatment results from the combination of light transmission, photosensitizer distribution, tissue oxygenation, and intrinsic tissue sensitivity to reactive oxygen species damage. As the light shines, several mechanisms, such as clearance of the photosensitizer from the tissue during exposure, photobleaching of the drug due to the chemical reaction, and depletion of oxygen might introduce time dependent therapeutic effect (Ficheux 2009). In summary, PDT is a complex physical and biochemical process; and measurement of treatment effect will be extremely valuable for clinical application of PDT.

1.3.4. Photodynamic therapy with Pc 4

A fairly large number of photosensitizers are under preclinical development at the present time. PDT with Photofrin is FDA approved for treating early and advanced lung cancer, esophageal cancer and Barrett’s esophagus (Selman et al. 1996; Chang et al. 2008). Second-generation photosensitizers have emerged which have advantages over Photofrin by chemical purity and absorption wavelength of 650-850 nm for better tissue penetration. PDT using second-generation photosensitizers is being evaluated for treating a variety of cancers, including prostate cancer (Huang et al. 2007; Josefsen and Boyle 2008; Juarranz et al. 2008). The photosensitizer TOOKAD and its water-soluble derivative WST11 have been tried on locally recurrent prostate cancer (Framme et al. 2006; Huang et al. 2007; Woodhams et al. 2006; Tremblay et al. 2003). They are considered as vascular-targeted photodynamic therapy (VTP) because of preferentially targeting blood vessels. Vascular-targeted photosensitizers tend to accumulate in cells of tumor vasculatures and cause stasis, vessel collapse, and vessel leakage in tumors.
Another type of second generation photosensitizing drug, the silicon phthalocyanine Pc 4 [HOSiPcOSi(CH$_3$)$_2$(CH$_2$)$_3$N(CH$_3$)$_2$], seems to preferentially localize into the tumor parenchyma (Whitacre et al. 2000). Pc 4 in therapeutic range did not produce cutaneous photosensitivity that is a major side effect of many photosensitizers being used for PDT. Pc 4 has maximal absorption peak at about 675 nm that is substantially longer than the 630 nm wavelength of Photofrin. The longer wavelength allows deeper damage in the targeting tissue. Compared with the impure mixtures of porphyrins found in Photofrin, Pc 4 can be prepared in a high purity. The drug was originally synthesized at Case Western Reserve University (He et al. 1997), and under evaluation for treatment of variety cancers at Case Comprehensive Cancer Center (Colussi et al. 1999; George, III et al. 2005; Miller et al. 2007; Kim et al. 2008). Clinical trials have shown PDT is encouraging for treatment of various cancers in terms of safety and possible biological response (Miller et al. 2007).

1.4. Contributions and organization

We are investigating treatment of prostate cancer using Pc 4-PDT. This dissertation demonstrated the potential to treat prostate cancer using PDT with the photosensitizer Pc 4 by Pc 4-PDT experiments on prostate tumor-bearing mice. A MR imaging marker for quantifying the early therapeutic efficacy of Pc 4-PDT to prostate cancers was present. High-resolution MR images can reveal more detail information, such as spatial distribution of necrotic and viable tumor regions. An image classification method was proposed to directly classify necrosis from tumor in MRI. In order to monitor the biological response of tumors to drugs or therapies, images of tumors at different time points usually are acquired. As the mouse body is quite small and very flexible, it is very
difficult to maintain the mouse at the same position for each imaging session. A nonrigid registration scheme for longitudinal image analysis is implemented in this thesis. PET visualizes tissue metabolic activity. However, PET images are much blurred and quantification of tumor radioactivity is heavily biased by its partial volume effect. A MRI-guided PET partial volume correction method was designed to improve tumor metabolism quantification. In summary, the thesis implemented and verified the diffusion weighted MRI imaging technique and a number of image processing methods for quantification of the early therapeutic response of prostate cancer to Pc 4-PDT in mouse models.

In the next chapter, Chapter 2, the treatment of prostate tumors on mice has been described, and imaging techniques applied to monitor treatment effect to PDT with different photosensitizers have been reviewed. MR imaging experiment is described and a potential sensitive imaging marker is evaluated for detecting early therapeutic response of prostate tumor to Pc 4-PDT.

An image classification method for MR images is presented in Chapter 3. The method was used to directly measure necrosis region from diffusion weighted MRI and apparent diffusion coefficient of tumors. We verified the method by correlating the classification results with histology images.

Chapter 4 outlines a whole body mouse image alignment strategy to align mouse images acquired at different time points. The scheme includes a nonrigid bone surface matching and following intensity-based volume registration. We proposed and evaluated a surface matching algorithm for non-rigid registration of bones extracted from mouse images.
PET images have been performed to evaluate the treatment effect of Pc 4-PDT. In Chapter 5, we show a PET partial volume effect correction method which effectively improves PET image quality and provides more accurate quantification of interrogated metabolic activity.

The last chapter, Chapter 6 summarizes the work and discusses the possible directions of further exploration in order to translate the study from mouse experiments to real clinical applications.
Chapter 2. DW-MRI for Monitoring PDT Tumor Response

Photodynamic therapy is complicated with a number of parameters which need to be determined before the treatment, such as drug selection, drug dose, light dose, light delivery rate, and light delivery pattern. It is extremely valuable if a technique can be used to monitor tumor response and predict treatment efficacy at an early time. Thereby, immediate prognosis and early consideration of adjuvant treatment might be possible. In the case of prostate cancer treatment, inspection of tumor response to a therapy often relies on serum PSA level. But time elapse of several weeks to months is usually required for a measurable PSA response. MRI provides in vivo information about tissue morphology and metabolism, and so allows assessment of tumor response to treatment by monitoring the induced tissue properties change. In this chapter, diffusion-weighted MRI was studied for monitoring CWR22 prostate tumor response to Pc 4-PDT.

2.1. MR imaging for PDT

A number of MRI techniques have been employed for assessing early therapeutic response of prostate cancer to PDT. T1, T2, diffusion and proton density weighted MRI was used to study the effects of Photofrin II PDT on normal rat brain (Jiang et al. 1991). Both T1 and T2 increasing 24 hours after PDT were observed and corresponded to induced histological necrosis in the study of PDT to murine mammary tumor (Dodd et al. 1989). However, in the study of Photofrin II PDT to RIF tumors implanted on mice feet (Liu et al. 1995), although prolongation of T1 relaxation time was observed several hours after PDT, T2 value did not show such change. Thereby, MRI characterization of tumor response to PDT might depend on tumor types and applied photosensitizers. High field
MRI has been used to evaluate PDT-induced hemorrhagic necrosis in the murine M1 tumor model (Winsborrow et al. 1997) in which tumor response to three photosensitizing drugs was imaged by a 9.4 T MR scanner. T2-weighted MRI using a high field small-animal MR scanner has been evaluated to monitor Pc 4-PDT to prostate tumor model PC-3 in mice (Fei et al. 2007a; Fei et al. 2007b). The significant increasing of T2 relaxation time 24 hour after PDT might relate to the treatment effect.

Contrast-enhanced MRI (CE-MRI) is sensitive to the alternation of tissue perfusion that is lower in necrotic regions compared with viable portions. Gadolinium CE-MRI revealed decrease of tissue perfusion 5 hours after PDT to rat borne tumors (Kennedy et al. 1994). 7 days Gadolinium T1-weighted MR showed avascular lesion formation of a recurrent localized prostate cancer treated by PDT after the failure of external beam radiotherapy (Trachtenberg et al. 2008). When T2-weighted and Gd-enhanced MR examined a vascular targeted PDT to locally recurrent prostate tumors (Haider et al. 2007), the patients study showed CE-MRI depicted irregular margins of treatment effect but T2-weighted MRI did not clearly show the boundaries 1 week after the treatment. Dynamic contrast enhanced MRI (DCE) has been employed to study Pc 4-PDT to U87 brain tumors in rats (Varghai et al. 2008). Significant tumor enhancement from DCE signal was detected 6 days after the PDT.

PDT consumes oxygen to generate reactive singlet oxygen for cell injury. Blood oxygenation level-dependent (BOLD) contrast MRI was attempted to real-time monitor PDT efficacy at a solid tumor model (Gross et al. 2003). Treated tumor site has shown 25–40% signal attenuation by the BLOD contrast MRI, and the attenuation was independent to the ensuing blood flow change. Magnetic resonance spectroscopy (MRS)
measures the level of metabolites in tumor cells which might vary as response to a treatment. A relationship between $^{31}$P MRS measurement and the therapeutic effect of zinc phthalocyanine-based PDT to RIF-1 murine fibrosarcoma in mice was demonstrated (Bremner et al. 1999). Ramaprasad et al. (2007) employed $^{19}$F MRS to study the pharmacokinetic profile of a fluorinated photosensitizer in RIF tumor model.

Different from T1- or T2-weighted MRI that images alternation of tissue water content, diffusion weighted MRI (DW-MRI) visualizes displacement of water molecules that has sharp discrimination between viable and necrotic tissues. A biphasic change in the apparent diffusion coefficient ($ADC$) derived from DW-MRI has been reported within the first 24 hours after TOOKAD-PDT to prostatic adenocarcinoma in mice (Plaks et al. 2004). $ADC$ decreasing 7 hours after the treatment might provide a unique marker for detection of successful tumor treatment at very early time. In a study of TOOKAD-PDT to normal canine prostate gland (Huang et al. 2006), a marked $ADC$ decrease in regions of necrosis was found 7 days after the PDT, which was counterintuitive since necrosis induces more water diffusion thus presumably higher $ADC$. DW-MRI with a high $b$-value sequence was studied for imaging early colon tumor response to PDT and chemotherapy (Roth et al. 2004).

We have studied RIF-1 tumor response to Pc 4-PDT using both MRI and positron emission tomography. The study showed assessment of therapeutic effect was improved by fusion of MRI anatomical and PET functional information (Fei et al. 2006). We are developing non-invasive imaging and quantitative analysis techniques to identify biomarkers associated with prostate tumor early response to Pc 4-PDT. Our previous study demonstrated T2 relaxation time changes 24 hours after Pc 4-PDT to PC-3 prostate
tumor at mice (Fei et al. 2007b). In present study, we evaluate the ability of DW-MRI and derived $ADC$ to identify early therapeutic efficacy of Pc 4-PDT to CWR22 prostate tumor in animals. Different from PC-3 prostate tumor model, CWR22 tumor is androgen dependent, and serum PSA level increases as tumor growing like what happens in human patients. This study suggests a specific $ADC$ pattern of prostate tumor response to Pc 4-PDT that could eventually be useful for monitoring Pc 4-PDT of prostate cancer clinically.

2.2. Diffusion-weighted MRI

Water molecules randomly move in the extracellular space from agitation of thermal energy. The movement or diffusion is affected by cellularity conditions and the integrity of cellular membranes, such as the restricted space of tumor tissue limits water molecules diffusion in Figure 2-1 (Ross et al. 2003). A variety of study showed the diffusion is sensitive to tissue cellular size, extracellular volume and membrane permeability (Kauppinen 2002). DW-MRI produces in vivo tissue MR images weighted by the water diffusion; thereby, provides indirect information regarding tissue cellular and subcellular microstructures that might precede macroscopic change in response to a treatment. Treatment-induced cell vitality change causes cellularity alternation and is often associated with water diffusion change; therefore, DW-MRI has been applied for detection of tumor response to different therapies (Hamstra et al. 2007; Harry et al. 2008; Plaks et al. 2004; Ross et al. 2003).
Figure 2-1 Illustration of water molecules diffusion in extracellular space. Left figure shows dense cells restrict water molecules diffusion in comparison with more free diffusion in right figure. So diffusion coefficient in left figure is less than the one in the right figure.

Diffusion-weighted MRI is obtained by adding diffusion gradients to magnify water diffusion effect during the preparatory phase of a MR spin echo imaging sequence (Vlaardingerbroek and den Boer 1999). The diffusion gradient consists of a dephasing pulse and an inverse rephasing pulse. As the first linearly varied dephasing pulse is applied, protons begin precession at different rates proportional to the magnet strength, therefore generate phase shift in water molecules. The second pulse with opposite magnitude refocuses the spins and removes the phase shift if the water molecule remains at its original position. However, protons that diffuse with water molecules in the time between the two pulses cannot be completely rephrased, which results in MR signal reduction that directly relates to the water mobility. DW-MRI is MR images weighted by water diffusion, in which higher water diffusion leads to lower MR signal and more restricted diffusion results in greater MR intensity. MR signal in DW-MRI is usually described by the equation: 

$$S = S_0 \exp(-\gamma^2 G^2 \delta^2 (\Delta - \frac{\delta}{3} D))$$

$S$ and $S_0$ is MR signal with and without the diffusion gradients; $G$ is the diffusion pulse strength, $\delta$ is the gradient pulse duration, $\Delta$ is time interval between the two gradient pulses, $\gamma$ is gyromagnetic ratio.
that is a constant for a nucleus type. $D$ is apparent diffusion coefficient ($ADC$) which characterizes water molecules diffusion in the location.

### 2.3. Cancer and cancer angiogenesis

Cancers are created by malfunctioning cells they uncontrollably grow beyond normal tissue forms, invade into adjacent tissue, and sometimes spread to other locations in body. According to the presumed tissues of origin, cancers mainly include carcinoma derived from epithelial cells, sarcoma from connective tissue and leukemia originated in hematopoietic cells. Among them, the majority are carcinoma arising from epithelial tissue, such as skin cancers, lung cancers, breast cancers and prostate cancers.

Almost all cancers begin with genetic abnormalities in the cell DNA which may arise from environment stimulus, randomly acquired errors in DNA replication, or inherited gene defect. Typically, two types of gene mutation are associated with the transformation of a normal cell into a tumor one (Weinberg 2006). An oncogene results in hyperactive and uncontrolled cell growth. Growth factor oncogene causes a cell to continuously secrete growth factor that induces uncontrolled cell proliferation. The other is tumor suppressor gene mutation that liberates the cell from its own growth-inhibiting mechanism. Tumor suppressor genes code proteins to regulate cell cycle or promote apoptosis in response to cell signaling. Inactivation of the gene by mutation will cause unregulated cell division.

Carcinoma usually consists of abnormally proliferating cells and supportive stroma (Weinberg 2006). Stroma is made up of connective tissue and blood vessels. Connective tissue provides supportive framework for the tumor; and blood vessels supply oxygen and
nutrients for tumor growth. The maximal distance which oxygen and nutrients can go across from blood vessels is 1~2 mm; therefore a tumor cannot grow beyond the distance without further vascularization (Kumar et al. 2004). During tumor growth, tumor cells and/or inflammatory cells release angiogenic factors that recruit cells for tumor vasculature and activate the events for formation of new capillaries. The capillaries not only supply nutrients and oxygen, but also stimulate tumor growth by secreting polypeptides (Aragon-Ching and Dahut 2008). Therefore, carcinomas are not only a group of tumor epithelial cells, but also include various stromal cells, such as endothelial cells, pericytes, and smooth muscle cells for tumor vasculature.

2.4. DW-MRI for Pc 4-PDT

2.4.1. Animal tumor model

An androgen-dependent prostate tumor xenograft model, CWR22 was originally derived from a primary human prostatic carcinoma (Wainstein et al. 1994). For growing tumor at mice, liquid nitrogen stored cells were thawed in 37°C water, washed with tissue culture medium that was RPMI 1640 (Hyclone Laboratories, Inc., Logan, UT) with 20% calf serum (Hyclone Laboratories, Inc., Logan, UT), and then filtered through a single layer of Nitex with 100 µm porosity (Tetko, Inc., Briarcliff Manor, NY). The cells were suspended in Matrigel (BD Biosciences, Bedford, MA) with a volume at least equal to the cell volume. The suspension was drawn into 1.0-cc syringes with a 19-gauge needle for 0.2ml volume per injection. Athymic mice of 4-8 weeks old were housed under controlled conditions (12 hour dark light cycles; 20 - 24°C temperature) and freely reachable sterilized mouse chow. 12.5-mg sustained-release testosterone pellet
(Innovative Research of America, Sarasota, FL) was implanted in each nude mouse 1 week before cell injection. Each animal was given an injection of cell suspension subcutaneously on the side opposite to the implanted testosterone and far from the lung and heart in order to minimize motion effects during imaging of the mouse.

After having mice with CWR22 tumors, the cells for the following transplantation were prepared from tumors dissected from the mice. The tumor was quickly and carefully dissected after mouse euthanization, and then minced to fragments no more than 1 mm maximal dimension in tissue culture medium. The minced tumor was digested serially with 0.1% pronase (Roche Diagnostics GmbH, Mannheim, Germany) in Joklik-modified minimum essential media (SMEM; BioWhittaker, Walkersville, MD). The medium of each digest was filtered and suspended in the tissue culture medium. Fresh CWR22 cells were obtained by centrifuging all the cell suspension at 800 rpm for 7.5 min. At last, the cells were suspended with Matrigel and injected to athymic mice for tumor growth. The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University and conformed to the guidelines of the National Institutes of Health for the care and use of laboratory animals.

2.4.2. Photosensitizer formulation

A photosensitizing drug, the silicon phthalocyanine Pc 4, \([\text{HOSiPcOSi(CH}_3\text{)}_2(\text{CH}_2\text{)}_3\text{N(CH}_3\text{)}_2]\) was used in our study. The drug was synthesized using the protocol of Oleinick et al. (1993) and obtained from Dr. Malcolm E. Kenney, Chemistry Department, Case Western Reserve University. Stock solution (1 mg/mL) was prepared in 50% Cremophor EL and 50% absolute ethanol, and then diluted with 9
volumes of normal saline. For treatment, Pc 4 solution was administered at a concentration of 0.05 mg/mL (0.07 mM) which was obtained by further mixing the solution with an equal volume of 5% Cremophor EL, 5% ethanol and 90% saline.

2.4.3. PDT protocol

PDT was performed when the subcutaneous tumors reached 8-10 mm in diameter (typically in 2-4 weeks). The mouse was constrained and injected with 0.6 mg/kg Pc 4 solution (e.g., 240 µL to a 20g mouse) intravenously via tail vein. The dose was found to be optimal for treatment in the xenograft model of OVCAR-3 ovarian epithelial carcinoma (Colussi et al. 1999) and was used in our previous study (Fei et al. 2007b). 48 hours after the Pc 4 injection, treatment and imaging of the animals were done in a small-animal imaging facility. For PDT, the tumor was illuminated for 25 min with light of a total fluence of 150J/cm² and at an irradiance of 100mW/cm² using a diode laser (Applied Optronics Corp., Newport, CT). The light was delivered at a wavelength of 672 nm at which Pc 4 obtains maximal absorption. Treatment of PC3 prostate tumors (Fei et al. 2007b) and other tumors (George, III et al. 2005) have shown Pc 4-PDT with the given parameters produced effective response and some cures without significant thermal effects. The light was adjusted to illuminate the whole tumor and spare the surrounding skin which was covered with black tape to avoid possible damage. Total 27 tumor-bearing mice were treated and imaged in this study. Two types of control experiments were conducted. One was light control (n=3) where the tumor-bearing mice were illuminated with laser light but excluding Pc 4 injection; the other was dark control (n=4) in which the mice had Pc 4 injection but were not treated by the laser light. All parameters for control were same to those used in PDT of the treated mice. Tumor
response was recorded by MRI, serum PSA levels and tumor sizes at different time points relative to the treatment.

2.4.4. **MR imaging experiments**

Animals were imaged using a dedicated mouse coil in a high field 9.4 T small animal scanner (Bruker BioSpin GmbH, Rheinstetten, Germany). The mouse was continuously supplied with 2% isoflurane (EZAnesthesia, Palmer, PA) in air for anesthesia. The anaesthetized mouse was constrained on a plastic holder and horizontally moved into the scanner until the tumor was at the center of the coil. We monitored the animal respiration rate and body temperature throughout the entire imaging. The body temperature typically was maintained between 35°C and 37°C using a feedback system that blew warm air to the mouse via a plastic pipe. The respiration rate was monitored and manually controlled by adjusting the isoflurane ratio. MR acquisition was triggered by the respiration signal to minimize motion effects. Therefore, one respiration cycle need be long enough for a complete MR signal acquisition defined by the parameter of time to repetition (TR). We maintained the mouse respiration rate not greater than 40/min, which allowed maximal TR of 1500 ms.
Prior to DW-MRI scan, a coronal scout image was obtained to determine position and size of the tumor. A diffusion gradient linearly incremented in the range of 0 to 400 mT/m with $\Delta = 35$ milliseconds and $\delta = 3$ milliseconds yielded five $b$ values (16.5, 133.35, 409.269, 759.643, 985.108 sec/mm$^2$), thereby, to generate diffusion weighted MRI and enable ADC calculation. ADC was only along a single direction assuming water diffusion in the tumor is isotropic. The other parameters for acquisition were TR=1282 ms, TE=52.5 ms, field of view 3.5 cm $\times$ 3.5 cm, matrix size 128 $\times$ 128, slice thickness 0.5 mm, receiver bandwidth 25000 Hz and no average. The total number of slices depended on tumor size; typically, 15-20 coronal slices were acquired to cover the whole tumor. The total DW-MRI scan time for a mouse was around 15 min. The mouse was MR scanned sequentially before, immediately after and 24 hour after PDT in order to characterize the early tumor response. Figure 2-2 presents pictures of PDT to a tumor bearing mouse (left) and the MRI scanner (right) for imaging at different time points. Figure 2-3 shows the treated tumor shrunk and became necrotic 7 days after the treatment.
Figure 2-3 Pictures of a treated mouse Pre-PDT, 24 h and 7 days after PDT. From top to bottom, the pictures were a mouse before, 24 h, and 7 days after Pc 4-PDT. The tumor was eradicated at 7 days after the treatment.

2.4.5. Minimization of motion effects in DW-MRI

DW-MRI visualizes the thermally driven displacement of water molecules on the order of micrometers (Charles-Edwards and deSouza 2006). So any motion other than water diffusion can adversely affect image quality and ADC measurement (Conturo et al. 1995). During MR imaging, the mouse was constantly anesthetized and immobilized in a holder. In this case, the most significant motion is mouse respiration whose amplitude is much
greater than the motion DW-MRI detects. We used several strategies to minimize the respiratory motion. First, the tumor was implanted subcutaneously on the flank of a mouse and as below as possible to the leg in order to minimize the respiration motion. Second, we also tightly taped the tumor to the wall of the holder for further immobilization during imaging. Third, other than careful immobilization of the tumor during imaging, MR acquisition was triggered by respiration signal.

The respiration motion was detected by a surface patch attached to the skin near to the tumor and monitored by a small animal monitoring & gating system (SA Instruments, Inc., Edison, NJ). A cycle of the signal consisted of an inspiration and expiration period. We have observed that the short inspiration was associated with significant abdomen deformation; but the relatively long and slow expiration corresponded with much small body motion. Therefore, Acquisition of MR signal was restricted to the expiration period through the respiratory signal gating. The mouse respiratory rate was controlled within 30-40 per minutes (2000-1500 milliseconds per respiration). Time of repetition of MR imaging was limited to less than 1700 ms to allow one RF signal acquisition within a respiration cycle. For the 9.4 T bruker scanner, the gating signal was set to be high during the respiration period, and became low when the low expiration signal was detected. The signal acquisition was triggered by the active low gating signal. Much better image quality and $ADC$ computation owing to the triggering have been visually confirmed.
2.4.6. MRI data analysis

We used the software package of Paravision 3.1 (Bruker BioSpin GmbH, Rheinstetten, Germany) for \(\text{ADC}\) computation. The \(\text{ADC}\) was calculated by linear least square fitting of the 5 \(b\) MR signal to monoexponential decay at each voxel, which is denoted as

\[
S = S_0 \exp(-b \times \text{ADC})
\]

\(S_0\) and \(S\) are proton signal intensities without and with the presence of diffusion-sensitizing gradient respectively. Apparent diffusion coefficient (\(\text{ADC}\)) characterizes water molecules diffusion at the location. \(b\) is the diffusion weighting factor and determined by imaging parameters setting according to

\[
b = \gamma^2 G^2 \delta^2 (\Delta - \delta/3)
\]

\(G\) is the gradient pulse strength, \(\delta\) is the gradient pulse duration, \(\Delta\) is the separation time of gradient pulses, and \(\gamma\) is gyromagnetic ratio that is a constant for a nucleus.

We segmented the tumors slice by slice from the DW-MRI using the image analysis software of Analyze (AnalyzeDirect, Inc., Overland Park, KS). The tumor boundary was manually drawn on the image and saved as an object map. The boundary was copied to \(\text{ADC}\) maps to calculate statistical properties of the tumor \(\text{ADC}\) including mean, standard deviation and histogram. The segmentation was examined and verified by a trained person.

2.4.7. Histological analysis

In order to measure tumor response histologically, 7 treated mice and 5 controls were euthanized 24 hours after PDT and the tumors were dissected and prepared for
histological slides. The other mice were kept in order to observe the long term treatment effect. The tissue preparation was described earlier (Fei et al. 2007b). After quickly removing from the body, the tumor was fixed in a large volume of 10% formalin for 1 hour. Then, the tumor was carefully cut into 3-5 mm thick slices along the same coronal plane as the direction in which MR images were taken. Typically 2-4 sections were obtained for one tumor. The slices were placed into cassettes and fixed in 10% formalin for at least 24 hours to allow complete tissue fixation. The slices were made into histological slides at Case Comprehensive Cancer Histology Core Facility. Usually, 1-2 slides with 3 µm thickness for each section were obtained and stained with hematoxylin and eosin (H&E). The histological slides were examined using a light microscope (BX40, Olympus, Japan) by a pathologist specifically trained in genitourinary pathology. We also digitized the slides using a video microscopy system with a motorized stage (Breen et al. 2004) to acquire color histological images.

2.4.8. Measurement of prostate specific antigen

Blood samples were collected to measure serum PSA level at the time points of before, 24 hours after and 7 days after PDT. Tail end of the anesthetized mouse was clipped off, and blood droplet formed at the tail tip was collected into a heparinized capillary tube by tail squeezing. 0.15~0.2 cc blood was collected for one tube and 5 samples were obtained for one mouse at each time point. As soon as possible, the capillary tubes were centrifuged for 10 minutes at 5000 to 5500 RPM to separate the blood into red blood cells and blood serum. The serum was collected into a 2 ml centrifuge tube and stored on ice, then sent for PSA measurement by a chemiluminescent immunometric assay.
2.4.9. Statistical analysis

Tumor ADC values at three time points (pre-PDT, post-PDT, and 24 h after PDT) were compared statistically. A two-tailed two-sample Student’s t-test in Microsoft Excel 2007 (Microsoft, Seattle, WA) was used to test the difference of ADC at the three time points. Statistical significance was defined by a \( p \)-value of 0.05.

2.4.10. DW-MRI and ADC classification

In order to evaluate the ability of DW-MRI revealing treatment effect and examine the inhomogeneity of tumor response, a multivariate image classification algorithm called multiscale fuzzy c-means method (Wang and Fei 2009a) was applied to classify DW-MRI and ADC of the treated tumors into necrotic and viable regions. The method constructed a multi-scale description of the images by diffusion filtering. Fuzzy c-means classification was performed at each scale with local smoothness constraints, and the result at low scale image was used to regularize the classification in the next scale. The method demonstrated to be effective for MR image classification and robust to noise. The classification assigned tissue type (viable or necrotic) to each voxel of the segmented tumor based on the 2-element vector consisting of DW-MRI intensity and ADC value at the position. The result present necrosis distribution within a treated tumor and provide a measurement for the treatment effect.

Histology usually is considered a gold standard to characterize treatment outcome. The high resolution histology image was down sampled to a pixel resolution of 0.1 mm; and then we manually segmented the tumor regions from no-tumor tissues in the images. The necrosis regions were also manually delineated from the tumor histology and verified
by a histologist. Since we carefully maintained the tumor orientation during dissection and cut the tumor along the coronal imaging direction, the histology around the tumor center was nearly matched with the center slice of tumor MR image. We checked the correspondence between the histological images and tumor MRI, and correlated the results from MR classification and histological segmentation.

2.5. Imaging Results

2.5.1. DW-MRI results

Figure 2-4 shows diffusion weighted MR images of CWR22 tumors before (Pre-PDT) and 24 hours after PDT in the first row and the corresponding ADC maps for each MRI in the second row. (a) (b) are the MR images of the treated mouse Pre-PDT and 24 h after PDT; and (c) (f) are MR images of a light control mouse for Pre-PDT and 24 h after. DW-MRIs and ADCs contrast the tumors for all the images, and ADC map of the treated tumor 24 h after PDT enhances the tumor contrast compared with the DW-MRI. The ADCs are displayed in a same scale. The brighter ADC of treated tumor 24 h after PDT indicates the higher water molecules diffusivity compared with Pre-PDT; the ADCs of the control have not such visible intensity change. Fluid swelling was observed surrounding the treated tumors immediately after treatment, which exhibited very bright in the ADC maps. It is a typical phenomenon for PDT to all the 27 treated mice and lasted for several days. The swelling also appeared on the image 24 h after PDT (Figure 2-4d). In comparison, control mice did not show such tissue or ADC change surrounding the tumor 24h after (Figure 2-4h). The edema phenomena might be the earliest visible response to Pc 4-PDT of CWR22 tumor. On both DW-MRI and ADC maps, there are
intensity variations within the treated tumors 24h after PDT that indicates heterogeneity of tumor response to the therapy. In addition, the variation of images at Pre-PDT suggests the inhomogeneity of tumor growth.

Figure 2-4 DW-MRI and ADC of tumor-bearing mice Pre-PDT and 24h after PDT. The images were acquired using a DWISE sequence and 5 $b$ value. The present MR image was reconstructed from the first echo. ADC was obtained by fitting the 5 $b$ MR intensity with an exponential function. (a), (b) were the MR images of the treated mouse Pre-PDT and 24h after, and (c), (d) were the corresponded ADC maps for the two MR images. (e), (f) were the MR images of a light control mouse (having Pc 4 injection but without laser illumination) Pre-PDT and 24h after PDT, and (g), (h) were the corresponded ADC maps for the two MR images.

We manually segmented the tumors from MR images at each time point and computed ADC statistical properties at these regions. Histogram of tumor ADC plots the number of voxels in the volume at each ADC value and is employed to characterize ADC distribution. Figure 2-5 shows ADC histograms of tumors Pre-PDT and 24 hours after Pc 4-PDT. The histograms were generated by counting voxel number with discrete ADC
value in a level of $4 \times 10^{-5}$ mm$^2$/s, which showed the main trend of ADC distribution and minimized noise fluctuation. The Gaussian-shape histogram denotes the major voxels having an ADC value around the peak. For the treated tumor in the upper figure, ADC histogram at 24h shifts to the right relative to the one Pre-PDT, which indicates increasing of ADC values 24 hour later in term of the whole tumor region. The light control of the middle figure and dark control of the bottom figure have not shown substantial histogram displacement 24h after relative to Pre-PDT. We did not observed significant change of ADC histograms immediately after the treatment in comparison to pre-PDT for either the treated or control tumors.
Figure 2-5 Histograms of ADC in tumors Pre-PDT and 24h after PDT. A histogram plots the pixel number in tumor region having an ADC value, thereby shows ADC distribution. The histograms of a tumor Pre-PDT and 24h after PDT are overlapped in a figure to show the ADC change. The top is for a treated tumor, second is for a light control mouse which was illuminated with laser light but without Pc 4 injection, and the bottom is for a dark control mouse that had Pc 4 injection but without laser treatment.
2.5.2. **ADC results**

A total of 27 CWR22 tumor-bearing mice were treated and imaged in the study. The average ADC value Pre-PDT and 24h after PDT were calculated from the histogram of each tumor. Three mice were excluded from statistical analysis because two had MR images with unacceptable quality and the other had a too big size tumor when treated. 5 control mice (2 dark controls, 3 light controls) were imaged and analyzed for average ADC. There is consistently increasing of average ADC in the treated tumors at 24 h after compared to the value of Pre-PDT. The average ADC for these treated mice were $0.000511 \pm 0.000119 \text{ mm}^2/\text{sec}$ before PDT and $0.000754 \pm 0.000181 \text{ mm}^2/\text{sec}$ 24 h after. Student's paired t-test showed significant ADC increase ($p = 2.35\text{e}-8$) 24 h after PDT with respect to Pre-PDT. For controls, no significant ADC change between the two time points were observed. The mean ADC of these 5 control mice is $0.000664 \pm 0.000168 \text{ mm}^2/\text{sec}$ Pre-PDT and $0.000683 \pm 0.000154 \text{ mm}^2/\text{sec}$ 24 h after ($p=0.20$).

2.5.3. **Treatment results by tumor size and PSA**

The treatment effect was verified by tumor size and serum PSA level. The tumor size was measured by a caliper and calculated as $0.5 \times \text{ width} \times \text{ length} \times \text{ height}$. Tumor size and serum PSA level of the 24 treated tumors and 5 controls were obtained for time points of Pre-PDT, 24 h and 7 days after PDT. The top of Figure 2-6 showed that the average tumor size of treated tumors almost shrunk half in comparison with twofold volume increasing of control tumors 7 days after PDT. Corresponding PSA level was shown in the bottom of Figure 2-6. PSA level falling was observed at 24 h after and more than 75% decreasing happened for the treated mice 7 days later. PSA level of control mice
continued increasing until the tumor size reached a point when the mouse should be euthanized. PSA level directly relates to CWR22 tumor cell proliferation. The synchronized PSA level and tumor size change characterize the therapeutic effect which might correlate with the ADC change at 24 h after.
Figure 2-6 Tumor size and PSA level of mice from treatment. The top figure shows average tumor sizes at different time points (Pre-PDT, 24 h, 7 days after PDT) of treated mice (n=16) and control mice (n=5, 2 dark controls, 3 light controls). The tumor size was measured by a caliper and calculated as \(0.5 \times \text{width} \times \text{length} \times \text{height}\). The bottom figure is the average PSA level at different time points of the treated mice and 5 controls. The PSA level of treated mice decreased along the times relative to the treatment, and the significant PSA decreasing at 7 days was synchronized with the tumor size shrinkage. Control mice had distinct PSA increasing 7 days after the procedure that corresponded to the rapid tumor growth in size.
2.5.4. Histological results

Seven treated tumors and two controls were dissected immediately after 24 h MR imaging, and prepared for H&E stained histology. Typical representative histology was shown in Figure 2-7a for a treated tumor and Figure 2-7b for a dark control. Edema surrounding the treated tumor was visible in the histology and was seen during dissection of all the treated tumors rather the controls. The sub-region of the treated tumor was characterized with broken cells, cell debris, increased inter-cell space and necrotic sub-regions in Figure 2-7c. On the contrary, integrated tumor cells were densely distributed in histology of control tumors at Figure 2-7d. Cell morphology in histology describes the therapeutic effect that impacts MR presence so as to give MR the ability as a biomarker of early tumor response. Substantial variation of histological appearance was observed in the treated histology, which was consistent with intensity variation in DW-MRI and ADC. PDT ablates tumors through the interaction of light, photosensitizer, and oxygen. Therefore, any inhomogeneity of light transmission, photosensitizer distribution, oxygen availability, and tissue microenvironment could lead to the heterogeneity in the tumor histology.
Figure 2-7 Histological images of treated and control tumors 24 hour after PDT. (a) is for the tumor from a representative treated mouse (M146), and (b) is for a dark control mouse (M52). Significant morphological difference illustrates the therapeutic effect 24 hours after the treatment. The rectangular areas on images (a) and (b) are magnified and shown in images (c) and (d), respectively. Massive areas of tumor cells damage are shown on (c) in comparison with the intact tumor cells in (d).
2.5.5. Classification results

21 treated tumors were segmented from DW-MRI 24 hours after Pc 4-PDT, and classified into necrotic and viable regions. Each voxel was characterized by a vector consisting of DW-MRI intensity and an $ADC$ value. The class with a higher ADC was assigned as necrotic region. Figure 2-8 showed a typical classification result of a tumor DW-MRI and $ADC$ from a middle coronal slice of a treated tumor. The tumor response heterogeneity appeared in the tumor MR and $ADC$. The classification differentiated the tumor into two classes corresponding to viable and necrotic regions respectively (Figure 2-8c); therefore, indicated the therapeutic heterogeneity. The histological image at Figure 2-8d was segmented, and Figure 2-8f was the result by manually drawing the histological tumor image into two classes. Necrotic region was attributed with disrupted tumor cells and enlarged extracellular space. The similarity between the results of MRI classification and histology segmentation indicated the effectiveness of quantifying tumor response using the image classification technique.
2.6. Discussion and conclusion

2.6.1. Prostate tumors treated by Pc 4-PDT

Our previous study has demonstrated that Pc 4-PDT induced T2 relaxation time increasing in PC3 prostate tumors at mice 1 day after the treatment (Fei et al. 2007b). The T2 change maybe relate to tissue water content change as tumor early response to the treatment. In this study, a human prostate tumor model CWR22 with PSA response was treated by Pc 4-PDT. Acute edema appeared immediately after the treatment and necrosis was visible a few days later. Distinctive tumor size shrinkage and PSA level decreasing were observed 7 days after the treatment. 2 mice were cured with complete tumor eradication in more than 6 month monitoring. These observations demonstrate the therapeutic role of Pc 4-PDT to CWR22 prostate tumors on mice. We imaged the mice
before, immediately after, 24 h after the treatment using diffusion weighted MRI, and evaluated the potential of using water molecule diffusion to assess the treatment outcome. Our results show significant ADC increasing was observed 1 day after PDT, and indicate ADC parameter is sensitive to CWR22 tumor response to Pc 4-PDT at an early stage. The finding of this study suggests tracking diffusion weighted MRI and ADC change might provide a useful tool to monitor the early stage tumor response and to determine the effectiveness of the therapy.

2.6.2. Tumor therapeutic response monitored by DW-MRI

Diffusion of water molecules in tissue is restricted by cell membranes and organelles. Therefore, water molecule diffusion allows characterization of tissue microenvironment that is associated with cellular integrity and pathology conditions (Chenevert et al. 2002). Diffusion weighted MRI produces in vivo tissue images weighted by water molecules diffusion. Thereby, it has been used to discriminate between healthy and malignant tissue (Song et al. 2002), assess tumor responses to chemotherapy, radiation and gene therapy (Kauppinen 2002). Diffusion weighted MR signal at a voxel is described as $S = S_0 \exp(-b \cdot ADC)$. MR intensity inversely depends on ADC, and so a voxel with a high intensity in DW-MRI image has a small value of ADC when specific $b$ are used. However, although edema surrounding the treated tumor 24 h after in Figure 2-4b appears similar intensities with the tumor, it shows much higher ADC in the map. DW-MRI signal depends on not only the diffusion but also the signal $S_0$ that is T2 weighted. Edema has longer T2 value than tumors (Hoehn-Berlage et al. 1992), which possibly explains the reason for the similar even higher intensity of surrounding edema in DW-
MRI but with a higher $ADC$. $ADC$ was computed by a function fitting which removed T2-weighted effect. Therefore, $ADC$ map might have better contrast differentiation of tumors from edema, and might be more reliable to characterize cellular properties than diffusion-weighted images.

2.6.3. *DW-MRI reveals tumor early response to Pc 4-PDT*

PDT generates reactive oxygen species that lead to tumor cell injury, vascular damage and possible immune response for tumor ablation. Although the principal mechanism of tumor ablation by Pc 4-PDT has not been completely understood, mitochondrial damages appear to play a major role in cell killing because Pc 4 primarily localizes in the mitochondria as well as other intracellular organelles (Xue *et al.* 2007; Oleinick *et al.* 2002). Therefore, Pc 4-PDT might lead to immediate cell necrosis or apoptosis in the first step of cell damage. Tumor necrosis is characterized by massive cell damage, reduced cell distribution density, increased inter-cellular space, and liberation of water molecules from cell membrane restriction. All the responses lead to elevation of water molecule mobility in comparison with the restricted diffusion in viable tumors. This probably results in the diffusion coefficient increasing 24 hours after Pc 4-PDT. Our results show the ADC augment at 24 h after is simultaneously developed with tumor necrosis in histology as well as PSA level decreasing. The diffusion response of treated tumors probably supports the major rule of nonvascular cell injure in the early therapeutic effect of Pc 4-PDT (Plaks *et al.* 2004). Diffusion weighted MRI and $ADC$ have been reported to monitor brain tumors treated by radiation therapy and others (Cui *et al.* 2008; Harry *et al.* 2008). The present study suggests the ADC change at 24 h may also serve as a marker for early therapeutic effect of Pc 4-PDT to prostate cancer.
Our study shows that water molecule diffusion measured by diffusion weighted MRI reveals tumor response to Pc 4-PDT by increasing of $ADC$ 24 hour after the treatment. Our $ADC$ evaluation was based on segmentation of whole tumors. Heterogeneity of treatment effect was observed in the tumors either on the MRI or from the histology slides. This suggests MRI could provide more in vivo therapeutic information in voxel or subregion level. Image classification was performed to assign image voxels to be necrotic or viable based on DW-MRI intensity and $ADC$ value. DW-MRI and $ADC$ have shown the ability to differentiate treatment effect of a therapy (Li et al. 2005). Our classification was correlated with histology. The correlation indicates the classification might reveal the spatial distribution of the treatment outcome and provide more detailed information for therapy assessment.

2.6.4. Applicability of DW-MRI for Pc 4-PDT

Non-invasively monitoring tumor response to a therapy allows prognosis at a very early time and timely plan for follow-up management if the treatment is not adequate. Our results indicate DW-MRI and $ADC$ may effectively describe tumor response to Pc 4-PDT in an animal model. However, the $ADC$ change of tumor xenograft in mice might not be extrapolated directly to human prostate cancers. Due to the difference of species and MR scanners, different $ADC$ change should be anticipated when translating the study from mice to human. The treatment might also start with prostate cancer at different stages. Therefore, relative $ADC$ change might be more important than the absolute value for assessing therapy induced response. Water diffusion is sensitive to tissue cellular change, and tissue response several hours after treatment has been reported (Plaks et al. 2004). It is likely the time point post therapy is important for effective treatment monitoring.
Because we treated very fragile athymic mice and imaged them for more than half hour in one session, this study focused on the early tumor response to Pc 4-PDT in 24 hours. Notwithstanding these limitations, the study suggests the potential of DW-MRI as a noninvasive methodology of rapid monitoring tumor response to PDT within 24 hours.

We have showed diffusion weighted MRI and derived $ADC$ might provide an imaging marker for assessment of early therapeutic effect of Pc 4-PDT to CWR22 prostate tumors at mice. The imaging techniques might eventually be useful for monitoring Pc 4-PDT of prostate cancer in a clinical setting.
Chapter 3. Multiscale Fuzzy Image Classification

Chapter 2 shows that CWR22 tumor responses to Pc 4-PDT with increasing ADC 24 hours after the therapy. The quantification was obtained from the whole tumor regions, and so the significant inhomogeneity of tumor response is ignored in the analysis. Tumor response is characterized by cell necrosis, and DW-MRI has shown the capability of detecting necrosis. Therefore, an image classification method was developed in this chapter to classify DW-MRI of treated tumors into necrotic and viable regions. Thereby, the treatment effect can be quantified by measuring necrosis area.

3.1. MR image classification

MRI can provide unique tissue contrasts such as diffusion weighted imaging and quantitative T1, T2 measurements, which are promising as in vivo imaging markers that can detect early therapeutic response of tumors to therapy (Carano et al. 2004; Li et al. 2005; Jacobs et al. 2001). Image classification is an important step for quantitative analysis in order to detect pathology or quantify disease response to therapy.

Image classification is a process to partition an image into a set of distinct classes with uniform or homogeneous attributes such as textures or intensity. Classification methods can be categorized as supervised and unsupervised methods (James 1985; Lu and Weng 2007). Supervised classification methods depend on the examples of information classes in the images and derive a prior model or parameters from the training sets (Cocosco et al. 2003). Unsupervised methods examine the images without specific training data and divide the image pixels into groups presented in the pixel values according to classification criteria. Unsupervised methods have the advantage of
being fast and not requiring training information which is sometimes unavailable (Young et al. 1986).

Classification of MR images can be challenging because they can be affected by multiple factors such as noise, poor contrast, intensity inhomogeneity, or partial volume effects. Partial volume effects occur where pixels contain a mixture of multiple tissue types, thus making it difficult to assign a single class to the boundary regions. The conventional ‘hard’ classification method restricts each pixel exclusively to one class and often results in a very crisp result; therefore, fuzzy classification or “soft” segmentation has been extensively applied to MR images in which pixels are partially classified into multiple classes.

A variety of fuzzy classification methods have been reported. First, statistical model-based methods have been used for quantification of brain tissues in MR images (Cuadra et al. 2005; Van Leemput et al. 1999). These methods typically employ a Gaussian finite mixture model to estimate the class mixture of each pixel by trying to fit the image histogram. The intensity of a single tissue type is modeled as a Gaussian distribution, and the classification problem is solved by using the expectation-maximization (EM) algorithm (Marroquin et al. 2002). Because of partial volume effects and intratumor heterogeneity, the intensity distribution may deviate from the Gaussian model. The intrinsic limitation of this method is that it assumes the independence of all the pixel intensity values and that it fails to take into account the spatial correlation between pixels. Such a limitation might cause this method to be sensitive to noise and thus to produce unreliable results for MR images (Choi et al. 1991). Markov random field (MRF) is developed to model the spatial correlation and is used as prior information in the EM
optimization (Held et al. 1997; Zhang et al. 2001). However, it is difficult to estimate parameters for an MRF model from the images. Meanwhile, as the MRF-EM method is computationally intensive and could easily be trapped into a local minimum, a good initial guess regarding tissue clustering is highly needed.

Second, fuzzy c-means (FCM) classification methods employ fuzzy partitioning and allow one pixel to belong to tissue types with different membership graded between 0 and 1 (Bezdek 1980). FCM is an unsupervised algorithm which allows soft classification of each pixel which possibly consists of several different tissue types (Pham and Prince 1999). Although the conventional FCM algorithm works well on most noise-free images, it does not incorporate the spatial correlation information which would cause it to be sensitive to noise and MR inhomogeneity. A different, modified FCM has been proposed to compensate for MR field inhomogeneity and to incorporate the spatial information. (Tolias and Panas 1998) imposed the spatial continuity constraints by post-processing the results obtained from conventional FCM classification where a set of rules describing regional homogeneity were applied to update the fuzzy membership. A geometry-guided FCM (GC-FCM) method was proposed by (Noordam et al. 2000) where local neighborhood information was incorporated into the optimization process. Recently, some approaches directly added regularization terms to the objective function, thereby showing increased robustness of the classification to noisy images. A regularization term has been introduced into the conventional FCM cost function in order to impose a neighborhood effect (Ahmed et al. 2002). A pixel is likely to be the same class if the majority of neighborhood pixels belong to one class. In this way, the final solution is
guided to a piecewise-homogeneous clustering. This method was demonstrated to be useful for MR images corrupted by salt and pepper noise.

Inspired by the Markov random field and expectation-maximization algorithm (Zhang et al. 2001), various modified FCM methods were proposed incorporating different regularization terms to overcome the noise sensitivity of FCM (Chen and Zhang 2004; Liew and Yan 2003). These regularizations penalized the FCM cost function using the spatial dependence between neighboring pixels. However, high-level spatial information other than neighboring pixels could be useful for classification. Similar to the statistical model-based methods as mentioned above, these FCM methods are computationally expensive, especially for multispectral 3D MR volumes. An FCM method also depends on the initial guess which greatly affects the speed and stability of the classification.

We propose a fully automatic, multiscale fuzzy c-means (MsFCM) classification method for MR images (Wang et al. 2007; Wang and Fei 2009a). We use a diffusion filter to smooth the MR images and to construct multiscale image series. A multiscale fuzzy C-means classification method is applied along the scales from coarse to fine levels. The objective function of the conventional FCM is modified to allow multiscale classification processing where the result from a coarse scale supervises the classification of the next fine scale. The multiscale fuzzy c-means classification is described in the following section. Results on synthetic data and real MR images are reported.
3.2. MsFCM image classification

3.2.1. Multiscale space by anisotropic diffusion filtering

As image classification algorithms can be sensitive to noise, image filtering can improve the performance of classification. Due to partial volume effect, MR images often have blurred edges. Linear low-pass filtering gives poor results as it incurs even more edge blurring and detail loss. Anisotropic diffusion filtering can overcome this drawback by introducing a partial edge detection step into the filtering process so as to encourage intra-region smoothing and preserve the inter-region edge. Anisotropic diffusion filtering, introduced by Perona and Malik (Perona and Malik 1990), is a partial differential diffusion equation model described as

\[
\frac{\partial x(i,t)}{\partial t} = \text{div}(c(x(i,t))\nabla x(i,t))
\] (1)

\(x_i\) is the MR image intensity at the position \(i\), \(x(i,t)\) stands for the intensity at the position \(i\) and the time \(t\) or the scale level \(t\); \(\nabla\) and \(\text{div}\) are the spatial gradient and divergence operator. \(c(x_i, t)\) is the diffusion coefficient and is chosen locally as a function of the magnitude of the image intensity gradient

\[c(x_i, t) = e^{-\left(\nabla x(i,t)\|/\omega\right)^2}
\] (2)

The constant \(\omega\) is referred as the diffusion constant and determines the filtering behavior. As shown in Figure 3-1, the local flow function \(\Phi(x_i, t) = c(x_i, t)\nabla x(i, t)\)

\(\Phi(x, t) = c(x, t)\nabla I(x, t)\) is plotted as a function of the diffusion constant. Maximal flow presents where the gradient strength is equal to the diffusion constant (\(\nabla x \approx \omega\)); the flow rapidly decreases to zero when the gradient is close to zero or much greater than \(\omega\), which
implies that the diffusion process maintains a homogeneous region (where $\nabla x \ll \omega$) and preserves edges where $\nabla x \gg \omega$. To reduce noise in the image, $\omega$ is chosen as the gradient magnitude produced by noise and is generally fixed manually or estimated using the noise estimator described by (Canny 1986).

The multiscale approach represents a series of images with different levels of spatial resolution. General information is extracted and maintained in large-scale images, and low-scale images have more local tissue information. The multiscale approach can effectively improve the classification speed and can avoid trapping into local solutions. Anisotropic diffusion is a scale space, adaptive technique which iteratively smoothes the images as the time $t$ increases. Our multiscale description of images is generated by the anisotropic diffusion filter. The time $t$ is considered as the scale level and the original image is at the level 0. When the scale increases, the images become more blurred and contain more general information. Figure 3-2 illustrates the scale space which was constructed using anisotropic diffusion filtering. Unlike many multi-resolution techniques where the images are down-sampled along the resolution, we kept the image resolution along the scales.
Figure 3-1 Diffusion filtering removing noise and preserving edges. Diffusion flow (Y-axis) is plotted as a function of image gradients (X-axis). When the gradient $\nabla x$ is close to zero (homogeneity regions) or when the flow is much greater than $\omega$ (the edges), the flow rapidly decreases to zero which indicates no filtering processing. This implies that the diffusion filter processing maintains a homogeneous region and preserves edge information. The flow is maximal when the gradient strength is near to the diffusion constant $\omega$. To reduce noise in the image, $\omega$ is carefully chosen so that the flow is maximal and the filter will remove the noise at that gradient.
Figure 3-2 Scale-space constructed by anisotropic diffusion filtering. The scale space is composed of a stack of the images filtered at different scales where t=0 is the original image. The greater the scale level, the less local information appears.

3.2.2. Conventional fuzzy C-means method

The conventional fuzzy c-means (FCM) algorithm is an iterative method that produces optimal c partitions for the image \{x_i\}_{i=1}^N by minimizing the weighted inter-group sum of the squared error objective function \(J_{FCM}\)

\[
J_{FCM} = \sum_{k=1}^{c} \sum_{i=1}^{N} u_{ik}^p \|x_i - v_k\|^2
\]  

(3)

Where \(\{v_k\}_{k=1}^c\) is the characterized intensity center of the class k and c is the number of underlying tissue types in the images. \(u_{ik}\) represents the possibility of the pixel \(i\)
belonging to the class $k$ and requires $u_{ik} \in [0,1]$ and $\sum_{k=1}^{c} u_{ik} = 1$ for any pixel $i$. The parameter $p$ is a weighting exponent on each fuzzy membership and is set as 2.

As the FCM objective function is minimized, each pixel is assigned a high membership in a class whose center is close to the intensity of the pixel. A low membership is given when the pixel intensity is far from the class centroid. The FCM is minimized when the first derivatives of Equation (3) with respect to $u_{ik}$ and $v_k$ are zero. The final classes and their centers are computed iteratively through these two necessary conditions. In the end, a hard classification is reached by assigning each pixel solely to the class with the highest membership value.

3.2.3. Modified fuzzy C-means method

A conventional FCM method only uses pixel intensity information and results in crisp segmentation for noisy images. In order to incorporate spatial information, different modified fuzzy c-means methods are proposed to allow the neighbors as factors and thus to attract pixels into their cluster. The modified fuzzy c-means method (MFCM) proposed by (Ahmad and Todd-Pokropek 2006) has an objective function

$$J = \sum_{k=1}^{c} \sum_{i=1}^{N} u_{ik}^p \|x_i - v_k\|^2 + \frac{\alpha}{N_R} \sum_{k=1}^{c} \sum_{i=1}^{N} u_{ik}^p \left( \sum_{x_r \in N_i} \|x_r - v_k\|^2 \right)$$  \hspace{1cm} (4)

Where $N_i$ stands for the neighboring pixels of the pixel $i$ and $N_R$ is the total number of neighboring pixels, which is 8 for a 2D image and 26 for a 3D volume. $\alpha$ controls the effect of the neighboring term and is inversely proportional to the signal-to-noise (SNR) levels of the MR images.
3.2.4. *Multiscale fuzzy C-means algorithm*

After anisotropic diffusion filtering processing, our multiscale Fuzzy C-means algorithm (MsFCM) performs classification from the coarsest to the finest scale, *i.e.* the original image. The classification result at a coarser level $t+1$ was used to initialize the classification at a higher scale level $t$. The final classification is the result at the scale level 0. During the classification processing at the level $t+1$, the pixels with the highest membership above a threshold are identified and assigned to the corresponding class. These pixels are labeled as training data for the next level $t$.

The objective function of the MsFCM at the level $t$ is

$$J = \sum_{k=1}^{c} \sum_{i=1}^{N} u_{ik}^p \|x_i - v_k\|^2 + \frac{\alpha}{N} \sum_{k=1}^{c} \sum_{i=1}^{N} \sum_{x_r \in N_i} u_{ik}^p \left( \|x_r - v_k\|^2 \right) + \beta \sum_{k=1}^{c} \sum_{i=1}^{N} \left( u_{ik}^p - u_{ik}' \right)^p \|x_i - v_k\|^2 \quad (5)$$

Similarly, $u_{ik}$ stands for the membership of the pixel $i$ belonging to the class $k$, and $v_k$ is the vector of the center of the class $k$, $x_i$ represents the feature vectors from multi-weighted MR images, $N_i$ stands for the neighboring pixels of the pixel $i$. The objective function is the sum of three terms where $\alpha$ and $\beta$ are scaling factors that define the effect of each factor term. The first term is the objective function used by the conventional FCM method, which assigns a high membership to the pixel whose intensity is close to the center of the class. The second term allows the membership in neighborhood pixels to regulate the classification toward piecewise-homogeneous labeling. The third term is to incorporate the supervision information from the classification of the previous scale. $u_{ik}'$ is the membership obtained from the classification in the previous scale. $u_{ik}^p$ is determined as:
\( u_{ik}^t = \begin{cases} u_{ik}^{t+1}, & \text{if } \max_k (u_{ik}^{t+1}) > \kappa \\ 0, & \text{otherwise} \end{cases} \) \hspace{1cm} (6)

Where \( \kappa \) is the threshold to determine the pixels with a known class in the next scale classification and is set as 0.85 in our implementation. The classification is implemented by minimizing the objective function \( J \). The minimization of \( J \) occurs when the first derivative of \( J \) with respect to \( u_{ik} \) and \( v_k \) are zero.

From

\[
\frac{\partial J}{\partial v_k} = \sum_{i=1}^{N} u_{ik}^2 (x_i - v_k) \alpha \sum_{r \in N_i} u_{ik}^2 (\sum_{x_i \in N_i} (x_r - v_k)) \beta \sum_{i=1}^{N} (u_{ik}^t - u_{ik}^t)^2 (x_i - v_k) = 0
\]

the class center is updated as:

\[
v_k = \frac{\sum_{i=1}^{N} u_{ik}^2 (x_i + \frac{\alpha}{N_R} \sum_{r \in N_i} x_r) + \beta \sum_{i=1}^{N} (u_{ik}^t - u_{ik}^t)^2 x_i}{(1 + \alpha) \sum_{i=1}^{N} u_{ik}^2 + \beta \sum_{i=1}^{N} (u_{ik}^t - u_{ik}^t)^2}
\] \hspace{1cm} (7)

Since \( \sum_{k=1}^{N} u_{ik} = 1 \) for every pixel, we used the Lagrangian method to converts this constraint optimization to an unconstraint problem, the Lagrangian \( (Fm) \) is defined as

\[
Fm = \sum_{k=1}^{N} \sum_{i=1}^{N} u_{ik}^2 \| x_i - v_k \|^2 + \frac{\alpha}{N_R} \sum_{k=1}^{N} \sum_{i=1}^{N} u_{ik}^2 (\sum_{x_i \in N_i} \| x_r - v_k \|^2) + \beta \sum_{k=1}^{N} \sum_{i=1}^{N} (u_{ik}^t - u_{ik}^t)^2 \| x_i - v_k \|^2 + \lambda (1 - \sum_{k=1}^{N} u_{ik})
\]

For optimization with respect to \( u_{ik} \), it requires

\[
\frac{\partial Fm}{\partial u_{ik}} = u_{ik} \| x_i - v_k \|^2 + \frac{\alpha}{N_R} u_{ik} (\sum_{x_i \in N_i} \| x_r - v_k \|^2) + \beta (u_{ik}^t - u_{ik}^t) \| x_i - v_k \|^2 - \frac{\lambda}{2} = 0
\]

Therefore
As long as $\sum_{k=1}^{c} u_{ik} = 1$, the membership of every pixel $i$ belong to the class $k$ is updated according to the equation below:

$$u_{ik} = \frac{\lambda}{2} + \beta u_{ik}^2 ||x_i - v_k||^2 \left( \frac{1}{||x_i - v_k||^2} + \frac{\alpha}{N_R} \sum_{x_r \in N_i} ||x_r - v_k||^2 \right)$$

$$+ \frac{\alpha}{N_R} \sum_{x_r \in N_i} ||x_r - v_k||^2$$

$$1 + \beta \sum_{m=1}^{c} \frac{u_{ik}^2 ||x_i - v_k||^2 - u_{im}^2 ||x_i - v_m||^2}{(1 + \beta) ||x_i - v_m||^2 + \frac{\alpha}{N_R} \sum_{x_r \in N_i} ||x_r - v_m||^2}$$

$$= \sum_{m=1}^{c} \frac{(1 + \beta) ||x_i - v_m||^2 + \frac{\alpha}{N_R} \sum_{x_r \in N_i} ||x_r - v_m||^2}{(1 + \beta) ||x_i - v_m||^2 + \frac{\alpha}{N_R} \sum_{x_r \in N_i} ||x_r - v_m||^2}$$

MsFCM is an iterative algorithm that requires an initial estimation of the class types. In general, proper selection of the initial classification will improve the clustering accuracy and can reduce the number of iterations. As noise has been effectively attenuated by anisotropic filtering at the coarsest image, the k-means method is used on the coarsest image to estimate the initial class types. As MR tumor images have low contrast, the edges between the clusters can be recognized as a class, and a real cluster could thus be missed by k-means. After intra-region smoothing by anisotropic diffusion filtering, the edge pixels have a significantly higher gradient than the pixels within the clusters. A gradient threshold is set to remove the edge pixels, and the remaining pixels go to the K-means classification for initialization.

The first step of the proposed MsFCM algorithm is to generate the multiscale description of the images using the anisotropic diffusion filter. The filter is implemented in the discrete image domain using the forward Euler numerical approximation.
\[ I_{x}^{t+1} = I_{x}^{t} + dt(F_{x+1}^{t} - F_{x}^{t}) \]

Where \( t \) denotes the discrete scale step, \( dt \) is selected to assure stability, \( x \) is the pixel position in the images, and \( F_{x} \) is the diffusion representing the flow between the location \( x \) and \( x+1 \):

\[ F_{x}^{t} = c(x,t)(I_{x+1}^{t} - I_{x}^{t}) \]

Starting from the original image \((t=0)\), diffusion filtering progressively generates a series of images from the scale 0 to the scale \( N \) using Equation (13). At the coarsest image, the K-means method is applied to produce the initial hard classification of the image. Using the mean intensities of every class as the initial value of the next scale image classification, the algorithm iteratively computes the intensities of each class and the membership for every pixel according to Equations (8) and (12) until the characterized intensities of each class between two iterations are less than a threshold. Repeating this procedure to the image in the next scale, the classification stops as the procedure is done on the original image. The pseudo-code of the algorithm is expressed in Table 3-1.
Table 3-1 Pseudo-code of MsFCM algorithm

**Filter** and generate the multiscale description of the image using the diffusion filter

**Perform** a k-means method in the coarsest scale \((t = N)\) to obtain initial class prototypes

**Set** membership regulation matrix \(\{u_{ik}\}\) zero as initial

**For** Image from Scale N-1 to 0

**Repeat** the following step at the current scale

- Update the membership using Equation (12)
- Compute the class centroids \(v\) using Equation (8)
- Compute the Euclidean distance of the class centroids \(|v_{new} - v_{old}|\)

**Until** the centroids distance less than a threshold (0.01)

**Threshold** the membership and get the regulation matrix \(\{u'_{ik}\}\)

**End**

**Assign** each pixel the class which has maximal membership

---

The weighting parameters for the scale information \((\beta)\) and the neighboring information \((\alpha)\) generally depend on the noise level. For example, images with high noise need higher level spatial regularization from neighboring information and scale images. \(\alpha\) and \(\beta\) are set as 0.85 in all our synthesized experiments. The conventional FCM is implemented by setting the constants \(\alpha = \beta = 0\) in the criteria function and by only performing the classification iteration in the original images. Similarly, the MFCM method is applied on the original images with \(\alpha = 0.85\) and \(\beta = 0\) in the criteria function. These two methods are also initialized using the K-means method.
### 3.3. Experiments for validation

Our MsFCM classification method has been evaluated by synthetic images and McGill brain MR database (McConnel Brain Imaging Center, McGill University, Quebec, Canada) (Kwan et al. 1996; Kwan et al. 1999; Aubert-Broche et al. 2006). We also applied the method to classify real brain MR images and compared the results with manual segmentation. Meanwhile, MFCM and FCM methods were applied to these dataset in order to compare the performance of these three fuzzy classification methods.

#### 3.3.1. Synthesized images for classification

As shown in Figure 3-3a, the synthetic images represent a sphere-shape tumor with three tissue types (labeled as Class I, II, and III). In order to test the algorithm on images with poor contrast, we synthesized the images with different image quality. Contrast is defined as the relative intensity difference between one class and its surroundings.

\[
\text{Contrast(\%)} = \left| \frac{I_o - I_b}{I_b} \right| \times 100
\]

Where \( I_b \) is the background intensity and \( I_o \) is the intensity of the object. We first give a preset intensity to the center sphere (Class II) and defined it as \( I_b \). Intensities of Classes I and III are defined as \( I_o \) and are computed as \( I_o = I_b(1 - \text{contrast}) \) for Class I and \( I_o = I_b(1 + \text{contrast}) \) for Class III when a contrast is given.

After the image is created, 10% Gaussian noise with a mean of 0 is added to the images. The standard deviation of the added noise is 10% of the intensity of Class III. The SNR = 10. The image has a size of 128 x 128 pixels. For five different intensities (\( I_b = 30, 40, 50, 60, \text{and} 70 \)) and six different contrasts = (60%, 50%, 40%, 30%, 20%, and 10%)
10%), a total of 30 images were synthesized to test the classification methods. For the three methods, MsFCM, MFCM, and FCM, 90 classification experiments were performed on the synthesized images. The overlap ratios of the classified results with the pre-defined truths are computed for each classification of each image.

3.3.2. **MR brain phantoms for classification**

We obtained the brain MR images from the McGill phantom brain database for comprehensive validation of the classification methods. The MR volume was constructed by subsampling and averaging a high-resolution (1-mm isotropic voxels), low-noise dataset consisting of 27 aligned scans from one individual in the stereotaxic space. The volume contains $181 \times 217 \times 181$ voxels and covers the entire brain. A realistic brain phantom was then created from manual correction of an automatic classification of the MRI volume. Based on the realistic phantom, an MR simulator is provided to generate specified MR images. The MR tissue contrasts are produced by computing MR signal intensities from a mathematic simulation of the MR imaging physics (Kwan et al. 1996). The MR images also take the effects of various image acquisition parameters, partial volume averaging, noise, and intensity non-uniformity into account.

Using the MR simulator, we obtained T1-, T2-, and PD-weighted MR volumes with an isotropic voxel size of 1-mm and 20% intensity inhomogeneity at different noise percentages (3%, 5%, 7%, 9% and 15%). The intensity inhomogeneity was implemented by the MR volume multiplying a synthetic inhomogeneity field shape with the specified non-uniformity level. Prior to the classification, the extracranial tissues, such as skull, meninges, and blood vessels, had been removed manually so that the MR images for
classification consisted of only three types of tissue, i.e. gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). The database also provides the ratio of each tissue type at each voxel. By assigning the voxel with the tissue type with a maximal ratio, the database also provides the ground truth for evaluation. We selected 2D transverse slices (Slices No. 70, 98, 100, 102, and 130) for the classification evaluation. These MR data were applied to the three classification methods.

3.3.3. Real MR Images for classification

The classification method also was applied to real T1-weighted MR images of human brain. The MR images were acquired with a 4.0 Tesla MedSpec MRI scanner (Bruker BioSpin GmbH, Rheinstetten, Germany) on a Siemens Syngo platform (Siemens Medical Systems, Erlangen, Germany). T1-weighted magnetization-prepared rapid gradient-echo sequence (MPRAGE) (TR=2500 ms and TE=3.73 ms) was used for the image acquisition. The volume has 256 × 256 × 176 voxels covering the whole brain yielding 1.0 mm isotropic resolution. First, non-brain structure such as the skull was removed manually. Then, significant field inhomogeneity was corrected using a local entropy minimization and a bicubic spline model method (LEMS) (Salvado et al. 2006). Finally, manual segmentation of brain structures was performed to evaluate the classification. The MsFCM classification was performed with parameters \( \alpha = \beta = 0.25 \).

3.3.4. Classification evaluation

We used a variety of methods to evaluate the classification methods. First, we use overlap ratios between the classified results and the ground truth for classification evaluation. For synthesized images, the ground truth was known. For brain MR image data, the true
tissue classification maps were obtained by assigning each pixel to the class to which the pixel most probably belongs. We used the Dice similarity measurement (DSM) (Cuadra et al. 2005) to compute the overlap ratios. The DSM for each tissue type is computed as a relative index of the overlap between the classification result and the ground truth. It is as defined as:

\[
DSM^c = \frac{2(A \cap B)^c}{A^c + B^c}
\]

Where \(A^c\) and \(B^c\) are the numbers of the pixels classified as Class \(c\) using our classification method and the ground truth, respectively; \((A \cap B)^c\) is the number of pixels classified as Class \(c\) in both results. This metric attains a value of one if the classified results are in full agreement with the ground truth and is zero when there is no agreement at all.

Secondly, the classification is evaluated by a confusion table, a table with two rows and two columns that reports the number of True Negatives (TN), False Positives (FP), False Negatives (FN), and True Positives (TP) for predictive analytics (Jaeschke et al. 2002). Similar to (Cuadra et al. 2005), the confusion table is computed as a matrix whose cells represent the ratio of the classified class on rows over the class of the ground truth on columns. Therefore, the diagonal entry represents the true positives (TP) for each class. The False Negative (FN) is defined as the percentage of the class of the ground truth mistakenly classified as the other classes. The False Positive (FP) of each class is computed as the percentage of the pixels incorrectly classified as the class over the pixels that do not belong to the class in the ground truth. The sensitivity of the classification method is calculated as \(TP/(TP+FN)\), and the specificity is defined as \(TN/(TN+FP)\).
3.4. Classification results

Figure 3-3 demonstrates the effectiveness of the anisotropic filtering processing for the synthesized noisy image. In (a), the synthesized image consists of three tissue types labeled as “Class I”, “Class II”, and “Class III”. (b) shows the intensity profile of the labeled sample line. (c) and (d) represent the intensity after the diffusion filtering processing at the scale levels 5 and 7, respectively. As the scale increases, the noise is smoothed within the each region, but the intensity difference at the edge has been enhanced so as to facilitate the classification.

Figure 3-3 Anisotropic filter processing for a simulated noisy image. (a) is the original image labeled with three classes (Class I, II, III). The image is corrupted with noise. (b) is the signal profile along the labeled center line on Image (a). (c) and (d) are the signal profiles at Scale 5 and Scale 7 by diffusion filtering, respectively. The noise is removed and the line is smoothed. Furthermore, the edges are enhanced as shown by the arrows in (d).
Figure 3-4 illustrates the visual assessment of the classification results on synthesized tumor images and the comparison of the three methods. The synthesized images all have the same class distribution but different image contrasts labeled in each row. For the images with a high image contrast (60% and 40%), the three classification methods can successfully restore the class distribution. The MFCM and MsFCM methods have more homogeneous results than the FCM approach. However, our MsFCM achieved acceptable results even on images with very low contrast (10%) where the other two methods failed.
Figure 3-4 Classification of synthesized images using the three methods. The first column contains the original images with the designed contrast level. The 2nd, 3rd and 4th columns are the classification results using the FCM, MFCM, and MsFCM methods, respectively. The MsFCM method performs better than the other two methods for images with different contrast levels (10%-60%).

Figure 3-5 shows the overlap ratios between the classification results and the ground truth for the three types of tissue (Class I, II and III). The ratios decrease as the image contrast decreases. When the image contrast is higher than 40%, the three methods achieve accurate classification. When the contrast decreases, the performance of the FCM and MFCM decreases, but the MsFCM method can still achieve a high overlap ratio of more than 80%. The error bar represents the standard deviation of the DSM.
measurements computed from the classification of five images generated with a different mean intensity $I_b$. A standard deviation of less than 1% indicated consistent results for all three methods.
Figure 3-5 Quantitative evaluation of classification of synthesized images. The Y axis is the overlap ratio between the ground truth and the classification results. The X-axis represents the contrast levels. From top to bottom, the figures are results for class I, II and III respectively. Each data point represents 5 classification results from 5 images.
Figure 3-6 demonstrates the classification results on T1-weighted brain MR images. Compared to the ground truth (c), our MsFCM method (f) performed better than the FCM (d) and the MFCM (e). The MsFCM method was applied to the images with different noise levels. Table 3-1 shows the overlap ratios for the different noise levels. The overlap ratios are greater than 90% for different noise levels indicating that our MsFCM method is not sensitive to noise.

Figure 3-6 Classification of brain phantom MR images. The original MR image (a) is smoothed after the anisotropic filter processing (b). (c) is the ground truth of the classification. (d), (e), and (f) are the classification results using the FCM, MFCM, and MsFCM methods, respectively. Compared to the ground truth, the MsFCM performs better than the other two methods. The image was obtained from the McGill brain database with 9% noise and 20% intensity inhomogeneity.
Table 3-2 Overlap ratios between classification and the truth. The overlap ratios are between the classification results and the ground truth for the brain data with respect to different noise levels for three types of tissue, i.e. cerebrospinal fluid (CSF), gray matter (GM), and white matter (WM).

<table>
<thead>
<tr>
<th></th>
<th>3%</th>
<th>5%</th>
<th>7%</th>
<th>9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>0.93 ± 0.02</td>
<td>0.93 ± 0.02</td>
<td>0.92 ± 0.03</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>GM</td>
<td>0.94 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td>WM</td>
<td>0.96 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.95 ± 0.02</td>
<td>0.94 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 3-7 shows the comparison results of the three methods for the images at different noise levels. Although the three methods have good results for images with low noises, MsFCM achieved overlap ratios of 88% ± 1% and 84% ± 1% for gray matter at the noise levels of 12% and 15%, respectively. However, for the same type of tissue, the overlap ratios of the MFCM method were 78% ± 5% and 70% ± 6% for the same images at the noise levels of 12% and 15%, respectively. The overlap ratios of the FCM were 77 ± 3% and 70 ± 5%, respectively (b). Our MsFCM method consistently performed better than the other two methods for different tissues and for images at different noise levels.
Figure 3-7 Overlap ratios verse image noise for three classification methods, i.e. FCM, MFCM, and MsFCM. From top to bottom, the figures are classification of cerebrospinal fluid, gray matter and white matter. The images were obtained from the McGill brain database with different noise levels and 20% inhomogeneity. Each bar represent 5 classifications from 5 images, Mean and standard deviation are plotted. The MsFCM achieved higher overlap ratios compared to the other two methods.
Table 3-3 Evaluation of classification using confusion tables. Tables from top to bottom represent the results for the MsFCM, MFCM, and FCM methods, respectively. On each table, the top row indicates the ground truth and the left column indicates the classified results. The value in each cell is the percentage against the ground truth. For example, the table at the top represents the results from the MsFCM method. For the column “CSF”, 96.77% pixels of the true CSF were correctly classified as Class “CSF”; 3.23% were incorrectly classified as Class “GM”; and none pixel was classified as Class “WM”. Thus, the false negative value is 3.23%. The images are T1-weighted MR images from McGill brain database with 9% noise and 20% field inhomogeneity.

<table>
<thead>
<tr>
<th>MsFCM</th>
<th>Ground Truth</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CSF</td>
<td>GM</td>
<td>WM</td>
<td>FP</td>
<td></td>
</tr>
<tr>
<td>Classified</td>
<td>CSF</td>
<td>96.77±0.93</td>
<td>6.68±1.70</td>
<td>0.00±0.00</td>
<td>3.08±0.77</td>
</tr>
<tr>
<td>Results</td>
<td>GM</td>
<td>3.23±0.93</td>
<td>87.22±2.36</td>
<td>5.38±1.27</td>
<td>4.86±0.94</td>
</tr>
<tr>
<td></td>
<td>WM</td>
<td>0.00±0.00</td>
<td>6.10±2.10</td>
<td>94.62±1.27</td>
<td>4.33±1.15</td>
</tr>
<tr>
<td>FN</td>
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<td>3.23±0.93</td>
<td>12.78±2.36</td>
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<table>
<thead>
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<th>MFCM</th>
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<td>CSF</td>
<td>GM</td>
<td>WM</td>
<td>FP</td>
<td></td>
</tr>
<tr>
<td>Classified</td>
<td>CSF</td>
<td>99.30±0.61</td>
<td>22.39±11.16</td>
<td>0.03±0.06</td>
<td>9.99±4.30</td>
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<tr>
<td>Results</td>
<td>GM</td>
<td>0.69±0.61</td>
<td>68.48±7.77</td>
<td>6.34±1.53</td>
<td>5.07±1.28</td>
</tr>
<tr>
<td></td>
<td>WM</td>
<td>0.01±0.02</td>
<td>9.13±4.86</td>
<td>93.63±1.56</td>
<td>6.44±3.01</td>
</tr>
<tr>
<td>FN</td>
<td></td>
<td>0.70±0.61</td>
<td>31.52±7.77</td>
<td>6.37±1.56</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Ground Truth</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF</td>
<td>GM</td>
<td>WM</td>
<td>FP</td>
<td></td>
</tr>
<tr>
<td>Classified</td>
<td>CSF</td>
<td>97.62±1.21</td>
<td>16.75±7.01</td>
<td>0.03±0.04</td>
<td>7.52±2.60</td>
</tr>
<tr>
<td>Results</td>
<td>GM</td>
<td>2.37±1.21</td>
<td>72.47±4.23</td>
<td>13.19±2.52</td>
<td>10.76±2.29</td>
</tr>
<tr>
<td></td>
<td>WM</td>
<td>0.01±0.02</td>
<td>10.78±4.70</td>
<td>86.78±2.55</td>
<td>7.64±2.85</td>
</tr>
<tr>
<td>FN</td>
<td></td>
<td>2.38±1.21</td>
<td>27.53±4.23</td>
<td>13.22±2.55</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-3 is the confusion table for the classification of the three methods. Although the false positive values of the MsFCM classification for CSF are higher than the other two methods, MsFCM achieved lower false negative and false positive values for gray matter and white matter. The FCM and MFCM methods incorrectly assign more pixels to Class CSF. As shown in Figure 3-7. The overlap ratios of our MsFCM method are consistently higher than those of the other two methods for the MR images with different noise levels. The sensitivity and specificity of the MsFCM classification method are higher than those of the other two methods.

Classification results on real MR brain data were showed on Figure 3-8. Significant intensity inhomogeneity was observed in the MR images (a), which was corrected and shown in (b). MsFCM image classification (c) was performed on different slices and the overlap ratios were 85% ± 3% for CSF, 82% ± 5% for gray matter, and 88% ± 4% for white matter (d).
Figure 3-8 Classification of a real MR brain volume. Results of two slices are showed in rows, the first column (a) is the original MR images, second (b) is the image after intensity inhomogeneity correction, third column (c) is the MsFCM classification on corrected images where 3 gray levels represent CSF, gray matter and white matter respectively. Manual segmentations in last column (d) show the accuracy of MsFCM classification.

3.5. Discussion and conclusion

We developed and evaluated a multiscale fuzzy c-means classification method for MR images. We used an anisotropic filter to effectively attenuate the noise within the images but to preserve the edges between different tissue types. A scale space was generated by anisotropic filtering, and the general structure information was kept in the images at a coarser scale. The classification was advanced along the scale space to include local information in the fine-level images. The result from a coarser scale provides the initial parameter for the classification in the next scale. Meanwhile, the pixels with a high probability of belonging to one class in the coarse scale will belong to the same class in the next level. Therefore, these pixels in the coarser images are considered as pixels with
a known class and are used as the training data to constrain the classification in the next scale. In this way, we obtain accurate classifications step-by-step and avoid being trapped into local minima. Furthermore, we also include a regulation term that constrains the pixel so that it can be influenced by its immediate neighborhoods. Using the multiscale classification, higher spatial information is also included in the MsFCM method. This approach can achieve a piecewise-homogeneous solution. Our method was evaluated using synthesized images, a brain MR database, and our real brain MR data. The classification method is accurate and robust for noisy images with low contrast.

Our method was compared with FCM and MF CM and achieved consistent better results. We also compared our method with Gaussian Markov random field classification on brain phantom data with 15% noise and 20% inhomogeneity. MsFCM got a marginal improvement with overlap ratios of 85% ± 5% for CSF, 84% ± 1% for gray matter, and 92% ± 2% for white matter while the overlap ratios from the Gaussian Markov random field classification were 83% ± 2% for CSF, 81% ± 3% for gray matter, and 88% ± 2% for white matter. This improvement is marginal because Gaussian function is a good model for intensity distribution of normal brain structure. We expected better results from MsFCM for those images such as tumor images that deviate from a Gaussian distribution.

The algorithm is implemented in Matlab 7.1 (MathWorks, Natick, MA) with a desk computer (3GHz Pentium IV processor and 3G bytes RAM). As the classification is run in multiscale images from course to fine levels, the centroids of the initial classes from the coarser image improves the speed of the convergence. The classification result from a course level was used as an initial for the classification in the fine level. At the course level, the threshold $\kappa$ in Equation (6) was set to 0.85 for our MR images. This value may
vary for other images with different noise levels. However, our simulation data demonstrated that classification performance was not sensitive to the threshold when it is between 0.8 and 0.9. Normally, the classification on one scale converges within 3~4 iterations. The classification takes approximately four minutes that can be significantly reduced if C++ and a high-performance workstation are used for the computation. The method can be applied to 3D volume data where 26 neighboring pixels are considered. This could include more spatial regularization and lead to a better result. However, a fast 3D diffusion filtering algorithm is required.

If there is significant field inhomogeneity on MR images, it could possibly affect the classification results. Fortunately, various inhomogeneity correction methods can be used as a pre-processing step before classification (Liew and Yan 2003; Salvado et al. 2006). We applied inhomogeneity correction on real brain MR data and achieved satisfied results.

The classification has been applied to classify tumor DW-MRI and ADC into necrotic and viable regions. Instead of classifying one channel image, the classification was used to classify the combined vector of DW-MRI and ADC at each voxel of tumors. The classification correlated well with the histological results in Chapter 2 and provided a method for quantitatively monitoring tumor response to Pc 4-PDT.
Chapter 4. B-spline Point Matching for Surface Registration

In order to monitor biological response of CWR22 tumors to Pc 4-PDT, we acquired MR images before, immediately after and 24 hours after the treatment. Image classification in Chapter 3 provides region-based quantification of tumor response. Comparatively, tracking the dynamic process of each tumor voxel will offer more accurate and subtle information about the response. However, significantly nonrigid deformation between mouse images at different times prevents the longitudinal comparison at voxel level. This chapter presents a nonrigid image registration method for whole body mouse image alignment (Wang and Fei 2008) which will allow alignment of mouse images for voxel-based analysis.

4.1. Surface registration

Surface registration is extensively studied and applied in machine vision and biomedical imaging. The applications vary from object detection and tracking (Dufour et al. 2002; Malassiotis and Strintzis 2007) to alignment of medical images with atlases for analysis and quantification (Chui et al. 2003; Liu et al. 2004), and multimodality images integration for diagnosis or surgery planning (Betke et al. 2003). Surface registration is to find spatial mapping between a floating surface and a reference surface that have semantically spatial correspondence. The mapping usually aligns two surfaces in terms of an optimization criterion defined on actual structures or content expected to be aligned. The mapping can be classified as rigid and nonrigid transformation based on the assumption about the motion between the two surfaces (Audette et al. 2000). Lots of real-world applications require nonrigid transformation rather than a rigid alignment because
of object or shape variation between the two surfaces, such as the tasks like recognition of human faces, tracking cardiac motion from series cardiac images (Wierzbicki et al. 2004) and registration of brain images between different subjects for brain modelization (Ganser et al. 2004; Gee et al. 1993).

Surface matching is a subset of the general image registration problem which as well as includes landmark-based and volume-based registration (Maintz and Viergever 1998). Landmark-based registration requires manual identification of landmarks which is tedious and varies with different operators. Moreover, registration based on a limited number of landmarks may be difficult to accurately recover the nonrigid deformation which is distinct at each location. Generally, intensity-based registration has a limited capture range of the seeking transformation and typically requires a rigid transformation to roughly align the two images for registration at a preprocessing step (Hill et al. 2001; Pluim et al. 2003). Some structures, such as bones in images, might not have enough intensity information for accurate structure alignment. In comparison, anatomical surfaces usually can be extracted from images by various semi-automatic or automatic segmentation methods. The point redundancy of surfaces could yield more accurate registration compared with landmarks especially for nonrigid matching. The prior knowledge of extracted surfaces could provide advantage over volume registration for restoring large whole body motion and avoiding local optimization.

Surface registration can be implemented by representing the surfaces as points, features (Maurer et al. 1996) and physical modeling (Ferrant et al. 2001; Stammberger et al. 2000). Point based registration aligns surfaces based on relatively dense points that could be each surface point or subset points on the surfaces. The registration offers a
solution for free-form matching without feature extraction or determination of point correspondence before registration. There are two problems need be resolved for point based surface registrations: point correspondence and transformation. The registration could be solved only by determining right correspondence, in which high level information of the points like lines, shapes (Felmar and Ayache 1996) and spatial relationships (cross and Hancock 1998) was used as point attributes to locate point correspondence. These methods usually are restricted to affine or projective transformation and difficult to handle outliers. The other popular approach is solving the correspondence and transformation iteratively. Most methods in this category fall into a similar framework that iterates between two steps: seek correspondence and solve transformation. These methods are distinguished by the types of transformation to be recovered, the way to define the correspondence and the approach to solve the transformation.

Besl and Mckay (1992) proposed the iterative closest point (ICP) method to find rigid transformation between two point sets. The point correspondence was established by the nearest points between two datasets, and the transformation was solved by minimizing the sum of squared distance between the closest point pairs. Different methods were proposed to either accommodate outliers exclusion or improve algorithm efficiencies by modified closest point searching strategies (Zhang 1994; Stewart et al. 2003). These methods are guaranteed to converge to a local minimum for a rigid transformation. In order to achieve a global result, the two surfaces are assumed to be relatively close or have sufficient numbers of initial poses to start with. Apparently, large nonrigid motion invalidates the assumption, and point correspondence established by the
closest points will generate lots of local minima and fail the registration for a global nonrigid transformation.

The one-to-one correspondence of ICP methods hinders the solution for a global nonrigid transformation between point sets. An alternative strategy is to relax the binary correspondence to fuzzy matching where a point in one set is partially corresponding to each point in the other set with a ratio. One category of the methods models the point alignment as matching of probability density distribution based on statistical modeling of point sets. The point set was modeled by a mixture of gaussian distributions and the similarity between the distributions was quantified by an information measurement (Roy et al. 2007; Wells 1997). Approximation of Kullback-Leibler (KL) divergence was used by Jian and Vemuri (2005) in which the transformation was solved by directly optimizing the statistical similarity measurement. Because of the probabilistic modeling of point sets, one-to-one correspondence cannot be accomplished finally.

The other way to seek the transformation is to use the well-known Expectation Maximization (EM) algorithm (Wells 1997). E-step estimates the probabilistic correspondence under a given transformation, and M–step updates the transformation according to current correspondence estimation. Robust point matching framework (RPM) was proposed to enforce the correspondence using a softassign technique, and jointly estimate the transformation and correspondence by deterministic annealing scheme (Gold et al. 1998). Chui and Ranagarajan (2000; 2003) extent the framework into thin plate spline based nonrigid registration (TPS-RPM). The point matching was solved in a way similar to the expectation-estimation algorithm: TPS transformation was solved by least square fitting as the fuzzy point correspondence was given, and the fuzzy correspondence
was updated according to the TPS transformation. The method is limited by computational constraints because of the TPS motion model that involves computation of all the surface points. Generally the number of points could not exceed about 200, which is clearly not enough for common real applications. The method was modified using clustering technique (Chui et al. 2003) in which the original points was first clustered down to several clusters and then the clustering centers were used as the point sets for registration. The clustering method might over simplify the surfaces and lose the ability to handle outliers. TPS-RPM method constrains global affine transformation by its distance to a unit matrix which might limit the freedom of global transformation. Meanwhile, any possible prior knowledge about global transformation cannot be effectively incorporated into the optimization.

Based on the robust point matching framework, we proposed a point based nonrigid surface registration method in this chapter. The transformation is modeled as a combination of a rigid transformation and volumetric B-spline local deformation; and the RPM framework is modified to seek the transformation as well as the point correspondence. The method was validated by registration experiments of 2D and 3D synthesized data and real anatomical surfaces sampled from biomedical images.

4.2. B-spline point matching for surface registration

4.2.1. Transformation model

Assume we have two point sets $R$ and $V$ sampled from two surfaces for registration, and we want to register the floating surface point set $V$ to the reference $R$. the point sets are one-to-one corresponded and denoted as $r_a (a=1, 2, ..., N)$ and $v_a (a=1,2,..N)$. $r_a$ or $v_a$
represent the coordinate of the point in the image that the surface is extracted from, and is a $1 \times 2$ vector in two dimension image or $1 \times 3$ vector in three dimension volume. In the following, we describe the points in three dimensional space. We define $f$ is the spatial transformation that deforms the point $v_a$ to match the corresponding point $r_a$, and $f(v_a)$ is the absolute position result from the transformation instead of the displacement of $v_a$. Rigid or affine global transformation alone is not sufficient to describe nonrigid surface deformation for lots of real applications. Therefore, the transformation is modeled as a combination of a global transformation and a local deformation written as

$$f(v_a) = f_{\text{global}}(v_a) + f_{\text{local}}(v_a)$$ (1)

The global motion $f_{\text{global}}$ describes the overall position change. Affine transformation was chosen for global motion in (Chui and Ranagarajan 2003) as a part of thin plate spline. However, they have difficulty to constrain the global transformation. We select a transformation which consists of 6 degree of freedom rigid transformation and a 3 degree of freedom scaling for 3 dimension points. The rigid transformation explicitly describes 3 axial rotation ($\theta_x, \theta_y, \theta_z$), translation ($t_x, t_y, t_z$) and scaling ($s_x, s_y, s_z$).

The global transformation is written as

$$f_{\text{global}}(v_a) = \text{scale}(v_a)\text{rot}(v_{a,x})\text{rot}(v_{a,y})\text{rot}(v_{a,z})\text{Trans}(v_a)v_a$$ (2)

$\text{rot}(v_{a,x}), \text{rot}(v_{a,y})$ and $\text{rot}(v_{a,z})$ is the transformation matrix to rotate $v_a$ in $x, y$ and $z$ direction, $\text{scale}(v_a)$ is a 3D scaling matrix. 9 parameters need be solved for the global transformation, which is different from 6-parameter rigid body or 12-parameter affine transformation. Global translation, rotation and scaling are explicitly present in the model. Therefore, these parameters can be explicitly constrained according to the application.
Additional local deformation $f_{local}$ is required to account for local shape change between surfaces. Local deformation could vary significantly between applications, and there might be infinite ways to describe the mapping. We selected the B-spline based free form deformation model, which is widely applied for medical image registration and image-based organ motion tracking (Rueckert et al. 1999). The model deforms a volume by manipulating a mesh of control points equally distributed across the volume. The control point grid produces a smooth and $C^2$ continuous non-linear transformation. Unlike TPS, B-spline is local-supported where a control point only exerts local influence on the transformation which could greatly simplify the computation and enable surface matching with a great number of points.

We select cubic B-spline for local deformation model, which can be written as a 3-D tensor product of 1-D cubic spline

$$f_{local}(v_a) = \sum_{i=0}^{3} \sum_{m=0}^{3} \sum_{n=0}^{3} B_i(u)B_m(v)B_n(w)\phi_{i+l,j+m,k+n}$$

(3)

Assume the volume domain is spaced with $n_x \times n_y \times n_z$ uniform grid of control points, and where $i = \left\lfloor \frac{x}{n_x} \right\rfloor - 1, j = \left\lfloor \frac{y}{n_y} \right\rfloor - 1, k = \left\lfloor \frac{z}{n_z} \right\rfloor - 1$ are integer position of point $v_a$ in the control points grid, and $u = \frac{x}{n_x} - \left\lfloor \frac{x}{n_x} \right\rfloor, v = \frac{y}{n_y} - \left\lfloor \frac{y}{n_y} \right\rfloor, w = \frac{z}{n_z} - \left\lfloor \frac{z}{n_z} \right\rfloor$ are the fractional part of the position. $\phi_{i,j,k}$ is a B-spline coefficient defined at the control points, and $B$ represents cubic B-spline basis function.

Partial derivative of the local motion with respect to each control point coefficient can be analytically computed by evaluating (3), and a control point coefficient change
only affects the motion within the neighboring $4 \times 4 \times 4$ grid point region. The gradient is written as

$$\frac{\partial f_{\text{local}}(v_a)}{\partial \phi_{p,q,r}} = B_i(u)B_m(v)B_n(w)\delta_{(p,q,r),(i+1,j+m,k+n)} (4)$$

Here $v_a$ is located at the control point grid with integer position $(i, j, k)$ and fraction of $(u, v, w)$, and the derivative is not zero only at the control points of $(i+l, j+m, k+n)$ with $l, m, n \in \{-1,0,1,2\}$.

The local motion ought to be characterized as a smooth function to discourage arbitrary unrealistic shape deformation. A smoothness penalty term is introduced to regularize the local motion by the second order spatial derivatives written as

$$L_{f_{\text{local}}}(v) = \sum_{a=1}^{N} \left[ (\frac{\partial^2 f_{\text{local}}}{\partial x^2})^2 + (\frac{\partial^2 f_{\text{local}}}{\partial y^2})^2 + (\frac{\partial^2 f_{\text{local}}}{\partial z^2})^2 + 2(\frac{\partial^2 f_{\text{local}}}{\partial x \partial y})^2 + 2(\frac{\partial^2 f_{\text{local}}}{\partial x \partial z})^2 + 2(\frac{\partial^2 f_{\text{local}}}{\partial y \partial z})^2 \right] (5)$$

Thank to the analytical formulation of B-spline in (3), the second spatial derivatives in the smooth constraint can be computed as

$$\frac{\partial^2 f_{\text{local}}(v_a)}{\partial x^2} = \sum_{i=0}^{3} \sum_{m=0}^{3} \sum_{n=0}^{3} \frac{dB_i^2(u)}{du^2} B_m(v)B_n(w)\delta_{i+1,j+m,k+n} (6)$$

The other second derivative entries in (5) have analogy format.

4.2.2. Surface registration by robust point matching

In general, object surfaces are extracted by 3D volume segmentation, and then surface point sets are obtained by down sampling the surfaces. It is difficult if not impossible to extract exact corresponding point sets from two surfaces, and even existence of point correspondence for some points cannot be ensured. We incorporate our motion model
into the robust point matching framework (RPM) (Chui and Ranagarajan 2003; Gold et al. 1998) which does not require one-to-one point correspondence and is robust to noise and outliers. RPM models point matching as a linear assignment least square problem, and point correspondence is constructed using fuzzy logic and enforced to be one-to-one correspondence using softassign and deterministic annealing techniques.

For registering two point sets \( v_a (a=1,2,...,N) \) and \( r_i (i=1,2,...,M) \) with unknown point correspondence, we would like to map the two point sets as closely as possible while removing the impact of outlier points that have not corresponding partners in the other set at all. The common way for matching is to minimize the Euclid distance between corresponding points. However, we do not have the correspondence information before the final alignment. Using the fuzzy logic, a point \( v_a \) is not considered to be one-to-one corresponding to a point in \( R \), but \( v_a \) relates to all the points in \( R \) with a fuzzy ratio \( m_{ai} \)

\[
( v_a \rightarrow \sum_{i=1}^{M} m_{ai} r_i ) \quad \text{where} \quad m_{ai} \in [0,1] \quad \text{and subjects to}
\]

\[
\sum_{a=1}^{N} m_{ai} = 1 \quad \text{for} \ i=1,2,...,M, \quad \text{and} \quad \sum_{i=1}^{M} m_{ai} = 1 \quad \text{for} \ a=1,2,...,N.
\]

In order to handle the outlier situation where a point in \( v \) does not correspond to any point in \( R \) or vice verse, an extra row and column are added to the corresponding matrix \( M \). Once a point is identified as an outlier, the point will be assigned to correspond to this extra row or column and will be taken away during transformation computation. In this way, \( M \) becomes a matrix with a dimension of \((N+1) \times (M+1)\).

As \( M \) gives the point correspondence ratio between two point sets, we minimize the following cost function for the matching

\[
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\]
\[ E = \sum_{a=1}^{N} \sum_{i=1}^{M} m_{ai} \| r_i - f(v) \|^2 + \beta \| Lf_{local} \| ^2 + \alpha \sum_{a=1}^{N} \| f_{local}(v) \| ^2 + \kappa \sum_{a=1}^{N} \sum_{i=1}^{M} m_{ai} \log m_{ai} \]  

(7)

Subjecting to the normalization requirement of

\[ \sum_{a=1}^{N+1} m_{ai} = 1 \text{ for } i=1,2,...,M \quad \text{and} \quad \sum_{i=1}^{M+1} m_{ai} = 1 \text{ for } a=1,2,...,N \]

And the bounded restriction \([lb, ub]\) for the global transformation of rotation \(\theta\), shift \(t\) and scaling \(s\) denoting as

\[ lb_{\theta} < \theta = (\theta_x, \theta_y, \theta_z) < ub_{\theta}, \]

\[ lb_{t} < t = (t_x, t_y, t_z) < ub_{t}, \]

\[ lb_{s} < s = (s_x, s_y, s_z) < ub_{s}. \]

The cost function first evaluates the point sets distance weighted by the fuzzy matrix \(M\), then gives the constraints for the motion, and finally makes the point correspondence to be as binary (one-to-one corresponding) as possible. We give the range for each parameter in the global transformation (scaling, rotation and translation) explicitly according to possible prior information. The local transformation is constrained to be smooth by second order derivatives as (5). However, the local deformation could be an arbitrary function to fit the cost function (7) even with the smoothness restraint; and the transformed surface could lose its main structure when \(M\) is significantly fuzzy at the beginning of optimization. Therefore, we constrain the absolute local deformation \( \sum_{a=1}^{N} \| f_{local}(v) \| ^2 \) to ensure predominance of the global transformation. According to well-known entropy theory (Yao 2003), entropy of matrix \(M\) increases as the matrix becomes less fuzzy, and the entropy of matrix \(M\) reaches maxima when \(M\) becomes permutation.
(i.e., either 1 or 0 for each entry). At the final matching, point sets from both surfaces are expected to be aligned as near as possible so $M$ should be as binary as possible. Therefore the cost function minimizes the negative entropy of $M$ using $\sum_{a=1}^{N} \sum_{i=1}^{M} m_{ai} \log m_{ai}$ in order to reach one-to-one correspondence finally.

The energy function consists of optimization for both point correspondence and the transformation. Similar to EM method, the point matching function can be minimized by iterating two the interlocking optimizations for the correspondence matrix $M$ and non-rigid transformation $f$ respectively.

4.2.3. Update fuzzy corresponding matrix $M$

The matrix $M$ is updated by evaluating the first derivative of energy equation (7) with current transformation $f$

$$m_{ai} = e^{\frac{-\|v_i - f(v_a)\|^2}{\kappa}}$$  \hspace{1cm} (8)

For $i=1,2,...,M$, and $a=1,2,...,N$. A constant $\exp(-9)$ is given to the extra row and column for $i=M+1$ or $a=N+1$ to account for the outlier correspondence. Here each point is assumed to have a small probability $\exp(-9)$ to be an outlier point, which means an outlier point has not any corresponding partner from the other surface within the region of $3\sqrt{\kappa}$.

In order to satisfy the normalization requirement, $M$ is normalized along row and column by the Sinkhorn’s method which iterates row and column normalization of $M$ for a number of times (Gold and Ranagarajan 1996). After the normalization, if a point is far
away from any reference point, the extra entry will domain the correspondence so the point can be considered as an outlier and removed from the following transformation computation. \(\kappa\) is called as temperature parameter which weights the fuzziness of \(M\) to the distance measurement in the cost function. Equation (7) indicates that \(\kappa\) provides a distance range in which two points from both sets have significant corresponding ratio. Therefore, when \(\kappa\) is a higher temperature, \(M\) is fuzzier and the objective function becomes more convex for optimization; as \(\kappa\) gradually decreases, only points very close to each other can have a significant \(m_{ai}\), and \(M\) becomes less fuzzy, in other words, more close to one-to-one point correspondence.

4.2.4. Solve the transformation

Once \(M\) is determined, \(v_a\) is considered to correspond to point \(\bar{r}_a = \sum_{i=1}^{M} m_{ai} \bar{r}_i / \sum_{i=1}^{M} m_{ai}\). As the two point sets \((v, \bar{r})\) become one-to-one corresponding, the problem becomes a constraint least-square fitting of (7) for the transformation \(f\). Transformation \(f\) is resolved in two consecutive steps: first the global transformation is solved by a constraint linear least square fitting between \(v\) and \(\bar{r}\) using preconditioned conjugate gradient method (Horn and Johnson 1985). Each unknown variable of the 9-parameter global transformation in (2) is bounded by \([lb, ub]\) according to prior information of the motion between point sets.

The next step is to solve B-spline coefficients at each control point by fitting the remaining square distances after removal of the global motion, which is written as minimization of below cost function

\[
E = \sum_{a=1}^{N} \left\| \left( \bar{r}_a - f_{\text{global}}(v_a) \right) - f_{\text{local}}(v_a) \right\|^2 + \beta \left\| L f_{\text{local}} \right\|^2 + \alpha \sum_{a=1}^{N} \left\| f_{\text{local}}(v_a) \right\|^2
\]  

(9)
Where $L_{\text{local}}$ and $f_{\text{local}}$ are defined in (5) and (3) respectively. Now the function $E$ is quadratic for B-spline coefficients, the minimum occurs when all its first-order partial derivatives are zero. The partial derivatives are given in (4) and (5), so setting the partial derivatives equal to zero leads to a system of equations as $A\phi = b$. The B-spline can be directly solved by Cholesky decomposition (Barrett et al. 1994).

4.2.5. Deterministic annealing

Iteration of optimization for $M$ and transformation $f$ depends on a given temperature $\kappa$ which specifies the weight of the correspondence fuzziness. At the initial stage, a more widely search range or more fuzzy correspondence is expected for more convex optimization with a higher temperature $\kappa$; finally one-to-one correspondence is desired for surface registration which asks for a small $\kappa$. This is analogous to a deterministic annealing scheme where a global solution gradually reaches as temperature decreases (Chui and Ranagarajan 2003). Therefore, the energy function is minimized in a deterministic annealing schedule. The temperature is linearly decreased according to $\kappa^{\text{new}} = 0.90 \cdot \kappa^{\text{old}}$. At each temperature of $\kappa$, the interlocking optimizations are performed until convergence. Our experiments show 3~5 iterations are enough to get satisfactory results. We terminate the annealing as there is no further correspondence change if decreasing the temperature parameter.

4.2.6. Algorithm of B-spline surface registration

Minimization of the cost function (7) becomes two interlocking optimizations consisting of the estimation of correspondence matrix $M$ and a least square fitting for the transformation $f$ under deterministic annealing scheme. Table 4-1 is the pseudo code for
our B-spline surface matching algorithm. Before registration, surface points will be extracted from both volumes and the coordinates of both point sets are scaled into [0, 1]. Then we initialize the transformation as unit matrix indicating no transformation initially, and start the annealing scheme with $\kappa_0 = 0.2$. 
Table 4-1 Pseudo code of B-spline non-rigid surface registration algorithm

| Prepare floating and reference surface point sets |
| Initialize temperature parameter $T = 0.2$ |
| Initialize the transformation as unit matrix |
| While maximal $M$ change is greater than a threshold (0.0001) or $T > 0.0001$ |
| Repeat cost function minimization at current $T$ |
| Update correspondence matrix $M$ based on current transformation |
| Compute global transformation $f_{global}$ based on $M$ |
| Compute B-spline local deformation $f_{local}$ based on $f_{global}$ and $M$ for 3 iterations |
| Compute $M$ change from previous $T$ to current $T$ |
| Decrease $T = T \cdot 0.9$ |
| End While |
| Obtain the final transformation $f = f_{global} + f_{local}$ |
| Transform the floating surface using the final transformation |

The parameters $\beta$ and $\alpha$ balance point matching and transformation constraints. When matrix $M$ is fuzzy at high temperature $\kappa$, the transformation need be more rigid for a global matching and maintain the surface geometry, so greater local motion constraints are expected; as $\kappa$ becomes smaller, flexible nonrigid transformation is desired for nonrigid alignment. Accordingly, the parameters are set to adaptively decrease to follow the temperature annealing schedule by $\beta = 50\kappa$ and $\alpha = 10\kappa$. Our experiments show the
design can generally used for 2D and 3D surfaces without significant influence on the final outcome.

4.3. Registration experiments

Our experiments design first demonstrates the principle of the method on 2D-curve point matching, then validates the approach on synthesized 3D surfaces with respect to noise and point outliers. The experiments of 2D face matching and 3D biomedical surface registrations allow us to evaluate the method in real applications.

4.3.1. 2D surface registration

In order to test the algorithm performance for recovering a given transformation, we scaled the 5 simulated 2D data set used in Chui and Ranagarajan (2003) into curve points in an image with a dimension of $256 \times 256$, then applied the point matching method to the point sets for registration. The B-spline control points grid was set as an $8 \times 8$ mesh to cover the whole image. Visually exact one-to-one point matching was achieved for all five data pairs in both directions. We also run extensively registration experiments on the data by adding a range of rotation (from $-100^\circ$ to $100^\circ$) and translation $([-40, 40] \times [-40, 40])$ to the data pairs. The method consistently achieved exact point matching and exhibited much wide search range of the global transformation.

The algorithm was applied to register 2D faces where the feature boundaries of face, eyes, mouth and nose were manually segmented from both face images for point sets registration. The $256 \times 256$ face image was sampled with around 1025 points from the segmented boundaries and was gridded by $8 \times 8$ B-spline model. The final
transformation gave the motion of each location in floating image to reference image. Therefore, the reference image was wrapped back to floating image space when applying the surface transformation for image registration. The image wrapping was implemented by linearly interpolating image intensity for each transformed location in the reference image.

4.3.2. Synthesized 3D surface registration

To test how the method behaved in 3D surface registration and in the presence of noise and outliers, we simulated a sphere in a $64 \times 64 \times 64$ volume with a diameter of 40 voxels and created a mesh for the surface using a commercial software AMIRA (Mercury Computer Systems, Inc., Chelmsford, MA). The 1501 triangle mesh vertices were sampled as the surface points for registration. We also down sampled the surface to get 400 mesh vertices as boundary points of the surface. The volume and point set were artificially transformed first by an affine transformation and then deformed with a smooth nonrigid motion. In order to simulate realistic motion, the nonrigid motion was generated from B-spline interpolation with $12 \times 12 \times 12$ control points over the volume, and the coefficients at control points were sampled from a Gaussian distribution with standard deviation of 8 voxels. The motion at the 400 boundary points was interpolated from the B-spline motion. These point displacements formed the boundary condition for a finite element method (FEM) based elastic deformation of the sphere. The displacement at the 400 boundary vertices were the force that drove the sphere which was modeled as linear isotopic elastic material with Young's modulus of 60kPa and Poisson's ratio of 0.45 (Fei et al. 2006). The deformation was computed using the finite element analysis software FEMLAB (COMSOL, Inc., Burlington, MA). Thereby, we generated two 3D surfaces
and obtained 1501 surface points from both surfaces with exactly known deformation. The registration was performed with a B-spline having $8 \times 8 \times 8$ control points. The distance between the corresponding point pairs were used to evaluate the method to recover the synthesized motion.

4.3.3. Real biomedical surface registration

In order to follow up disease progression, quantify tumor growth and monitor the biological response to drugs or therapies, images of experimental small animals at different time points or modalities need be aligned. As an animal body is quite small and very flexible, it is very difficult to maintain it at the same position for each imaging session, and the situation becomes more severe considering the uncontrollable animal respiration and heart beating. We are attempting to apply the nonrigid surface registration method for small animal image registration. Tumor-bearing mice were treated using photodynamic therapy and MR imaging was conducted before and 24 hour after the therapy for monitoring the therapeutic effect (Fei et al. 2007b). We tried to register tumor MR images before and 24 hours for quantifying tumor change from MR images. The whole body mouse MR images were first registered using a rigid-body registration method (Fei et al. 2006). Significant global and local deformation was observed. We manually segmented the tumors from MRI and then aligned the surface using the proposed method. Finally we applied the volumetric transformation to wrap the tumor MR images. 24 tumor MRIs from 24 mice experiments have been examined, and we focused on the surface registration by evaluating the tumor volume overlapping ratio result from the registration. The overlapping ratio was defined as
\[ \text{oratio} = \frac{2(\text{Ref} \cap \text{Def})}{\text{Ref} + \text{Def}} \]  

(10)

Where \(\text{Ref}\) and \(\text{Def}\) are the voxel numbers of the object at reference and deformation volumes respectively, and \((\text{Ref} \cap \text{Def})\) is the number of voxels spatially present in the both volumes simultaneously.

We also evaluated the proposed method for whole body registration of a mouse at different positions where the significant non-rigid bone motion makes intensity based registration difficult. The method was applied to mouse microCT images which were acquired at three different positions using a microCT scanner. The CT images with a resolution of \(0.1 \times 0.1 \times 0.1\) mm\(^3\) were down sampled to a resolution of \(0.2 \times 0.2 \times 0.2\) mm\(^3\) and cropped into size of \(120 \times 100 \times 200\). We first manually aligned the volumes with rigid transformation, then segmented the bones by a threshold (1100 unit), and finally down sampled the surfaces to point sets (4500–5000 points). The downsampling was implemented by keeping a surface point but removing all other points within \(3 \times 3 \times 3\) window centered at the point. We imaged a mouse at 3 different positions and registered the bone surfaces between them. So there were total 6 registration experiments. 5 anatomical feature points were manually picked from the images and used to evaluate the accuracy of bone surface registration.

The method was applied to sulcal point sets sampled from two human MR brain images. The two MR volumes were obtained from the McGill phantom brain database with dimension of \(181 \times 217 \times 181\) (Collins et al. 1998). After removing the skin and scalp, the brain surfaces were rendered using AMIRA (Mercury Computer Systems, Inc., Chelmsford, MA) and the sulci were manually tracked from the both surfaces. The
extracted 14 sulcal lines were sylvian fissure, central sulcs, postcentral sulcs, precentral sulcs, superior frontal and temporal sulcs at both sides of brain, medial frontal line and interhemispheric fissure. These lines were sampled into point sets which were 903 points for reference and 877 for the floating one. Registration of the sulci might demonstrate the potential of the surface registration method for neurologic applications.

4.4. Registration results

4.4.1. Study of 2D surface registration

Exact point matching has been achieved for all the five 2D datasets. Figure 4-1 shows the registration of a 2D point sets pair containing 60 points but added 40 pixels translation for both $x$ and $y$ axis, 100 degree of rotation and 0.6 scaling of axis $x$ and $y$. In this experiment, the bound of global transformation are set as $[-120^\circ, 120^\circ]$ for rotation, $[-60, 60]$ for translation and $[0.4, 1.5]$ for scaling. The intermediate results within the deterministic annealing are showed in row 2 ($\kappa=0.18$) and 3 ($\kappa=0.08$) in Figure 4-1, and the last row is the final point sets matching. The accompanying global transformation of the floating shape is showed in the second column and the last column is the result using the TPS-RPM algorithm. The figure shows the main structure geometry is maintained at the initial stage with higher temperature using the proposed method, which guides the optimization to the correct result as the local transformation gradually increases with temperature $\kappa$ decreasing. Whereas the registration using TPS-RPM shows the main shape has changed at temperature $\kappa=0.08$ in the last column of Figure 4-1, and finally the registration reaches a wrong local minima. This probably is because the constraint of the global affine transformation is not enough in TPS-RPM at the higher temperature.
Experiments using ICP fails the alignment of the dataset since ICP is known to be prone to local minimization.

Figure 4-2 shows the face matching result (Figure 4-2c) by wrapping back the reference image (Figure 4-2b) to the floating image (Figure 4-2a). The segmented boundary points are overlapped on the images, and matching of the floating boundary of Figure 4-2a with the deformed face image (Figure 4-2c) demonstrates a successful registration. The boundary overlapping of the floating surface to the reference before and after registration is displayed in Figure 4-2e and Figure 4-2f respectively. We also intentionally remove the nose curves in the floating image (labeled as arrow in Figure 4-2g) and try the matching method between the two faces by the point matching method. In this case, the missing noses in the floating boundary makes the reference nose boundaries have no corresponding points so they are outliers for registration. Figure 4-2i shows the alignment has not been affected by the missing portion, and thus illustrates the method is somewhat robust to the outliers.
Figure 4-1 Registration of 2D point sets. The alignment is from the empty squares to the solid dots using the proposed method and TPS-RPM. First row is initial point sets ready for registration. The second and third row are the intermediate results during deterministic annealing with $\kappa = 0.18$ and $\kappa = 0.08$ respectively, and the last row is the final results showing the points alignment from registrations. First column is registration result using the proposed method, the middle column is the resultant global transformation in the first column (scaling, translating and rotating the floating point set), the third column is the registration result using TPS-RPM method. TPS-RPM method fails on the data and the geometry of empty square set changes at $\kappa = 0.08$. 
4.4.2. Study of 3D synthesized surface registration

Registration of the synthesized 3D point datasets is shown in Figure 4-3. The two point sets are sampled from the surface of a synthesized sphere and the elastic deformed volume. Only equally sampled 150 points from the point sets are displayed in the figure for better visualization. Figure 4-3a is the two point sets with known simulated motion,
and Figure 4-3b is the overlapping of transformed empty circle points with the reference filled dots. The point sets are projected to X-Y plane, and the points before and after the registration are displayed in Figure 4-3c and Figure 4-3d respectively as 2D plotting. The 3D and 2D points overlapping clearly demonstrate the registration successfully restores the simulated motion.

Figure 4-3 Registration of 3D synthesized point sets. The two 3D surface points are overlapped before and after the registration in (a) and (b) respectively. The results are projected to X-Y plane and shown the surface difference in (c) and the alignment in (d).
Figure 4-4 Histogram of point distance on 3D surfaces before and after registration. The distance of 1501 point pairs before registration is 6.5 ± 0.9 voxel and becomes 0.7 ± 0.3 voxel after.

Figure 4-5 Mean registered point distance on 3D surfaces verse motion noise. Motion noise is sampled from a Gaussian distribution whose standard deviation is the percentage noise level of the maximal simulated motion.

Figure 4-4 plots the histogram of the corresponding point pairs distance before and after the registration, where the average distance decreases from 6.5 ± 0.9 to 0.7 ± 0.3 voxel.
voxels from the registration. After one-to-one corresponding 3D point sets were obtained, we added noise motion to perturb the displacement between each point pair. The noisy motion was sampled from a Gaussian distribution whose standard deviation was 2%, 5%, 8%, 10%, 15% and 20% of the maximal synthesized local motion. Figure 4-5 plots the average distance of the corresponding point pairs after the registration which illustrates the reliability of the method to motion noise.

4.4.3. Study of real biomedical surface registration

Figure 4-6 shows the alignment of a tumor surface before and 24 hours after a therapy. There is significant global and local motion between the two surfaces after whole body rigid registration (Figure 4-6c). Figure 4-6f indicates the effective registration of the two surfaces from the visible surface matching. We applied the resultant volumetric transformation to wrap the tumor MRI volume, and computed the overlapping ratio between reference and deformation tumor. The average overlapping ratio for 24 tumor experiments are $94 \pm 5\%$. 

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Figure 4-6 Registration of 3D tumor surfaces from a mouse MRI. The images were before and 24 hours after a therapy. (a) is the floating surface with the triangle surface elements, (b, e) is the reference surface, and (c) shows the global and local difference between the two surfaces. The B-spline point matching registration transforms surface (a) to (d), and the overlapping of transformed and reference surface in (f) indicates the alignment of both surfaces.

For the whole body mouse registration, our method succeeds in all six experiments on the bone surfaces at different positions according to visual inspection. The bone surface in Figure 4-7b is registered to the one in Figure 4-7a. Figure 4-7d indicates alignment of the bone surfaces after registration by the surfaces overlapping. We manually selected 5 anatomic feature points illustrated in the left figure in Figure 4-8. The selection was on the three surfaces and these points are one-to-one corresponding. The Euclidian distance between corresponding point pair was computed before and after the registration. We average the distance of each feature point pair on all the six experiments, and plot the mean and standard deviation of the point distance in the right figure of Figure 4-8, which shows the corresponding points become much closer owing to the
registration. The average distance of the five feature points improves from $4.6 \pm 2.2$ to $1.3 \pm 0.2$ voxels after the nonrigid registration as well as the standard deviation decreasing.

Figure 4-7 Registration of mouse bone surfaces at two different positions. (a) is reference bone surface, (b) is the floating surface, (c) is the overlapping of the reference and floating surface which shows the shape difference between two positions. (d) is the overlapping of transformed and reference surfaces after the proposed registration.

Figure 4-8 Distance of five feature points before and after registration. Left figure shows the five manually selected anatomical feature points identified on both surfaces. The mouse was CT imaged at 3 positions and there were total 6 registration experiments. The average distances of the feature points before and after registration are compared in the right figure.
Table 4-2 lists the 5 feature point coordinates in 3D volumes before and after registration in the Floating and Deformed row respectively. The deformed points are much closer to the Reference. The table shows both-direction registration of the bones between two positions.

Table 4-2 Coordinates of five feature points before and after registration. The coordinate \((x, y, z)\) in each table entry is the point location in 3D space. The Floating row is the coordinate of floating point, and the Reference row is coordinate of the corresponding feature point, the Deformed row is the transformed floating point that is close to the reference position. Experiment 2 is to register surfaces in inverse direction of Experiment 1.

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<tbody>
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<td>Floating</td>
<td>68</td>
<td>8</td>
<td>30</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>Reference</td>
<td>72</td>
<td>10</td>
<td>29</td>
<td>57</td>
<td>67</td>
</tr>
<tr>
<td>Deformed</td>
<td>72.2</td>
<td>11.3</td>
<td>28.8</td>
<td>57.6</td>
<td>65.6</td>
</tr>
</tbody>
</table>

For brain sulcs matching, 14 sulcs curves were extracted from the rendering brain MRI surfaces and displayed in Figure 4-9a and Figure 4-9b. Shape difference between the two sulcs sets are significant in Figure 4-9c according to the overlapping of solid dots and empty points. Figure 4-9d overlaps the reference and deformed 3D curves and shows the alignment of both sets after the registration.
Figure 4-9 Registration of sulci from two brain MRI volumes. (a) and (b) show the manual tracing of brain sulci from two 3D brain surfaces. 14 sulcal curves are segmented from both surfaces. The shape difference between two sulci set is showed in (c) with the solid point sets of (b). (d) is the alignment of both sulci using the point matching method.

### 4.5. Discussion and applicability

The method extends the TPS-based robust point matching method for nonrigid registration of surfaces with dense sampled points. The surfaces could be feature point sets, 2D curves, 3D surfaces, or surfaces consisting of multiple objects with outliers in various applications. The algorithm solves both the point correspondence and transformation without landmarks portraying or features extraction. Fuzzy correspondence and deterministic annealing techniques are employed to achieve global optimization and outliers rejection. We modify the robust point matching framework by integrating a motion model combining a global transformation and a B-spline local
deformation. Prior information about translation, rotation and scaling can be easily incorporated into the optimization to constrain local nonrigid motion. Meanwhile, the local support B-spline motion greatly decreases the degree of freedom for optimization and achieves better computation efficacy for surfaces with a great number of points than the TPS-based method.

The deterministic annealing scheme has shown to realize the registration in a scale-space behavior that enables much robust registration than the classic ICP method (Chui and Ranagarajan 2000). We have observed the global structures are registered at the high temperature, and nonrigid local structures are aligned at low temperature because of more binary correspondence and the synchronized decreasing of the local motion constraint weights ($\alpha$ and $\beta$). Our motion model enables straightforward restriction of the global motion and dynamical control of the local transformation. Classic rigid ICP method seeks a local optimal solution and assumes the approximate alignment of two surfaces. Thereby, the significant global motion might not be solved by the rigid ICP method before the nonrigid alignment. Our method emphasizes the global motion at high temperature; whereby the method is robust to the possibility to trap into local optimization especially when the correspondence is much fuzzy at the higher temperature.

The common factors affecting point based surface registration is noise and outliers arising from image acquisition and reconstruction. Temperature $\kappa$ weights the fuzziness of point correspondence and gives a search range for point correspondence. We assume each point has a small prior probability to be an outlier point and give the additional outlier row and column a constant of outlier probability. If a point is far away from each reference point by greater than $3\sqrt{\kappa}$ distance, the normalization of row or column of the
correspondence matrix $M$ makes the outlier row or column dominate the correspondence. By thresholding the outlier entry in $M$, the outlier point can be recognized and removed from transformation computation. Our approach to reject outliers is similar to the outliers rejection strategy that was proposed by Feldmar and Ayache (1996) in ICP registration. In their method, distances between nearest point pairs were fit to a Gaussian distribution, and point pairs with distance greater than 3 times of the standard deviation were rejected as outliers. Our experiments of the synthesized 3D data show the reliability of the method for point set matching in presence of noise and outliers.

Our point based surface registration aligns surfaces based on relatively dense point samples that can be considered as feature points of the surfaces. Another common surface registration method is to extract features expressing surface morphology and register the surfaces by quantifying similarity between the features. Intuitively, our method can be straightly applied to feature point registration which the robust point matching framework was originally proposed for (Gold et al. 1998). However, we believe surface matching directly using dense sampled points could provide more information redundancy especially for nonrigid surface matching where mapping for every point is expected. ICP and its various modifications have demonstrated the effectiveness of point based surface registration, as well as the robust point matching framework has been successfully applied to thin plate spline based nonrigid feature points registration (Chui et al. 2003). Although point based method handles much more points and computational complexity than feature based method, our method employs a global transformation and local support B-spline which decreases thousands of degree of freedom to the number of B-spline coefficients covering the whole volume. The global transformation and volumetric local
deformation yield smooth whole space transformation which not only includes the surface area. Therefore, the point matching of identifiable structures in medical images could be used to estimate the registration of the whole body images, and an intensity based registration will be followed for more subtle but localized volume registration. The strategy may be necessary for whole body image registration where many articulated structures and very large shape change may make an intensity-based registration really difficult (Li et al. 2008).

We have applied the registration method to align mouse bones in MicroCT images acquired at different positions. The alignment of bone structures is a good initial position for the following whole body intensity-based registration. For the mouse MR imaging, mouse spine can be easily extracted from MRI. Therefore, the densely sampled bone surface points at different imaging times can be aligned using the proposed algorithm without exclusively giving point correspondence. After removing the whole body deformation, the mouse MRI volumes can be registered by an intensity-based deformable registration (Li et al. 2008; Rueckert et al. 1999).
Chapter 5. MRI-guided Method for PET Partial Volume Correction

Positron emission tomography (PET) with a tracer of $^{11}$C labeled choline has been applied to evaluate prostate tumor response to Pc 4-PDT in animal models (Fei et al. 2008; Fei et al. 2009). The studies show that tumor standardized choline uptake decreased 48 hours after the treatment. However, PET present much blurred tumor images that were due to partial volume effect (PVE) of PET. PVE could largely bias tracer uptake quantification on the tumors. With the acquisition of mouse MRI and registration of MRI with PET, this chapter describes a MRI-guided partial volume correction method for PET (Wang and Fei 2009b). The correction not only improves the radioactivity quantification of region-of-interests but also yields a corrected PET image for display and analysis.

5.1. PET partial volume effect and correction

PET in vivo images the distribution of radiolabeled tracers in an absolute quantitative fashion. This allows in vivo monitoring of various physiological processes such as perfuse, metabolism, gene expressions, protein interactions and etc. PET is an essential tool for diagnosis and longitudinal study of several neurological diseases, and has been widely used to differentiate between benign and malignant tumors, determine tumor stages and assess therapies (Pan and Mawlawi 2008). Recently, PET becomes a molecular imaging technique to render molecular or protein interaction during biological processes (Riemann et al. 2008). However, despite the continual improvement of physical characteristics of PET machines, PET is still impaired by its low spatial resolution compared with other imaging modalities such as MRI or computed
tomography (CT). The limited spatial resolution induces partial volume effect (PVE) to the images, which largely biases the quantitative measurement of regional radioactivity (Soret et al. 2007; Prieto et al. 2006). Such bias could result in incorrect estimation of local tracer concentration and eliminate the subtle change in metabolism or physiology as a function of an event or time (Fazio and Perani 2000).

There are two distinct phenomena result in the partial volume effect in PET (Harri et al. 2007; Soret et al. 2007). First is the relatively low spatial resolution of PET so that an image voxel might consist of different structures and undermine the sub resolution heterogeneity. This tissue fraction effect can be attenuated by a higher-resolution PET system. However, PET imaging physics, the detector design and reconstruction impose a theoretical limitation of PET resolution. The second phenomenon is the blurring of PET scanner point spread function (PSF). The response of a scanner to a radioactivity point source shows a bell shape instead of a perfect impulse function. This point spreading effect causes radioactivity spillover between image pixels, so that the post-reconstructed radioactivity at a pixel is not only from the source at the position but also from its neighboring. Mathematically, PVE is modeled as a convolution of true radioactivity with the PSF, so it can be corrected principally by a deconvolution procedure if the PSF has been characterized. Deconvolution is an ill-pose problem and often leads to noise amplification and severe ringing artifacts. Therefore, prior information about the imaging objects and noise characteristics are usually required to regularize the process.

To date, a number of methods have been proposed for PET partial volume effect correction (PVC). Broadly, the correction can be categorized into two classes: correction during PET reconstruction from the sonogram of radioactivity events, and correction on
post-reconstruction PET images. Each type of these categories can be subdivided into voxels-based and region-of-interest (ROI)-based approach depending on the way to apply the prior information.

ROI-based post-reconstruction PVC attempts to recover true radioactivity in region level with the assumption of homogeneous radioactivity within each region. Therefore, the number of radioactivity value to be determined is equal to the number of defined ROIs. Usually, these ROIs are obtained from co-registered anatomical images. The development of image registration as well as the emergence of combined PET/CT and PET/MRI machines make possible to acquire aligned anatomical images for PET. ROIs can be delineated by segmenting the anatomical images into a number of non-overlapping compartments with distinct and uniform radioactivity. In the geometric transfer matrix (GTM) method (Frouin et al. 2002; Rousset et al. 1998), a regional spread function of each region is computed by forward projection or PSF convolution of the region with a unit radioactivity. The geometric transfer matrixes transfer the unit activity in each region to the reconstructed image and form a number of equations to solve the region-based correction. The method was initially proposed for brain where images can be classified into gray matter, white matter and CSF structures, and was extended to arbitrary numbers of ROIs (Aston et al. 2002). By solving the regional radioactivity in a least square fitting framework, a general theory for the ROI-based method considering explicit noise model and error estimation was discussed in Aston et al. (2002). These methods require not only registration and segmentation of the anatomical images, but also the knowledge about regionally structural and functional correspondence in order to satisfy the assumption of regionally homogeneous radioactivity.
With the knowledge of object shape and assumption of a homogeneous radioactivity, model fitting based methods simultaneously recover size and activity of the object (Chen et al. 1998; Chen et al. 1999; Gutierrez et al. 2006; Wassenaar et al. 2004). These methods mathematically modeled the shape of the interesting object with a limited number of parameters, such as describing spheroid or ellipsoid-shape tumor with the location and axis lengths (Wassenaar et al. 2004; Chen et al. 1998; Chen et al. 1999; Chen et al. 1999), or cylinder blood vessel with the position and widths (Gutierrez et al. 2006). The methods also assume the object in a uniform background; therefore, the object and its radioactivity are determined by minimizing the square distance between PET and PSF-convoluted object in a local area. These methods can be understood as a type of deconvolution with greatly simplified object geometry and radioactivity distribution. These simplifications make difficult to apply the methods for whole body PET images consisting of objects with various shapes and activities. Although the methods do not require anatomic information, initial knowledge about the object location is necessary for the optimization to converge to the correct solution.

Other than providing corrected radioactivity for ROIs, voxel-based PVC restores activity in each voxel and yields images which enables better visual assessment and allows further image process. By segmenting brain MRI into CSF, WM and GM tissue types, Bataille et al. (2007) attempted to correct the brain regions of WM and GM under the assumption of zero radioactivity in CSF. The methods have been extended to a 3-compartment method to accommodate more heterogeneous distribution of the tracer (Muller-Gartner et al. 1992). Meltzer et al. (1996) added the method another tissue compartment in order to consider brain atrophy. These compartment-based methods
required tissue segmentation and assumed uniform distribution in each compartment except the one for correction, thereby generated partially corrected PET images.

A novel multiresolution method (Boussion et al. 2006) was proposed to utilize MRI anatomical images but eliminate image segmentation and the assumption of uniform radioactivity within a region. The method decomposed high-resolution MRI into different scale images using a discrete wavelet transformation. True radioactivity was restored by synthesizing the detail information of MRI into PET images. Stemming from the methodology, a structural-functional synergistic resolution recovery method (Shidahara et al. 2009) was proposed to use anatomical probabilistic atlases instead of CT or MRI for wavelet decomposition. These methods did not rely on MRI segmentation or PET points spread function, but assumed the positive relationship of high frequency information between PET and MRI that might need more validation. Boussion et al. (2007) modified the method by replacing MRI with the deconvoluted PET and so eliminated the need for anatomical images.

Deconvolution based partial volume correction could generate voxel-based correction without the need of anatomical images. Several authors attempted the methodology with different forms of smoothing regularization to limit noise amplification. Tohka and Reilhac (2007; 2008) have evaluated several deconvolution methods (the iterative Richardson-Lucy, the reblurred Van Cittert, the linear Wiener and the reblurred Van Cittert with total variation regularization) for raclopride-PET correction. Their results showed more accurate radioactivity quantification than the uncorrected images, and the methods have almost similar performance with GTM method. But these methods were only evaluated on a Monte Carlo simulated PET database instead of clinic
data. Kirov et al. (2008) proposed an iterative deconvolution method using the one-step later regularization procedure. The method achieved recovery of radioactivity and contrast similar to the methods incorporating anatomical images.

We developed a MRI guided partial volume correction method but eliminated the segmentation of MR images (Wang and Fei 2009b). Our method corrected PET PVE by an iterative deconvolution with an edge-preserving smooth constraint defined between neighboring voxels. We hypothesized the true radioactivity image is not only the deconvolution version of the post-reconstruction PET but also has maximal structural similarity to aligned MRI. The method utilized edges information in MRI rather than tissue class information; therefore, it could yield partial volume corrected images for whole body PET scans.

5.2. MRI-guided PET partial volume correction

5.2.1. PET imaging model

Assume $i(r)$ is the post-reconstruction PET from imaging true spatial distribution of radioactivity $o(r)$ by a PET scanner with a 3D point spread function $h(r)$. $r$ is the 3D voxel coordinate. The image formation is assumed as a linear and space invariant process at the levels and distribution of radioactivity. Spatially independent Gaussian noise is considered for the present work. Under these conditions, PET imaging is a convolution process and is modeled as

$$i(r) = o(r) \otimes h(r) + n(r)$$  \hspace{1cm} (1)

Where $r$ is a 3-element vector $(x, y, z)^T$ representing a point in three dimensional space or a 2-element vector $(x, y)^T$ for two dimensional plane. $n(r)$ is Gaussian
distributed noise, and the operator $\otimes$ denotes convolution. Eq. (1) can be expressed in frequency domain as

$$I(k) = O(k)H(k) + N(k)$$  \hspace{1cm} (2)$$

Where capitalization denotes the Fourier transform of the variables, and $k$ is the conjugate spatial frequency. The convolution becomes a multiplication operation in frequency domain, so the computation can be greatly speeded up as a fast Fourier transform is available.

The goal of partial volume correction is to restore the true radioactivity $o(r)$ by deconvolution of the observed PET $i(r)$. It becomes a least square minimization denoting as

$$J^{\text{deconvolution}} = \sum_r (i(r) - o(r) \otimes h(r))^2$$  \hspace{1cm} (3)$$

Solving $o(r)$ by minimization of $J$ usually leads to noise amplification and severe ring artifacts. In order to find a unique and stable solution for $J$, prior knowledge about $o(r)$ is required to regularize the minimization.

5.2.2. Bayesian deconvolution framework

Viewing PET as a probabilistic mapping of the radioactivity to an intensity count sampled at each voxel of the image, partial volume correction can be modeled as maximization of a posteriori probability to obtain the true radioactivity $o$ providing the observed image $i$, PSF and prior information about $o$. According to Bayesian’ theorem, the posteriori probability can be expressed as
\[ p(o|i) = \frac{p(i|o)p(o)}{p(i)} \]  \hspace{1cm} (4)

\( p(o|i) \) is the posteriori probability of \( o \) accompanied with an observation \( i \). \( p(i) \) is the probability of observing PET \( i \) and is a constant here. \( p(o) \) is the prior information about true PET activity. \( p(i|o) \) is the posterior probability density of observing image \( i \) given true radioactivity \( o \). Based on the assumption of spatially independent Gaussian noise model, \( p(i|o) \) is denoted as

\[ p(i|o) = \exp\left(-\frac{1}{2\sigma_n^2} \sum_r (i(r) - o(r) \otimes h(r))^2 \right) \]  \hspace{1cm} (5)

\( \sigma_n^2 \) is the Gaussian noise variance which could be estimated from background or tissue regions with uniform radioactivity distribution and is assumed to be spatial independent.

5.2.3. Prior probability from MRI anatomical information

The prior information of the true radioactivity \( p(o) \) is important for partial volume correction. We modeled the true radioactivity as a Markov Random Field (MRF) and described the prior information as intensity interaction between voxels in order to account for the regularization of local smoothness. The prior information is described by Gibbs formulation as

\[ p(o) = \frac{1}{G} \exp\left(-\lambda \sum_{c \in C} U_c(o) \right) \]  \hspace{1cm} (6)

where \( U_c(o) \) is Gibbs potential defined on each possible set \( c \) of voxels, \( G \) is a normalizing factor. Cliques \( C \) determine the range of voxel interaction. In the proposed
method only the interaction between neighboring voxels is considered and the prior information is expressed as

\[
p(o) = \frac{1}{G} \exp(-\lambda \sum_{r \in N(r)} \sum_{k=N(r)} \frac{w_{rk}}{d_{rk}} (o(r) - o(k))^2)
\]

\(N(r)\) is the neighborhood around voxel \(r\) that is 26 voxels in 3D or 8 pixels in 2D. \(d_{rk}\) is the Euclidian distance between voxel \(r\) and \(k\). \(w_{rk}\) is a weighting coefficient. The prior information constrains the local smoothness between neighboring voxels by the square of intensity difference, and \(w_{rk}\) determines the weight to enforce this regularization. For example, \(w_{rk}=1\) will lead to equalize the intensity between two neighboring voxels and \(w_{rk}=0\) will release any intensity constraint.

We manipulated \(w_{rk}\) to generate an appropriate prior regularization for PET partial volume correction. In PET, we can assume the true radioactivity is smooth at two voxels once their intensity difference less than a small threshold \(\mu\) but discontinuous when the two voxels having intensity difference higher than a threshold \(\nu\). This prior is similar to edge preserving deconvolution (Mugnier et al. 2004). However, PET is contaminated by significant partial volume effect which blurs image edges; thereby it is difficult to identify tissue boundaries just through PET thresholds. Fortunately, aligned MRI provides edge information for PET with much higher contrast and resolution. True radioactivity edges will appear at the edges of MRI because a region belonging to one tissue class in MRI should have a same tracer activity in PET. Therefore, the smoothness regularization need be removed when a MRI edge presents. However, the regularization ought to be kept when PET signal shows smoothness via the small radioactivity threshold.
\( \mu \) although MRI expresses an intensity discontinuity. Based on these observations, \( w_{rk} \) is defined as

\[
w_{rk} = \exp\left(-\frac{(\Delta o_{rk})^2}{\mu}\right) + [1 - \exp\left(-\frac{(\Delta o_{rk})^2}{\mu}\right)]\exp\left(-\frac{(\Delta o_{rk})^2}{\nu}\right)\exp\left(-\frac{(\Delta l_{rk})^2}{\kappa}\right)
\]

\[\approx \begin{cases} 1 & |\Delta o_{rk}| < \mu \\ 0 & |\Delta o_{rk}| > \nu \\
1 & \mu < |\Delta o_{rk}| < \nu \text{ and } |\Delta l_{rk}| < \kappa \\ 0 & \mu < |\Delta o_{rk}| < \mu \text{ and } |\Delta l_{rk}| > \kappa
\end{cases}\]  

(8)

Where \( \Delta o_{rk} = o_r - o_k \) and \( \Delta l_{rk} = MRI_r - MRI_k \). \( \mu \) and \( \nu \) are radioactivity thresholds with \( \mu < \nu \). \( \kappa \) is a threshold to determine an edge present in MRI, by which an edge is considered to appear if the intensity difference between the neighboring voxels is greater than \( \kappa \). If \( |\Delta o_{rk}| \) is smaller than \( \mu \) which means there is no edge between voxel \( r \) and \( k \), \( w_{rk} \approx 1 \) will switch on the constraint for intensity smoothness. If \( |\Delta o_{rk}| \) is greater than \( \nu \) denoting there is an edge between \( r \) and \( k \), \( w_{rk} \approx 0 \) switches off the intensity constraint.

For neighboring voxels having radioactivity difference between \( \mu \) and \( \nu \), MRI edge information is employed to ensure that there is an edge in the corrected image \( (w_{rk} \approx 0) \) if MRI presents an edge by \( |\Delta l_{rk}| > \kappa \).

5.2.4. PVC with MRI-guided information

The true radioactivity is restored by maximizing a posteriori probability as below

\[
o = \arg \max_o p(o \mid i) = \arg \max_o [p(i \mid o) p(o)]
\]

(9)

With the definition of \( p(i \mid o) \) in (5) and \( p(o) \) in (8), the maximization becomes minimization of the following cost function
\[ o = \arg \min_o J(o) = \arg \min_o \left[ \sum_r (i(r) - o(r) \otimes h(r))^2 + \lambda \sum_{r, k \in N(r)} \frac{w_{rk}}{d_{rk}} (o(r) - o(k))^2 \right] \quad (10) \]

\( \lambda \) is a constant parameter to balance the convolution and the MRF smoothness constraint, which is chosen heuristically in this work but could be simultaneously estimated based on image noise distribution.

The correction \( (o) \) is sought by minimization of the cost function \( J \) using a conjugate gradient (CG) method. CG minimization requires analytical derivatives of the cost function with respect to the true radioactivity in each voxel, which is given as

\[ \frac{\partial J}{\partial o(r)} = 2(o(r) \otimes h(r) - i(r)) \bullet h(r) + 4\lambda \sum_{k \in N(r)} \frac{w_{rk}}{d_{rk}} (o(r) - o(k)) \quad (11) \]

Where \( \bullet \) denotes a correlation computation. Because PSF \( (h) \) is a symmetric Gaussian function, the correlation is equivalent to convolution operation. The convolution is computed in Fourier domain described in (2) to achieve a better computation speed.

A new conjugate gradient method with guaranteed descent is applied for minimization (Hager and Zhang 2006). The conjugate gradient scheme guarantees descent by satisfying a new descent condition and avoids jamming happened in Powell method. The minimization is performed by iterating update of the conjugate direction and the solution that is denoted as \( o^{m+1} = o^m + \alpha d^m \). \( m \) is the iteration number, \( d^m \) is the conjugate direction, and \( \alpha \) is a positive step size which is determined by a line search. We derive it according to \( \frac{dJ(o^m + \alpha d^m)}{d\alpha} = 0 \) and have the following updating formulation in iteration \( m \)
The CG method achieves a better performance than common conjugate gradient methods. We also restart the CG direction to the steepest descent if the CG cannot significantly improve the minimization or reach iteration number \((N=50)\). Post-reconstruction PET image is used as the initial for the minimization which is ended if no further improvement between two consecutive direction restarting.

5.2.5. PET point spread function and images preprocessing

It is widely accepted that PET PSF can be approximated as an anisotropic 3 dimensional Gaussian function that is written as

\[
h(r) = \frac{1}{(2\pi)^{1/2}\sigma_x\sigma_y\sigma_z} \exp\left(-\frac{1}{2} \left[ \frac{x^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2} + \frac{z^2}{\sigma_z^2} \right] \right)
\]

\(\sigma = [\sigma_x, \sigma_y, \sigma_z]\) is standard deviation in three directions. The point spread function can be measured by fitting the Gaussian function to PET image of a point radioactivity source. Full width at half maximum (FWHM) of different PET machines have been reported and the standard deviation can be related to FWHM as \(\sigma = \frac{\text{FWHM}}{2\sqrt{2\ln 2}}\).

In comparison with PET, we consider PSF of MR imaging is an impulse response. But MRI could be degraded by noise and tissue fraction partial volume effect (Salvado and Wilson 2006) that could affect MRI edge extraction for PET PVC. Therefore, MRI preprocessing is important for accurate PET correction. The segmented or classified
MRI presents the best boundary information for PET PVC, and MRI intensity-based classification might be enough for the purpose (Wang and Fei 2009a). A variety of image processing methods can be used to improve MRI quality. We use an edge-preserving diffusion filtering to attenuate image noise but maintain the edges in MRI (Perona and Malik 1990). The diffusion filtering smoothes region with a gradient lower than a threshold but preserves edges with gradients higher than the threshold. \( \kappa \) in (8) is used as the gradient threshold for MRI diffusion filtering.

### 5.2.6. The MRI-guided PET PVC algorithm

Based on the Bayesian deconvolution framework, PET PVC is implemented by minimization of the cost function. The cost function \( J \) consists of a square distance between PET and convoluted true activity as deconvolution and a weighted square neighboring intensity difference for local smoothness regularization. Our correction starts with MRI and PET images acquisition. Point spread function of the PET scanner also need be determined, and typically is approximated as an anisotropic Gaussian function which can be derived by fitting the Gaussian function to a post-reconstruction PET of a point radioactivity source. The next step is to register MRI to PET using a rigid image registration algorithm (Maes et al. 1997) if only rigid motion presents. For better edge extraction, MR images can be processed using a diffusion filtering or other edge enhancement methods. We interpolated PET to the MRI resolution using a sync interpolation in our experiments, and then defined the edge thresholds for both images and the weight (\( \lambda \)). The final correction is implemented by CG minimization of the cost function at (10) initialized with PET. The minimization is ended when there is no further improvement between consecutive restarting of searching direction or the maximal
iteration number was reached (n=500). Usually, a median filter is applied to the result in order to remove possible isolated voxels with very high magnitudes (Mumcuoglu et al. 1994).

5.3. PVC validation experiments

5.3.1. Synthesized image experiments

The algorithm was first validated using a synthetic dataset. The synthetic dataset started with a given true radiotracer distribution on a 256 × 256 image which consisted of 4 row of discs with different size and contrast ratio in a background intensity of 50. Each row had five horizontal discs of diameters of 30, 20, 16, 10 and 8 pixels from left to right, and each column had same size discs with decreasing intensity of 65, 60, 45, and 30 from top to bottom which were intensity contrasts of 30%, 20%, 10%, and 40% with respect to the background. A MR image was synthesized with the same size and discs distribution with the synthesized true tracer image, but the intensity given to a disc was different from each other in order to characterize different tissue types. The background of MR image was given an intensity of 5. The true radioactivity image was added with Gaussian white noise of different level (1%, 5%, 8%, 10%, and 12%) relative to the background. The noisy PET was convoluted with a Gaussian function having FWHM of 10 pixels in x and y axis. The convolution induced signal contamination between neighboring regions, and accordingly simulated the point spread function blurring of the true radioactivity. In order to account for tissue fraction effect, the convoluted PET image was down sampled by extracting a point every 2 pixels along each axis. The synthesized data was applied to evaluate the correction method to PET images with variable statistical quality and
resolutions. The PSF and image size were given with a unit of pixel which can be easily mapped to typical MRI and PET resolutions.

The restored intensity was compared with the true PET in lines traversing the centers of each disc in a row. The mean intensity in the discs was calculated before and after the correction in each one of the discs and then quantitatively compared with the given true tracer intensity. The comparison was performed by recovery ratio which was defined as percentage of \( o/I_{true} \) for correction and \( I/I_{true} \) for original PET. Therefore, the ratio before correction was greater than 100% if the object was in a hot background (object radioactivity is less than the background) and less than 100% if the object was in a cold background. Recovery error was calculated as absolute value of the difference between the recovery ratios to one, which characterized the percentage of error to represent the true signal. Our experiments demonstrated the quality of partial volume recovery of objects with different size (3.0, 2.0, 1.6, and 1.0, 0.8 times of FWHM) and different contrasts (10%, 20%, 30%, and 40%). We also performed PVC using the geometrical transformation matrix (GTM) method on the synthesized data with ROIs defined from the MRI. GTM method is considered as the reference numerical approach for partial volume correction in PET since we used the ideal ROI segmentation.

We also used the synthesized image to assess the proposed method in the cases where image content of MRI and PET were no longer correlated. For examples, two distinct structures in MRI have same radioactivity intensity or one region in MRI has an inhomogeneous radiotracer distribution in PET. We removed the discs in the middle row with an intensity of 45 in the true radioactivity image and generated a PET image for correction. MR image was unchanged, so different structures in MRI at the middle row
had a same true radioactivity. In the other case, without altering the PET image, the first row of discs in MRI was removed and set as the background intensity. Another configuration was present in which a sub region in the first row of MRI had structure information. The data was used to demonstrate whether the method can recover local regions in PET without affecting other areas that had clearly structure information available. This could be happened for tumor metabolic quantification from a whole body scanning where global anatomical information might not be salient in the aligned MRI but with identifiable tumors for correction. For these cases, the threshold for PET was carefully selected in order to recognize the boundaries in PET.

Alignment of MRI and PET is essential for correction of the partial volume in PET using MRI anatomical information. The robustness of the proposed algorithm to small spatial misalignment was studied by introducing artificial displacement of the synthesized PET with regards to MRI. A set of PET images was generated by 1, 2, 3 pixels shift to left and 1, 2, 3 degree of rotations clockwise. 5% Gaussian noise was considered for all the PET images. The recovery error was calculated for each disc using the ROIs in MRI and tailed them with the size.

5.3.2. Phantom imaging experiments

A phantom was designed to evaluate the performance of the partial volume correction method. The phantom consisted of a 10 × 10 × 40 mm³ square and a 30 mm diameter sphere in a plastic box container. FDG concentration of 25 uCi/cc and 16 uCi/cc were added to the square and sphere respectively. The phantom was first imaged by a microPET scanner system (R4, Siemens Preclinical Solutions, Knoxville, TN) and
reconstructed by 16 subsets and 4 iteration OSEM method. The PET was reconstructed with a dimension of $128 \times 128 \times 63$ and a resolution of $0.845 \times 0.845 \times 1.215 \text{ mm}^3$. After PET imaging, the FDG solution was removed and 0.1% Gd-DTPA solution was given to the square and sphere respectively. A T1-weighted MRI image of the phantom was acquired using a 9.4T small animal scanner (Bruker BioSpin GmbH, Rheinstetten, Germany). The MRI had a dimension of $198 \times 218 \times 45$ and a voxel size of $0.2 \times 0.2 \times 0.5 \text{ mm}$. Rigid registration of MRI and PET were performed using Analyze 6.0 (AnalyzeDirect, Inc., Overland Park, KS). The microPET R4 machine has FWHMs of 1.85 mm at the axial direction and 1.66 mm at the radial direction within the field of view of $6.3 \times 6.3 \text{ cm}^2$ size (Knoess et al. 2003). The PSF of the scanner was created by a 3D Gaussian function with the given FWHMs. Since the central transverse section of the sphere was much greater than the FWHMs, the average radioactivity within the center region of diameter 1 cm was considered as the true radioactivity for the sphere. We selected different transverse sections from the sphere to get ROIs with varied sizes, and evaluated the method based on the recovery of true radioactivity at these ROIs with regard to the circular diameters.

5.3.3. Simulated brain FDG-PET experiments

We also evaluated the proposed method on a publicly available dataset of 16 Monte Carlo simulated dynamic brain FDG-PET volumes (Reilhac et al. 2005) that has been used for PVC validation (Tohka and Reilhac 2008). The dataset was simulated by the PET-SORTEO Monte Carlo simulated software (Reilhac et al. 2004) that models PET imaging with the Exact-ECAT HR+ scanner operating in 3D mode. The simulation account for the specific scanner and most of the phenomena appeared in radioactivity signal acquisition,
and also allows faithful reproduction of PET tomography image formation. The realistic anatomical models were derived from human brain MR images, which were partitioned into white matter (WM), gray matter (GM) and CSF according to the properties of FDG uptake. Constant activity of 8.45kBq/cc and 22.99kBq/cc were assigned to WM and GM respectively, activity of zero was given to CSF and the background. Imaging of the FDG distribution model using the specific scanner was simulated, and the data was reconstructed by the filtered back projection with 0.3mm\(^{-1}\) frequency cutoff hanning filter. The filter produced images with a low noise level but a poor resolution compared with other filters. The resulting PET volumes had a size of 128 × 128 × 63 with a resolution of 2.11 × 2.11 × 2.43 mm\(^3\), and were perfectly aligned with the original MR volumes. The dimension of MR volume was 256 × 256 × 63, and the voxel size was 1.0 × 1.0 × 1.0 mm\(^3\). PSF of the simulated scanner was also approximated with a 3D Gaussian function. The FWHM of the PSF was derived from imaging a point source located at 5 cm from the center of field of view of the scanner. For the simulated FDG-PET data, the FWHMs were 9.2 mm in axial and radial direction. The original MRI for anatomical model, the true FDG activity model and the simulated PET volumes provide a reliable dataset for quantitative evaluation of the PET PVC algorithm. One of 16 PET volumes was used to tune the parameters in the method, and the other 15 volumes were corrected using the proposed method. The results were evaluated on four regions of interests which were defined based on the MRI segmentation.
5.4. PET correction results

5.4.1. Synthesized images study

The method is first evaluated on a simulated data in Figure 5-1. Figure 5-1a is a synthesized 2D image of true tracer distribution. Circular objects with different size and tracer intensity are in a background with an intensity of 50. From left to right, objects in a row have diameters of 3, 2, 1.6, 1 and 0.8 times of PSF HWFM. From top to bottom, the objects in a column have contrasts of 30%, 20%, 10% and 40% relative to the background. Figure 5-1b is the simulated PET by convolving PSF with Figure 5-1a and adding 5% Gaussian noise. Figure 5-1c is the synthesized MR image where different intensity is assigned to each circular object so that edge information is present. Figure 5-1d is the resultant partial volume corrected PET of Figure 5-1b guided by the structural information in Figure 5-1c. The image contrast has much improved and looks close to the true. The quantitative comparison of PET restoration is shown is in Figure 5-2 which displays the signal profile through the center lines. Signal of original PET, true intensity and corrected intensity cross the centers of circles at the first row and the last row are displayed in top and bottom figures respectively. The matching of corrected signal with the true illustrates the algorithm restores the true activity from the original PET blurred from the partial volume effect.
Figure 5-1 Partial volume correction of a simulated 2D image. (a) is a synthesized 2D image of true tracer distribution. Circular objects with different size and tracer intensity are in a background with an intensity of 50. (b) is simulated PET by convolving PSF with (a) and added with 5% Gaussian noise. (c) is the synthesized MR image where different intensity is assigned to each circular object so that edge information is present. (d) is the resultant partial volume corrected PET from (b) guided by the structural information in (c).

The synthesized MR image delineates the ROI for each circular object which is used for quantification of the correction in region base. Figure 5-3 compares average intensity in each object without correction (PET), with correction using the proposed algorithm (Corrected) and using geometrical transformation method (GTM). There are 4 different intensity contrast objects in 4 row objects, and each subfigure in Figure 5-3 shows the comparison of objects in a row with respect to their diameters. Figure 5-3a is for the 4 objects in the first row of the simulated image and has a contrast of 20%, b is for the objects in the second row with a contrast of 30%. In Figure 5-3a and Figure 5-3b, the objects are in a cool background (background intensity is lower than the objects), so the
correction increases the intensity to match the true intensity. Figure 5-3c and Figure 5-3d are for objects in the third and last row respectively. The object intensities are lower than the background, and so the background spills intensity into the objects and the correction decreases objects intensity so as to restore the true radioactivity. Accurate classification is given for GTM correction method, so its result can be considered as the gold standard for partial volume correction. The proposed method achieves very similar results with GTM method but without MR classification.
Figure 5-2 Signal lines of profile for PVC evaluation. Signal of original PET, true intensity and corrected intensity cross the centers of circles at the first row and the last row are displayed in top and bottom figures respectively. The matching of corrected signal with the true illustrates the algorithm restores the true activity from the original PET blurred due to the partial volume effect.
Figure 5-3 Comparison of average intensity in objects from correction. The results are without correction (PET), with correction using the proposed algorithm (Corrected) and using geometrical transformation method (GTM). Each figure shows the comparison of average intensity at a contrast and with respect to object sizes. (a) is for the 4 objects in the first row of the simulated image and has a contrast of 20%, (b) is for the objects in the second row with a contrast of 30%. The objects are in a cool background (background intensity is lower than the objects), so the correction increases the intensity. (c) and (d) are for objects in the third and last row in the image. GTM is considered as the standard method for partial volume correction when accurate classification is obtained from MRI.

The robustness of the proposed method to PET image noise and mis-registration between MRI and PET are evaluated using intensity recovery error. Figure 5-4 compares intensity recovery error at the circular objects with different PET noise level between the original synthesized PET, corrected images using our method and the GTM method.
Noise level of 1%, 5%, 8%, 10% and 15% are added to PET images. Recovery error of objects with sizes of 3, 2, 1 times of FWHM are shown in Figure 5-4a, Figure 5-4b, Figure 5-4c respectively. The error bar is derived from the four objects with different contrasts but the same size, and only the proposed method has the error bar shown. The noise less than 15% leads to less than 5% recovery error for different size object using the proposed correction method. The mis-registration between MRI and PET significantly affect the MRI based PET partial volume correction. We test the influence of the mis-registration to the correction using the proposed method in Table 5-1. MRI provides the ROI delineation for the recovery error calculation at each object. PET image is shift (0, 1, 2 pixels) or rotate (1, 2, 3 degrees) and then corrected using the MRI-guided method. Average intensity within each circular objects delineated from MRI is calculated and compared with the true intensity. The results are separated and compared according to the object sizes (Table 5-1.I), and the standard deviation is also derived from the four contrast objects with the same size. The recovery error of PET is calculated from the original image using the same ROIs definition in MRI (Table 5-1.II). Significant intensity restoration has been achieved using the method even with 2 pixels translation or 3 degree rotation for objects with a size no less than FWHM of the PSF.
Figure 5-4 Intensity recovery error of the circular objects. The results are from original PET, correction using the proposed method and GTM method verse image noise level. Recovery error is the percentage of signal difference to the true intensity. Noise level of 1%, 5%, 8%, 10% and 15% are added to images for evaluating the robustness of the proposed method to noise. Recovery error of objects of size 3, 2, 1 times of FWHM are shown in a, b, c respectively. The error bar is calculated from the four objects with different contrasts but a same size. Only the result of the proposed method has the error bar shown.
Figure 5-5 shows the partial volume correction of PET guided with a MRI having partial tissue without correlation. In first case, Figure 5-5a is a synthesized MRI and b is the PET for correction. There are not objects in PET corresponding to the ones in the second row of MRI. The corrected image (Figure 5-5c) has not been affected by the extra objects in MRI. In the second case, the synthesized MRI (Figure 5-5d) has no objects corresponding to the circles in the first row of PET (Figure 5-5e), so no structural information is provided for correction of these objects. By selecting an appropriate threshold, the proposed correction can restore the objects in PET without MRI information. Figure 5-5f is the corrected result of Figure 5-5e. Different from Figure 5-5d, the synthesized MRI in Figure 5-5g recognizes a small object in the first row to give local anatomical information for PET in Figure 5-5h. The result of Figure 5-5i illustrates the algorithm can recover the object when a local structural information is given either by post processing or manual segmentation.

Table 5-1 Recovery error of PVC with respect to the mis-registration. MRI provides the ROI delineation for the recovery error calculation in each object. The results are separated and compared verse the object sizes, and the standard deviation is from the four contrasts objects with the same size.

<table>
<thead>
<tr>
<th>Corrected Recovery Error (%)</th>
<th>Object size( × FWHM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>X shift(pixel)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.0 ± 3.8</td>
</tr>
<tr>
<td>2</td>
<td>7.3 ± 5.7</td>
</tr>
<tr>
<td>3</td>
<td>5.8 ± 5.0</td>
</tr>
<tr>
<td>rotate(degree)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.2 ± 2.1</td>
</tr>
<tr>
<td>2</td>
<td>6.9 ± 6.3</td>
</tr>
<tr>
<td>3</td>
<td>11.8 ± 10.5</td>
</tr>
</tbody>
</table>
Figure 5-5 PET PVC guided with no correlation of partial tissues in MRI. (a) is a synthesized MRI and (b) is the PET for correction. There are no objects in PET corresponding to the ones in the second row of MRI. The corrected image (c) has not been affected by the extra objects in MRI. A synthesized MRI (d) has no objects corresponding to the circles in the first row of PET (e), so no structural information is provided for correction these objects. By selecting a correct threshold, the proposed correction can restore the objects in PET without MRI information; (f) is the corrected PET. Different from (d), the synthesized MRI in (g) recognizes a small object in the first row to give local anatomical information for PET in (h). The result in (i) illustrates the algorithm can recover the object when a local structural information is given.

5.4.2. Phantom study

Figure 5-6 is the PET PVC of a phantom consisting of a square and a sphere with FDG tracer filled for PET and contrast agent filled for MRI. Two slices of the phantoms in MRI and PET are shown in the two rows. From the left to right, the figures in a row are a MRI slice, the aligned PET image, and the corrected result using our method respectively.
The image quality improvement from (c, f) to (b, e) are due to the proposed partial volume correction.

Figure 5-6 Partial volume correction of a PET phantom. The phantom is consisted of a square and a sphere with FDG tracer filled for PET and contrast agent filled for MRI. Two slices of the phantoms in MRI and PET are shown in the two rows. (a) is a MRI slice and (b) is the corresponding PET slice, (c) is the corrected result using the proposed method. (d) is another MRI slice and (e) (f) is the PET before and after the partial volume correction. The image quantity improvement from (c, f) to (b, e) are result from the proposed partial volume correction.

The radioactivity restoration of the sphere is plotted in Figure 5-7 verse different object size. The objects are selected from different slices of the sphere in the phantom MR image. They are circular shapes with different diameters in order to demonstrate the partial volume correction with respect to objects sizes. Average activity of the selected region before and after the correction are shown and compared with the true intensity. The significant improvement of FDG quantification demonstrates the effectiveness of PET PVC.
Figure 5-7 Radioactivity restoration for the objects in phantom experiment. The true activity is measured from the mean PET intensity within the center sphere area with a radius of 1 cm which was not affected by partial volume effect. The objects are selected from different slices of the sphere object in the phantom MR image with different diameters. Mean activity of the selected region before and after the correction were shown and compared with the true intensity.

5.4.3. FDG-PET brain data study

The correction of simulated human brain FDG-PET database is shown in Figure 5-8 where each row is a result for a slice in a 3D brain volume. The first column is the real MR images, and the second column is the aligned PET images interpolated to MRI resolution. Corrected results of the PET images are in the third column, which compares with true FDG distribution in the fourth column to indicate the accuracy of the correction. Four slices are displayed along the row, and each slice has a ROI defined on it which is delineated by the red lines. From top to bottom, they are objects of WM, GM, CSF and right thalamus. These objects are used for the following region-based evaluation of radioactivity recovery from the PVC.
Figure 5-8 Partial volume correction of simulated human brain FDG-PET. Each row shows the result for a slice in the 3D volume dataset. The first column is the real MR images, and the second column is the aligned PET images interpolated to MRI resolution. The third column is the correction results of PET images in the second column. The true FDG distribution in the images is displayed in the fourth column to indicate the accuracy of the proposed correction method. Four slices are selected, and each slice has a ROI defined on it which is delineated by the red line. From top to bottom, they are objects of WM, GM, CSF, and Right thalamus.

The weight of smoothness $\lambda$ balances deconvolution and image smoothness constraint. One MRI and PET is used to determine appropriate $\lambda$ that has optimal signal recovery for the dataset. The average activity within the four ROIs (white matter, gray matter, CSF and right thalamus) are calculated on the true, the original PET, and
corrected results with different smoothness weight. By considering the radioactivity restoration for all the objects in Table 5-2, $\lambda = 5$ is selected for our experiments in which the other 15 brain FDG-PET dataset are corrected using the method.

Table 5-2 Correction of simulated brain PET verse weight of smoothness $\lambda$. One MRI and PET data set is selected. The average activity within four ROIs (white matter, gray matter, CSF and right thalamus) are calculated on the true, the original PET, and corrected results with different smoothness weight. By considering the radioactivity restoration for all the five objects, $\lambda=1$ is selected for our experiments.

<table>
<thead>
<tr>
<th>ROI</th>
<th>TRUE</th>
<th>PET</th>
<th>Correction with $\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>WM</td>
<td>228</td>
<td>266</td>
<td>238</td>
</tr>
<tr>
<td>GM</td>
<td>616</td>
<td>442</td>
<td>549</td>
</tr>
<tr>
<td>CSF</td>
<td>0</td>
<td>190</td>
<td>45</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>621</td>
<td>537</td>
<td>604</td>
</tr>
</tbody>
</table>

16 FDG PET brain volumes are corrected and the average FDG radioactivity within the four ROIs on the true, original PET and corrected results are shown in Figure 5-9. The optimized $\lambda$ is used for the correction. The true activity is 0 for CSF, 228 uCi/cc for WM, 621 uCi/cc for GM, and 621 uCi/cc for right thalamus. The plot shows the regional radioactivity has been improved from $186 \pm 16$ to $30 \pm 7$ uCi/cc for CSF, $317 \pm 15$ to $236 \pm 10$ uCi/cc for WM, $438 \pm 4$ to $592 \pm 5$ uCi/cc for GM due to the partial volume correction.
Figure 5-9 FDG radioactivity within four ROIs from PVC. The optimized $\lambda$ is used for the proposed partial volume correction. The results are obtained from experiments on the 16 simulated FDG PET-MRI volumes. The true activity is 0 for CSF, 228 for WM, 621 for GM, 621 for right thalamus. The plot shows radioactivity in CSF has been improved from 186 ± 16 to 30 ± 7. The improvement for WM is 317 ± 15 to 236 ± 10, and 438 ± 4 to 592 ± 5 for GM result from the correction.

5.5. Discussion and Conclusion

The aim of the work is to develop a MRI-based methodology to efficiently correct partial volume effect at post-reconstructed PET images. It not only restores the true trace activity in region of interest, but also provides a PET image with attenuated partial volume effect. Automatic and reliable alignment of MRI and PET has been enabled through the rapid development of algorithms for multimodality image registration. Recently, anatomical images for PET data can be ever more easily acquired and directly superposed since the combined scanners such as PET/CT, SPECT/CT and PET/MRI come forth. Therefore, MR based methods become more natural choices for PET partial volume correction. However, most PVC methods focus on brain PET and employ tissue type information via
MR image segmentation or classification. These methods suffer from the accuracy of image segmentation and are difficult for other applications such as tumor metabolism quantification and whole body radioactivity studies because of the classification of MRI.

In this chapter, we propose a method which incorporates edge information in MRI to guide PET partial volume correction but without MRI segmentation. The method provides a corrected image by deconvolution of a reconstructed PET image with an edge-preserving smoothness constraint. It assumes true PET edge will present at the same position of MRI edge because MRI and PET are aligned. The assumption has been adopted in most region-based partial volume correction methods (Frouin et al. 2001; Frouin et al. 2002). However, these methods need prior information of PET tracer distribution in order to correctly combine or separate anatomical structures into regions having distinct tracer concentrations. The proposed method avoids the problem by a design of the edge-preserving constraint. The constraint removes edges present in MRI but not on PET using a small PET threshold and renders the edges present in PET but not on MRI by a much high PET threshold. Our experiments of the simulated images demonstrate the ability of the method to recover true tracer distribution from MR images. The simulation study shows the proposed method has similar performance with the conventional GTM method. The phantom experiments and FDG brain PET corrections show the improvement of radioactive tracer quantification due to the correction.

The correction tries to compensate both the system point spread function and the tissue fraction effect. MRI usually provides much higher resolution images than PET, so it can be reasonably assumed that the PSF is about equal or smaller than the size of a voxel and each voxel is a single tissue type compared with PET. Typically, MRI is
interpolated to PET resolution for correction which causes edge smoothness from tissue fraction effect. But Interpolation of PET to MRI resolution incurs inaccurate estimation of radioactivity from the interpolation. No significant quantification difference happens according to our experiments of different combination of interpolation between MRI and PET. A deconvolution-based method need full knowledge of the point spread function that describes the probability to detect an event occurrence within an image space when a point source presents. PSF usually depends on the position of the point source, but the variations across the field of view are somewhat small likely owing to the compton scatter effect. A result has shown very small change of PSF within a region centered at the field of view (Meltzer et al. 1999). In the current experiments, objects are located at the central region of PET FOV and we consider a constant PSF within the dimensions. Our experiments of the simulated dataset show we could perform partial volume correction in local regions within significant effect to other areas. Therefore, we might divide the whole space into small segments, and each segment has a constant PSF but the whole-image PSF is space-variant. Through correction in each segment with the local PSF, we might account for the effect of space-variant PSF. The other benefit of the local correction capability is to correct given regions surrounding tumors so as to improve tumor metabolic quantification as well as computation speed.

Several parameters need be determined before performing the correction. A threshold for MRI gradient is used to separate edge points from homogeneous MR regions. Good delineation of MRI edges will lead to better edge recognition and enhanced smoothness within regions. Various image enhancement methods can be used to improve MR images and edges delineation. We use a diffusion filtering to preprocess
MRI because diffusion filtering smoothes noise but preserves the edges. Image segmentation techniques can also be employed to intensify the edges in MRI. Binary parametric image of MR edges might generate best boundary restoration of radiotracer activity. However, because of possible MRI/PET mis-registration and MRI tissue fraction effect, a Gaussian smoothness of the binary edge might augment the robustness of the correction. Another two thresholds for PET gradient are also needed. A very small one is used to identify homogenous neighboring pixels and a greater one is to determine obvious discontinuous pixels from PET. Noise level positively affects the selection of the small threshold; thereby, PET images might be filtered before correction. The other important parameter is the weight of $\lambda$ that balances the ratio of smoothness constraint with deconvolution. The constraint regularizes regional homogeneity which is disturbed from noise amplification in deconvolution. So a bigger parameter is required for higher noise level. The threshold might be analytically derived with some assumption about image distribution in the future (Mugnier et al. 2004).

The restoration method is constructed in a Bayesian framework with MRF model for the prior information regularization. The cost function could equivalently derive from penalized maximum likelihood or deterministic least square approaches (Villain et al. 2003). We select two-element neighboring pixels for Gibbs formulation although more complicate pixels interactions might improve the result. The model ensures the convexity of the criterion and a unique minimum solution. The minimization is performed by a conjugate gradient method whose convergence to a stationary point is guaranteed because of the quadratic criterion function. The analytic solution for the step of line search also greatly speeds the minimization. Typically, 50 iterations are enough for correction of a
2D image with a size of 256 × 256. The solution is bounded between zero and a maximal uptake. The nonnegative constraint is implemented in iteration by a projective strategy (Nakamura et al. 1988). But a few isolated pixels of very high intensity might emerge because an intensity difference with very large magnitude incurs the same cost as smaller intensity difference (Mumcuoglu et al. 1994). Therefore, a median filer is applied finally to remove these isolated points.

Our study has shown the potential of using PET with $^{11}$C labeled choline as imaging marker for detecting prostate tumor response to Pc 4 PDT (Fei et al. 2008; Fei et al. 2009). The tumor images were with significant boundary blurring. The partial volume correction method could be employed to improve quantification of the tumor choline uptake. After PET imaging, an immediate MRI of the treated mouse could be acquired without significant mouse body motion since the mouse is maintained anesthetic. A rigid body registration brings alignment of MRI and PET. MRI presents tumors with high resolution, and thus provides anatomical boundary for PET correction. With the guidance of MRI, the method could be performed within a local region surrounding the tumor as long as only choline uptake in tumors is interested. The correction might offer more accurate measurement of tumor metabolism and better characterization of tumor response to Pc 4-PDT.
Chapter 6. Conclusion and Future Work

The study investigated treatment of prostate cancer using a novel therapy called photodynamic therapy with a photosensitizing drug, phthalocyanine Pc 4 in animal models. The potential of Pc 4-PDT of prostate tumors has been demonstrated on two types of human prostate tumor models (PC-3 and CWR22) at mice. The dissertation presents an imaging marker for early therapeutic efficacy quantification, and develops a number of image analysis techniques to monitor tumor response to the therapy. This chapter summarized the work in the study and discussed some future work.

6.1. Conclusion

6.1.1. DW-MRI for monitoring tumor response to Pc 4-PDT

Non-invasive monitoring of tumor response to a therapy allows immediate prognosis and consideration for adjuvant therapies at early stage in the cycle of cancer treatment. Longitudinal tracking of tumor change is also an essential approach to understand and optimize therapies in preclinical trials. MRI in vivo renders tissue morphological and functional information, and thus allows assessment of tumor response by quantifying the induced tissue properties change (Chenevert et al. 2002). Our imaging study examined DW-MRI and ADC for assessing early response of the prostate tumor xenograft CWR22 to Pc 4-PDT. Subcutaneous prostate tumor xenograft CWR22 was initiated on athymic mice and leaded to the increasing of serum PSA level. A high-field 9.4 T small animal MR scanner was used to acquire diffusion weighted MRI before, immediately after and 24 hours after the treatment. The experiments found that significant ADC increasing
within the treated tumors was observed 24 hours after PDT, and the change was synchronized with the tumor size decreasing and serum PSA level falling in 7 days. Therapy-induced cell damage was presented in the tumor histological images, which possibly led to the ADC change 24 h after PDT. The result indicates ADC parameter is sensitive to CWR22 tumor response to Pc 4-PDT at an early time, and suggests that tracking diffusion weighted MRI and $ADC$ might provide a useful tool to monitor the tumor response and to determine the therapy effectiveness.

6.1.2. *Multiscale classification of MRI for tumor quantification*

A multiscale fuzzy c-means classification method was developed to classify tumor MR images into viable and necrotic regions. Thus the treatment effect can be directly quantified by measuring the necrosis volume. We used a diffusion filtering to process MR images and constructed multiscale image series. The multiscale fuzzy c-means classification method was applied along the scales from the coarse to the finest. The objective function of the conventional fuzzy c-means method was modified to allow that the result from a coarse scale supervises the classification in the next fine scale. Accurate classification might be obtained step-by-step and without tapping into a local minima. The MsFCM method performed better than the conventional FCM or MFCM methods according to the experiments with simulated and synthesized datasets. The result on real MR images also demonstrated the effectiveness of the proposed method. The method was applied to classify tumor DW-MRI and $ADC$ 24 hours after PDT into necrotic and viable regions; thereby, offered quantitative measurement of tumor response in terms of necrotic volume and ratio to the whole tumor. The classification of DW-MRI and $ADC$ could be valuable in order to detect pathology or follow disease response to a therapy.
6.1.3. Nonrigid surface registration for whole-body animal images

In order to follow tumor response to a therapy, mouse images were acquired in a number of time points relative to the treatment. Nonrigid deformation of the mouse between the images at different times prevents longitudinal comparison of tumor anatomy or functionality in voxel level. A nonrigid image registration scheme was proposed to align the whole-body mouse images. The scheme first corrected the whole-body skeleton deformation using a B-spline based surface registration method, and then refined the volume alignment with intensity-based algorithms. The surface registration was implemented by aligning densely sampled surface points without landmarks portraying or features extraction. The registration integrated a motion model consisting of a global transformation and a B-spline local deformation with robust point matching framework (Chui and Ranagarajan 2000). The algorithm solved the point correspondence and transformation simultaneously by the fuzzy correspondence and deterministic annealing techniques. The B-spline function not only effectively modeled the complex body deformation, but also greatly decreased the degree of freedom for optimization, thereby achieved better computation efficacy for surfaces with a great number of points. Modification of the matching cost function allowed prior information about translation, rotation and scaling constraining the local deformation in the optimization. The performance was demonstrated and validated by registration experiments of synthesized 2D curves and 3D surfaces. We also applied the method to align anatomical surfaces from mice tumor MR images to human brain MR volumes, which indicated the applicability of the nonrigid surface matching for various biomedical applications.
6.1.4. MRI-guided PVC for PET quantification

PET with $^{11}$C choline radiotracer has shown the potential to detect tumor metabolic change resulting from Pc 4-PDT (Fei et al. 2008; Fei et al. 2009). However, low spatial resolution of PET induces partial volume effect which blurs the images and might bias the measurement of regional radioactivity. A voxel-based PET partial volume correction method was proposed which employed high-resolution MRI anatomical information to constrain PET deconvolution for radioactivity restoration (Wang and Fei 2009b). The correction was pursued by attenuating the blurring of PET scanner's point spread function and preserving edges present in both PET and aligned MRI. The edge preserving was described by the hidden markov random field modeling voxel interaction. The cost function was derived from the Baysian’s deconvolution framework and was minimized by a novel conjugate gradient method. The algorithm was evaluated on simulated data and phantom images acquired from a microPET and a 9.4 T MR scanner. The results showed that the method effectively restored the true PET activity of objects with a size of greater than the full-width at half maximum of the point spread function without MRI classification or prior knowledge about tracer distribution in tissue. The method not only enabled improved activity measurement in region of interests, but also yielded a corrected image. It might offer a partial volume correction method for various PET applications.

6.2. Future works

Alternative or adjunctive therapies for primary or recurrent prostate cancers are of great medical and economical significance. Our study has shown Pc 4-PDT may be an option
for prostate cancer treatment. We have identified a MR imaging marker for monitoring early therapeutic effect of Pc 4-PDT and developed several image processing methods for tumor response quantification. The future work will include seeking other imaging markers for earlier tumor response to the treatment, integrating the imaging techniques and image analysis methods to understand the short and long term therapeutic mechanism of the therapy, and exploring applications of the proposed image processing techniques for medical image analysis.

6.2.1. MRI parameters for monitoring tumor treatment

DW-MRI images water molecules diffusion which is associated with tissue cellular integrity and pathology conditions. Pc 4-PDT induced ADC change has been examined to detect tumor response in current work. In the future, Pc 4-PDT of prostate tumor cell spheroids will be performed as in vitro validation experiments. The spheroids will be preincubated with Pc 4 and washed to remove unbounded photosensitizers before laser irradiation. DW-MRI of the spheroids before and after the treatment will validate tumor cells damage from Pc 4-PDT and the correlation with water diffusivity change. Cell necrosis or apoptosis after the treatment can be examined using fluorescent techniques (Plaks et al. 2004). The experiments will intuitively demonstrate the relationship between the therapeutic outcome and ADC change. It might also reveal an earlier time point for tumors to have detectable MR parameters change resulting from Pc 4-PDT.

Tumor treatment might depend on initial tumor stages, for example, necrotic and aggressively growing tumor cells have different response to a treatment. As we imaged the treatment at a number of time points, the time-response curve can be generated to
characterize the dynamic processing. Thereby, the $ADC$ change with respect to initial tumor $ADC$ and long-term therapeutic outcome might be characterized by the curve. This investigation will offer clues to estimate final therapeutic results based on initial and current $ADC$ change obtained at early times.

Our DW-MRI assumed isotropic water molecules diffusion and imaged the diffusivity in one direction. However, depending on tumor cells arrangement and migration, water diffusion might be maximal in a specific direction. Diffusion tensor imaging (DTI) detects diffusion in several directions and provides a vector to describe intensity and direction of water molecule diffusion (Assaf and Pasternak 2008). DTI has been used to characterize the anisotropic diffusion in brain function imaging (Assaf and Pasternak 2008). A future work will be assessing the feasibility of diffusion tensor imaging of prostate tumor and evaluating the derived apparent diffusion coefficients and fractional anisotropy as parameters for treatment monitoring. The correlation between the change of diffusion fractional anisotropy and tumor histology after Pc 4-PDT will be evaluated to determine whether DTI offers another view of prostate tumor response to the treatment.

6.2.2. MRS of treatment induced chemicals change

Magnetic resonance spectroscopy (MRS) images resonance frequency shift of a nucleus due to its electronically immediate vicinity in a chemical, and so offers characteristic information about the chemical structure (Jiang et al. 1991). MRS study has shown malignant prostate cancer exhibits significant decreasing of phosphocreatine and increasing of phosphomonoester compared to the healthy prostate tissue (Mueller-Lisse
and Scherr 2007). Accordingly, it can be hypothesized that tumor metabolism of chemicals might change in response to the treatment in an early time; and the change of chemicals’ concentration imaged by MRS might provide another window to monitor Pc 4-PDT of prostate cancer.

In the future, proton MRS could be evaluated to track the change of tumor metabolic compounds resulting from Pc 4-PDT; and the concentration change will be correlated with the treatment outcome. MRS images a cubic voxel defined in the interesting tumors. The voxel need be carefully sized and orientated to cover most of the tumor volume but not outside. The proton spectrum will be acquired by the point resolved spectroscopy sequence (PRESS) to quantify chemicals concentration in the voxel. Variable pulse power and optimized relaxation delays sequence will be used for water suppression.

As choline is consumed by cells for membrane formation, a tumor is supposed to have high accumulation of choline; conversely, any cellular necrosis or apoptosis will cause decreasing of choline concentration. Accordingly, choline concentration might be a biochemical measurement of treatment effect. The ratio of choline concentration to water might be sensible for evaluating choline change resulting from Pc 4-PDT. Effective PDT is expected to decrease the ratio because tumor necrosis will induce choline concentration decreasing and water content increasing. The water signal could be obtained from another PRESS scan without water-suppression but with the same imaging parameters. MRS study of prostate tumor to Pc 4-PDT will reveal tumor metabolic process, and provide biochemical information about the treatment mechanism.
6.2.3. PET PVC for tumor metabolic response quantification

A MRI-guided PET partial volume correction method was described in Chapter 5. The method has been evaluated by phantom data and simulated FDG brain dataset. The method will be applied to correct PET of prostate tumors treated by Pc 4-PDT. MR images need be acquired for each session of PET in order to provide anatomical guidance information. MRI can be performed immediately before or after PET with minimal mouse body motion by keeping the animal anesthetized. Then a rigid body registration can be performed to align MRI and PET (Fei et al. 2002; Fei et al. 2006). The other way to get aligned MRI and PET is to use the combined PET/MRI machine obtaining both modalities images simultaneously (Wehrl et al. 2009). For animal study of tumor response, the major interesting region is the tumor. Therefore, the correction can be applied on the local region surrounding the tumor, which will greatly save computation time.

Instead of using the reported PSF of PET scanners, experiments measuring PSF of a specific scanner might be necessary for accurate PET correction. The experiment can be performed by imaging a point radioactivity source using the scanner, and then fitting a 3D Gaussian function (i.e., the point spread function) to the reconstructed PET. The spatial variation of PSF might also be inspected by imaging the source at different positions. Localized PSF can be used in the optimization since the method can be performed locally.

The correction will be experimentally evaluated by the following two approaches. First, the imaged tumor will be dissected from the animal body right after PET and MR imaging, then its radioactivity will be measured immediately using a well counter.
Thereby, the correction can be evaluated by examining how much radioactivity in the tumor has been restored before and after the correction in comparison with the well counter measurement. The other approach for validation is to examine whether the treatment-induced change of radiotracer uptake in tumors has improved due to the correction. The improvement could be evaluated with respect to different treatment parameters and tissue functional knowledge.

6.2.4. Clinical applications of the image processing methods

The imaging techniques and image processing methods are developed for small animal imaging of prostate tumor treatment. Undoubtedly, the techniques might also provide useful tools for monitoring efficacy of other therapies and optimizing treatment planning for clinical applications.

Image classification offers high level information for image interpretation. Its applications include anatomical visualization, quantitative measurement and tissue characterization. By removing uninteresting structures and assigning tissue with different types, image classification can also improve three dimensional display of image dataset. Image classification provides measurement such as organ or pathological tissue volumes; therefore, allows quantify pathology or therapeutic outcome. The proposed method does not strictly assume Gaussian distribution of tissue intensity in one class, so it might also be used on histological images. The experiments on MR brain classification indicated the potential of the method in neuroimaging applications.

The applications of surface registration vary from object detection and tracking to multimodality image integration for diagnosis or surgery planning. The B-spline based
point matching method allows nonrigid surface alignment without identification of point correspondence, and solves both point correspondence and nonrigid transformation. The experiments show that the nonrigid surface matching method can effectively register various surfaces from faces to anatomical structures from biomedical images. It also might offer a methodology for whole body biomedical image registration.

PET is an essential tool for diagnosis and longitudinal study of neurological diseases, and has been widely used to differentiate between benign and malignant tumors, determine tumor stages and assess therapies. Recently, PET also becomes a molecular imaging technique to characterize molecular or protein interaction in biological processes. PET partial volume effect largely biases the quantitative measurement of regional radioactivity. Such bias could result in incorrect estimation of local tracer concentration and eliminate the subtle change in metabolism or physiology as a function of an event or time. The MRI-guided partial volume correction method can improve both PET image contrast and quantification of radiotracer concentration. The method does not require MRI segmentation or prior information about tracer distribution; therefore, it might be useful for various PET applications as long as aligned anatomical images are available.

The dissertation demonstrated the potential of treating prostate tumors using Pc 4-PDT at human prostate tumor model CWR22. It evaluated the capability of MRI to monitor tumor early response to the treatment and provided image processing techniques to quantify the response at region and pixel levels. The developed imaging techniques and analysis algorithms might also offer useful tools for monitoring therapeutic efficacy and optimizing treatment planning in clinical applications.
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